

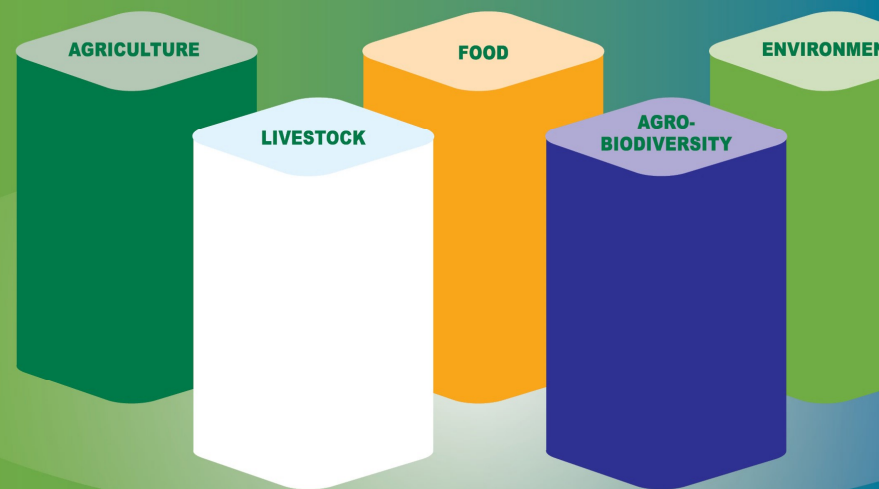
*"AGRO-BIODIVERSITY FOR LIFE AND ENVIRONMENT"*

The Journal of

# AGRICULTURE AND ENVIRONMENT

VOLUME: 24

JUNE 2023



GOVERNMENT OF NEPAL

MINISTRY OF AGRICULTURE AND LIVESTOCK DEVELOPMENT

SINGHADURBAR, KATHMANDU

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FOOD SECURITY AND FOOD TECHNOLOGY DIVISION

Singhadurbar, Kathmandu

***Telephone:*** +977-1-4211940

***Web:*** [www.moald.gov.np](http://www.moald.gov.np)

***Email:*** [info@moald.gov.np](mailto:info@moald.gov.np),  
[agrijournal@moald.gov.np](mailto:agrijournal@moald.gov.np) (manuscript submission)

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## EDITORIAL

Welcome to the 24th volume of The Journal of Agriculture and Environment. We are delighted to present a collection of research articles, review papers and insightful studies that highlight the crucial role of agriculture and environment for our nation's sustainable development in this issue of the journal in front of our readers.

Nepal boasts wealthy agro-biodiversity, with huge numbers of various accessions of genetic resources and wide range of ecosystem. However, unsustainable practices pose significant threats to biodiversity conservation causing degradation, losses and disappearance of local and indigenous genetic resources. Considering the importance of agrobiodiversity, Nepal celebrated year 2079 BS as Agro-biodiversity Year-2079 to conserve, protect and preserve the agro-biodiversity with the slogan, Agro-biodiversity for life and environment.

Agriculture forms the backbone of Nepal's economy, providing livelihoods for a significant portion of the population. As in many countries, smallholder farmers contribute a significant portion of population in Nepal. Nepal with its diverse agro-climatic, ecological regions and rich agricultural biodiversity, holds immense potential in ensuring food and nutrition security, enhance production and productivity of agriculture produce and livelihood improvement. However, we also face various challenges that demands our attention and concerted efforts for food system transformation. This journal aims to address challenges, foster knowledge exchange and inspire innovative solutions for the agriculture, livestock, food and environment sector.

The articles featured in this edition encompass wide range of topics, reflecting diversity and complexity of our agricultural landscape. Each articles contributes valuable insights to enhance the efficiency and resilience of our agricultural practices and advancement to our collective understanding from the studies on crop management, soil conservation, disease and pest management, livestock and fisheries production and management, food safety systems, agro-biodiversity, climate smart agriculture, climate change adaptation, agricultural policy and programs.

As the editorial team, our aim is to uphold the highest standards of academic quality, originality and relevance in the articles published. Each submission are rigorously reviewed by editorial members and undergoes a peer-review process by two reviewers, ensures the most impactful, scientifically sound and practical implications of the articles. We express our sincere appreciation to our esteemed reviewers, whose expertise and feedback have been invaluable in maintaining the journals' excellence.

We would like to extend our gratitude to the authors for sharing their valuable research findings and experiences. Your dedication to advancing the agriculture and environment sector is commendable, and your contributions are integral to shaping policies, informing and develop sustainable practices and creating positive impacts on agricultural communities.

Furthermore, as an editor-in-chief, I would like to acknowledge unwavering support of the Ministry of Agriculture and Livestock Development, all the members of Journal Publication Steering Committee and Journal Editorial Board members. It is through your vision and commitment to promoting research, innovation, views, experiences and sustainable development in agriculture and livestock due to which this journal came in this shape.

Finally, we express gratitude to the readers, researchers, scholars and all the stakeholders who supported The Journal of Agriculture and Environment. We hope that this edition of journal enriches the understanding, sparks meaningful discussions, and inspires further research and collaboration. Together, let us work towards a future where agriculture and the environment thrive in harmony, ensuring food security, ecological balance, and the well-being of our society. We welcome any feedback regarding the improvement of upcoming issues of the journal.

Thank you for continued support and trust in The Journal of Agriculture and Environment.

**Sanjeev Kumar Karn**  
Editor-in-Chief

## GUIDELINES TO AUTHORS: MANUSCRIPT PREPARATION AND SUBMISSION

The Journal of *Agriculture and Environment* is devoted to the cause of advancing understanding on the various aspects of Agriculture through literature review, theoretical analysis, research and practical experiences. Besides research and review papers, the journal may arrange spaces for case study, methodological approach, book review, report on seminar and meeting, short communication and letter to editor. Authors are requested to follow the following guidelines on preparation and submission of manuscript.

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# ADOPTION OF IMPROVED MANAGEMENT PRACTICES OF SUGARCANE IN BARA AND NAWALPARASI DISTRICTS

Anuma Bhattarai<sup>1\*</sup>, Udit Prakash Sigdel<sup>2</sup>, Pankaj Raj Dhital<sup>2</sup>, Ram Krishna Shrestha<sup>3</sup> and Srijana Timilsina<sup>1</sup>

<sup>1</sup> Ministry of Agriculture and Livestock Development, Government of Nepal

<sup>2</sup> Department of Agricultural Extension and Rural Sociology, Agriculture and Forestry University, Nepal

<sup>3</sup> Department of Agriculture, Ministry of Agriculture and Livestock Development, Government of Nepal

## ARTICLE INFO

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\*Correspondence:

bhattaraianuma@gmail.com

Tel: +977-9849758679

## ABSTRACT

*Agricultural technologies have an important role to improve productivity as well as the quality of the crops. However, the adoption of improved technology has remained poor for sugarcane in Nepal. A research was conducted in Bara and Nawalparasi (Bardaghat Susta west (BSw)) district of Nepal with a sample for 120 sugarcane growing farmers in the year 2019 to assess adoption of improved management practices of sugarcane. Descriptive statistics and inferential statistics were used to analyze the data. The logit model was used to identify the factors affecting adoption of improved management practices among farmers. Respondents were grouped into two categories, high adopter and medium adopter based on adoption index of each farmer. The average Adoption Index (AI) value obtained was 0.685. Around forty-nine percent of respondents were medium adopters and around fifty one percent were high adopters. The use of credit, ownership of agri-machine, and participation in training do have a significant influence on the adoption of improved practices. All variables had a positive relationship with the level of adoption of improved practices. The study revealed that farmers using credit, ownership of power-driven agri-machine, participation in crop related training increased the probability of farmer being high adopter. Agricultural machinery services, easy and cheap credit facilities and, easy access to extension services can help to increase in adoption of improved practices by sugarcane farmer.*

## 1. INTRODUCTION

Technology adoption is among the most revolutionary and impactful areas in the agricultural sector. Agricultural innovations also play a crucial role in fighting poverty, lowering per-unit costs of production (Berresaw, Shieferaw, & Muricho, 2011), and increasing rural income (Maertens & Barrett, 2013). Improving the livelihood of rural households through improving agricultural productivity would remain a mere wish unless the level of technology adoption is improved (Ajayi, Franzel, Kuntashula, & Kwesiga, 2003). In such regards, adopting agricultural technology becomes a key concern of agricultural extension workers, policymakers, agricultural researchers, and other stakeholders especially in developing country like Nepal.

Sugarcane is main commercial crops cultivated in tropics and sub-tropics region of Nepal (MoALD, 2018). It is cultivated in mid hills and Terai region but

is the major commercial cash crop in Terai region of Nepal. In Nepal, sugarcane is an important industrial crop, as it contributes 2.1% in AGDP and main source of income for 0.1 million farmers (MoALD, 2020; Pandey & Devkota, 2020). Many farmers of Terai are engaged in sugarcane cultivation. As it provides raw materials for a large number of industries, it is the major source of income in Terai (Neupane *et al.*, 2017). Before the establishment of Morang Sugar Mill in 1946; farmers were cultivating sugarcane in subsistence levels. Only after the establishment of Morang Sugar Mill, modern cultivation methods were used for commercial production of sugarcane in the country (Koirala, 1984).

Sugarcane industry is the only organized agro-industry in Nepal which is the source of income of thousands of farm families and hence contributes to poverty alleviation in rural areas (Nepal Agriculture Research

Council, 1997). Compared to the global average, Nepal's sugarcane yield is very low, most probably due to poor varietal selection (Sapkota *et al.*, 2019). Different biological and management factors like weeds, pests, disease, unscientific cultivation practices, selection of poor-quality sets are also major causes for low productivity of sugarcane in Nepal (Neupane *et al.*, 2017; NSMA, 2018). The average yield of sugarcane in the NARC research center with improved practices is very high compared to farmer's field (MoALD, 2020; Sapkota *et al.*, 2019). The productivity of sugarcane for Nepal is 49.48 t/ha and that of Nawalparasi and Bara district is 47.20 t/ha and 46.73 t/ha respectively (MoALD, 2022) whereas the productivity of India is 78.57 t/ha (FAOSTAT, 2021). This shows that there is poor adoption of improved technologies among the sugarcane growers, which is the main reason for the low level of productivity. Sugar recovery rate of Nepalese sugarcane is also lower than other global producers. The sugar recovery rate is around 10.5% in India and it is around 14 % in Brazil (Roy & Chandra, 2018). In Nepal, the recovery rate is 9.0 % (MoALD, 2020). The sugar recovery as a percent of cane crushed weight is affected by agronomic practices (cane quality) and post-harvest management (Roy & Chandra, 2018).

Low productivity of sugarcane and low sugar recovery rate are the two major problems being faced by sugarcane farmers and sugar factory, respectively, in Nepal. MoALD has identified a number of factors for the low productivity of sugarcane including low adoption of improved technology, farming for subsistence, unavailability of inputs, and limited investment (MoALD, 2018). Research stations under NARC have developed various improved technologies on sugarcane production in Nepal. Proper adoption of those technologies is expected to increase productivity and resultant income of sugarcane farmers. However, the adoption level of those technologies by the farmer is low as evidenced by the low productivity. Moreover, poor adoption of improved agronomic practices and post-harvest management techniques have also been associated with the low recovery rate in Nepal. This study was carried out to find the level of adoption and factors affecting level of adoption of improved management practices of sugarcane in Bara and Nawalparasi-Bardaghat Susta west (BSw) Districts.

## 2. MATERIALS AND METHODS

### 2.1 Study area and data collection

Ramgram Municipality and Triveni-Susta rural

municipality of Nawalparasi (BSw) districts were purposely selected to cover the command area of sugar mills. Similarly, Jitpur-Simara sub-metropolitan city and Parwanipur rural municipality were selected from Bara district considering the location of as National Sugarcane Research Center and Sugar mills.

From each rural/municipality two wards were randomly selected. Sampling frame of each ward was prepared with the key informant survey. Individual sample was selected randomly with the lottery method from each sampling frame. Fifteen individuals from each ward were selected. Altogether 60 samples from the Nawalparasi (BSw) district and 60 samples from the Bara district were selected. Both the primary and secondary data were collected for the study.

### 2.2 Selection of improved management practices

The practices which are responsible for an increase in productivity and quality of production were mainly considered as major improved management practices for this study. The improved practices were those which are recommended by NARC, Nepal Industrial Crop Development Program (NICDP), and other related responsible agencies viz industry. Altogether eight such practices were selected for the study.

#### 2.2.1 Varieties:

Score was given on the basis of the recommended variety was planted. The recommended varieties indicate the varieties recommended by NARC (Jitpur Lines) or the corresponding sugar industry Viz. Co 0232, Co 0238, selection of varieties for ratoon cropping.

#### 2.2.2 Quality of sets and sets treatment:

The quality of sets indicates the disease and insect free sets and the part of the plant used to prepare sets, numbers of buds per sets. Score was given on the basis of quality sets used and treatment of sets done by fungicide or hot water.

#### 2.2.3 Fertilizer application:

Fertilizer dose, time of application, split application of nitrogen, use of micronutrient and organic manure were considered under this practice. The recommended dose of NPK and Zinc from NARC was considered as the recommended fertilizer dose used as the standard for the study.

#### 2.2.4 Irrigation management:

It indicates the frequency of irrigation. The standard was

fixed as recommended by NICDP (2018) and NARC.

### 2.2.5 Weed management:

Number of weeding of the main crop was studied in this topic. The reference was taken from the recommendation made by NARC and sugarcane production manual of NICDP and NARC.

### 2.2.6 Intercultural operation:

The intercultural operation included gap filling, earthing up, cane propping. Score was given on the basis of the adoption of these activities.

### 2.2.7 Insect and disease management:

In this topic the regular monitoring of field to check the status of insect pest infestation was studied.

### 2.2.8 Postharvest management:

Storage of harvested cane in a shade place and milling on time were considered important post-harvest technology for sugarcane.

## 2.3 Score assign to each selected practice

The total score for selected eight practices was identified i.e.90, based on the discussion of sugarcane experts from NARC, staff of sugar mill, other experts and leader farmers. Out of the total score; 90, the distribution of the scores for the each practices was done with respect to the relative importance of the practices on improvement of productivity and quality of product (Table 1). The above mention eight practices were further sub-divided into sub-practices and scores for each sub-practices were provided based on the marks allotted to each of the main eight practices by the same member of experts and leader farmers.

**Table 1.** Score assigned to each selected practice

| S.N. | Improved Management Practices      | Score allotted |
|------|------------------------------------|----------------|
| 1    | Variety selection                  | 17             |
| 2    | Quality of sets and sets treatment | 10             |
| 3    | Fertilizer application             | 20             |
| 4    | Irrigation management              | 15             |
| 5    | Weed management                    | 10             |
| 6    | Intercultural operation            | 8              |
| 7    | Insect and disease management      | 5              |
| 8    | Post-harvest management            | 5              |
|      | Total                              | 90             |

## 2.4 Data analysis

Data entry and analysis were done by using computer software package; Stata (Version 13.0) and Microsoft Excel. Both descriptive and analytical methods were used to analyze the data. Binary Logit regression model was used to identify the factors affecting adoption of improved management practices of Sugarcane.

### 2.4.1 Technology adoption level

Rate of adoption refers to the full or partial use of recommended production technologies by the respondents during the last two year. The respondents were asked whether they adopted a particular practice or not, if they adopted, they were given a score, if not, they were not given any score. The individual farmer's overall adoption score was calculated by adding response components of improved technology. The extent of adoption of improved technology was determined by using the following formula (Dongol, 2004).

$$\text{Adoption Index} = \frac{\text{No of adopted technology in last two years}}{\text{Total no of technology recommended}}$$

Based on the level of adoption farmers were classified under two categories either medium adopter or high adopter based on their adoption index (Dhital & Joshi, 2016). From the adoption index, we can assess the level of adoption of each respondent.

**Medium Adopter:** For this study, medium adopter refers to the farmers who had got the value of Adoption Index below the average level of total farmers.

**High adopter:** High adopter means that the farmers who adopted the improved practice more than medium adopter does or whose AI is above or higher the average of the total farmer.

### 2.4.2 Factors affecting adoption of improved technology

The adoption of a new technology depends upon different factors such as age, gender, level of education, experience, land holding, nature of farming and participation in training, income, livestock holding, access of credit, tenure status and perception of farmers toward the crop. A logistic regression model was used to know the important factors responsible for adoption of improved sugarcane production technology. The level of adoption function is given as:

$$Y_i (\text{high adoption}=1) = b_0 + b_1 X_{1i} + b_2 X_{2i} + \dots + b_k X_{ki} + e_i$$

Where

$Y_i$  = ith observation of the dependent variables,  $Y_i$  = Adoption (dummy): 1 if high adopter and 0 for medium adopter.

$X_1, \dots, X_{14}$  = explanatory variables explained as below

$b_0$  = intercept term (constant)

$b_j$  = coefficient for each of the independent variables

$e_i$  = error term

$X_{ji}$  = ith observation of jth independent variables

$i=n$  = number of observations=120

**Table 2.** Description of variables and data used in the Logit model of regression

| Variables               | Description                                                       | Value                                           | Type       |
|-------------------------|-------------------------------------------------------------------|-------------------------------------------------|------------|
| Age                     | Age of farmer                                                     | year                                            | continuous |
| Education               | Years of education                                                | year                                            | continuous |
| Family size             | Family size                                                       | number                                          | continuous |
| Credit                  | Use of W credit                                                   | If yes=1, no=0                                  | Dummy      |
| Occupation              | Occupation                                                        | If only agriculture=1, agriculture+ off-farm =0 | Dummy      |
| Farm size /land holding | Total land holding                                                | Area in ha                                      | Continuous |
| Income                  | Annual HH income                                                  | Rs. in thousand                                 | Continuous |
| Experience              | Years of experience in sugarcane cultivation                      | years                                           | Continuous |
| Agriculture machinery   | Ownership of power-driven machine                                 | If yes=1, if no=0                               | Dummy      |
| Training                | Participation in sugarcane related training or workshop           | If yes=1, if no=0                               | Dummy      |
| Group                   | Involvement of farmers in farmer in farmers group or cooperatives | If yes=1, if no=0                               | Dummy      |
| Personal localities     | Contact with personal localite sources of information             | If yes=1, if no=0                               | Dummy      |
| Personal cosmopolite    | Contact with personal cosmopolite sources of information          | If yes=1, if no=0                               | Dummy      |

### 3. RESULTS AND DISCUSSION

#### 3.1 Demographic and Socio-economic Characteristics of Farmers in Study Area

Family size (7.5) of study area is more than national

average (4.37) (NSO, 2023), and land holding (2.25 ha) is also more than national average (0.68 ha) (CBS, 2011a). Credit use household (25%) is quite similar to national (21%) scenario (CBS, 2011b). Majority of respondent have only agriculture occupation (89.17%) which is also more than national average (NSO, 2023). Most of the respondents (86.67%) were literate with the average year

of schooling 6.74 years which is higher than the national literacy rate (76.2 %) in 2021 (NSO, 2023). The study sample comprised more Bramin, Chhetri and other caste group (61.67%) but the national scenario is quite different (Bramin: 12.2%; Chhetri: 16.6%, Yadav: 3.98%; Thakuri: 1.61%) (CBS, 2011b). Training participation of farmers (21.67%) was very low as compared to Pokherel *et al.* (2019) who found that 76.20% farmers were participated in sugarcane related training in eastern region of Nepal. Though group/cooperatives approach is promoted by government, only 40% respondent were associated with group/ cooperatives.

### 3.2 Adoption index and level of adoption

The average adoption index was 0.685 and it ranges from 0.403 to 0.916 having standard deviation of 0.1306. The respondents were categorized into two categories on the basis of average adoption index, i.e. high adopter having adoption index more than average and medium adopter having adoption index less than average.

Out of the total, high adopters and medium adopters were approximately equal, 50.83 percent of respondents were high adopters and 49.17 percent were medium adopters.

### 3.3 Characteristics of high and medium adopter

**Table 4.** Socioeconomic and demographic characteristics of high adopter (HA) and medium adopter (MA) of improved management practices of sugarcane

| Variables                           | HA n=61 | MA n=59 | T test              | Chi square           |
|-------------------------------------|---------|---------|---------------------|----------------------|
| Age                                 | 47.95   | 46.83   | 0.52 <sup>NS</sup>  |                      |
| Caste/ethnicity (BC=1)              | 43 (61) | 31(59)  |                     | 4.08 <sup>**</sup>   |
| Year of education                   | 7.91    | 5.52    | 3.48 <sup>***</sup> |                      |
| Family size                         | 7.47    | 7.69    | -0.32 <sup>NS</sup> |                      |
| Use of credit ( Yes=1)              | (25)61  | (5)59   |                     | 16.9 <sup>***</sup>  |
| Land holding                        | 2.85    | 1.64    | 2.28 <sup>**</sup>  |                      |
| Occupation (only agriculture=1)     | 54(61)  | 53(59)  |                     | 0.053 <sup>NS</sup>  |
| Total -income                       | 817.21  | 393.47  | 4.60 <sup>***</sup> |                      |
| Experience                          | 15.81   | 11.11   | 2.98 <sup>**</sup>  |                      |
| Ownership of Agri-Machine (yes=1)   | 46 (61) | 25 (51) |                     | 13.54 <sup>***</sup> |
| Membership ( Yes=1)                 |         |         |                     | 2.362 <sup>NS</sup>  |
| Training/workshop ( Yes=1) ( Yes=1) | 25(61)  | 1(59)   |                     | 0.0 <sup>***</sup>   |
| Personal _ Localite Yes=1           | 56      | 53      |                     | 0.14 <sup>NS</sup>   |
| Personal Cosmopolite ( Yes=1)       | 53      | 36      |                     | 10.47 <sup>***</sup> |

Figures in the small parenthesis indicate percentage. \*\*\* indicates significant at 1% level and \*\* indicates significance at 5% level

(Source: Field Survey, 2019)

**Table 3.** Distribution of farmers based on their location on the basis of their adoption index

| Districts         | Medium adopter | High adopter | Total    | Chi-square value    |
|-------------------|----------------|--------------|----------|---------------------|
| Bara              | 16(26.67)      | 44(73.33)    | 60(100)  | 24.3 <sup>***</sup> |
| Nawalparasi (BSw) | 43 (71.67)     | 17 (28.33)   | 60(100)  |                     |
| Total             | 59 (49.17)     | 61(50.83)    | 120(100) |                     |

Figures in the small parenthesis indicate percentage. \*\*\* indicates significant at 1% level (Source: Field Survey, 2019)

More than two third of the farmers of Bara were high adopters but about only one third of sugarcane farmers of Nawalparasi(BSw) were high adopter. The difference might be due to the existence of National Sugarcane Research Program in Bara districts, which facilitates farmers of Bara to have easy access for technical information and supports.

The average adoption index of study area was 0.685. It is lower than sugarcane farmers of Sri Lanka with average AI 0.72 (Peiris *et al.*, 2012). Similarly, it is also lower than average AI of couliflower farmers of Nepal with average AI 0.741 (Dhital & Joshi, 2016).

### 3.4 Factors affecting the adoption of improved management practices of sugarcane

This study was focused on the factors affecting the level of adoption of improved management practices. The association of selected variables (Table 2) and level of adoption was studied using logit model. To test the problem of multicollinearity, an estimate of the correlation between explanatory variables used in the regression model was carried out, showing no correlation between and among the variables that have

been used in linear regression. Overall, the model predicted 79.17 percent of the sample correctly. The Wald test (LR  $\chi^2$ ) for the model indicated that the model had good explanatory power at the 1% level. The Pseudo  $R^2$  was 0.3905. For the interpretation of the model, marginal effects were driven from the regression coefficients, calculated from partial derivatives as a marginal probability. The result from the Hosmer-Lemeshow's goodness fit showed a chi square with large P-value which indicates that this model presents a good adequacy and fits the data well.

**Table 5.** Factors affecting the level of adoption of improved management practices of sugarcane in the study area using logit model

| Variable                         | Coefficients | P> z  | Standard error | dy/dx <sup>b</sup> | S.E <sup>b</sup> |
|----------------------------------|--------------|-------|----------------|--------------------|------------------|
| Age                              | -0.0113      | 0.696 | 0.029          | -0.0015            | 0.0040           |
| #Caste/ethnicity                 | 0.7069       | 0.253 | 0.618          | 0.097              | 0.084            |
| Years of education               | 0.035        | 0.645 | 0.077          | 0.0049             | 0.0049           |
| Family size                      | -0.0472      | 0.531 | 0.075          | -0.0065            | 0.010            |
| #Use of Credit <sup>***</sup>    | 2.012        | 0.006 | 0.737          | 0.2788             | 0.089            |
| Landholding                      | -0.163       | 0.264 | 0.146          | -0.0225            | 0.019            |
| #Occupation                      | -0.611       | 0.530 | 0.814          | -0.0708            | 0.1121           |
| Total-income                     | 0.0014       | 0.227 | 0.0011         | 0.0001             | 0.0001           |
| Experience                       | 0.0492       | 0.184 | 0.037          | 0.0068             | 0.005            |
| #Mechanization <sup>**</sup>     | 0.9469       | 0.015 | 0.388          | 0.1311             | 0.048            |
| #Membership                      | 0.1195       | 0.831 | 0.559          | 0.0165             | 0.077            |
| #Training/workshop <sup>**</sup> | 2.835        | 0.018 | 1.19           | 0.3927             | 0.152            |
| #Personal_Localite               | -0.4108      | 0.629 | 0.850          | -0.0569            | 0.117            |
| #Personal cosmopolite            | 0.7868       | 0.216 | 0.635          | 0.1089             | 0.085            |
| Constant                         | -2.105       | 0.252 | 1.83           | -                  | -                |

#### Summary statistics

|                            |                                                        |
|----------------------------|--------------------------------------------------------|
| Number of observation (N)  | 120                                                    |
| Log likelihood             | -50.68                                                 |
| LR $\chi^2(14)$            | 64.95 <sup>***</sup> (Prob> $\chi^2$ = 0.000)          |
| Pseudo R <sup>2</sup>      | 0.3905                                                 |
| Goodness of fit test       | Person $\chi^2(105)$ = 102.20 (Prob> $\chi^2$ = 0.559) |
| Area Under RoC             | 0.8789                                                 |
| Overall Correct Prediction | 79.17%                                                 |

\*\*\*, \*\* and \* indicate significance at 1%, 5% and 10% levels, respectively. dy/dx indicates marginal change in probability (marginal effects after logit). # indicates dummy variables (1=Yes)

The result showed (Table 5) three variables were statistically significant for the level of adoption and they were; use of credit, ownership of agri-machine, and training participation. Eleven other variables namely age, ethnicity, year of education, family size, Occupation, landholding, annual income, year of experience, membership in groups, personnel localite, and cosmopolite source of information were statistically non-significant.

The study revealed that the use of credit for purposes of farming was positively associated with technology adoption and the level of association was highly significant (at  $p=0.01$ ). From the table 5 it is observed that the probability of a farmer being high adopter is increased by 27.88 percent when he/she have access of credit facilities for sugarcane cultivation. This finding was somewhat similar to the findings of Dixon *et al* (2006); Mohamed and Temu, (2008); Ullah, Khan, Zheng and Ali, (2018); Jerop *et al.* (2018), showed availability of credit increased the probability of adopting new technologies. It is believed that access to credit facilitates the adoption of risky technologies through relaxation of the liquidity constraints as well as increasing household risk-bearing abilities (Simtowe & Zeller, 2006). Sugarcane is long duration crop; farmers have to wait for long period to get income from crop. In present situation, Sugar mills are also not paying to farmers on time. In such situation, credit amount facilitates farmers to purchase of seed/set, fertilizer, payment to labour and to perform other activities, as credit improve the liquidity position of farmers.

Ownership of power-driven agri-machine was positively and significantly ( $p=0.05$ ) associated with adoption of improved management practices of sugarcane. The probability of farmers being high adopter is increased by 13.11 percent as they owned power driven agriculture machinery.

It was similar to Ullah *et al.*, who (2018) showed that ownership of machinery confirmed the positive and significant effect on improved cultivar adoption. Ownership of machinery increased the probability, which might be due to these machines facilitate in the operationalization of different management practices viz. weeding, irrigation management, intercultural operation as well as post-harvest management. Adoption of mechanization is itself adoption of innovation.

Participation in training/workshop related to sugarcane production also has positive relationship with technology adoption ( $p=0.05$ ). If a farmer participates in training/

workshop related to sugarcane, the probability of farmer being high adopter is increased by 39.27 percent.

Participation on training/workshop had positive and significant relation with adoption of improved practices. Several research studies found training has positive and significant impact on adoption of technology (Bhusal, 2012; Ghimire, 2013; Ghimire & Kafle, 2014; Li *et al.*, 2019). This is anticipated that training impacts knowledge and skill, and provide an opportunity to farmers to learn importance of improved technology and help to learn the way of best use of innovation.

The study revealed that though non-significant, age was negatively associated with technology adoption but the influence of age on technology adoption is very negligible. Similarly, family size, farm size, occupation, and personnel localite source of information were also negatively associated with adoption level of technology but their influences were non-significant. Moreover, ethnicity, year of education, year of experience, total income, membership in a group, and personal cosmopolite source have positive but non-significant relationship with the level of adoption.

#### 4. CONCLUSION

This research shows that farmers in study area were partially adopting the recommended improved practices of sugarcane production and post harvest management. On an average 68.5 percent of recommended practices were being adopted by the sugarcane farmers. This can partially explain the low productivity of sugarcane and low recovery rate prevailing in the study area. However, presence of most of the high adopters in Bara, reveals that location of sugarcane research station, indicates the technology spill overthrough demonstration effect in nearby farming population of agriculture research and development institution.

The study suggests that in study area, sugarcane farmers who have an access to institutional credit; owns farm machineries to perform basic farm operations including ploughing, planting, harvesting etc. and often participates in related training and workshop tend to adopt improved practices in sugarcane. So the policy related to agricultural credit, agricultural machinery services and knowledge and skill development program to farmers helps in the adoption of improved practices and hence improve the productivity and quality of sugarcane.

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# ANALYSIS OF RESOURCE USE EFFICIENCY AND PROFITABILITY OF WHEAT PRODUCTION IN KAILALI DISTRICT, NEPAL

Rajendra Bam<sup>1\*</sup>, Ganesh Mahara<sup>2</sup>, Kapil Khanal<sup>2</sup>, Rishiram Kattel<sup>2</sup> and Suman Bhattarai<sup>1</sup>

<sup>1</sup> Institute of Agriculture and Animal Science, Tribhuvan University

<sup>2</sup> Agriculture and Forestry University, Rampur, Chitwan

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\*Correspondence:  
rajendrabikrambom@gmail.com  
Tel.: +977-9848470991

## ABSTRACT

A study was done in 2021 in the Kailali rural municipality, Gauriganga municipality, Godawari municipality, and Dhangadhi sub-metro politian city of the Kailali district to analyze the economics of wheat production. A total of 200 samples (50 from each village) were selected by using a simple random sampling technique. Most respondents (91 percent) had agriculture as a primary occupation. The area under wheat (*Triticum aestivum*) cultivation per household was 0.6 hectare with no significant difference between super zone of the Prime Minister Agriculture Modernization Project and non-super zone areas. The average production per household was 1814.5 kg whereas the yield was 3.3 mt/ha. The average cost of production was NRs. 64,526.62 per hectare and the B:C ratio was found to be 1.492. The Cobb-Douglas function showed that fertilizer, pesticide, land preparation, irrigation, and threshing costs were significant contributing factors to wheat production. The return to scale was found to be 0.261. An Index of severity was constructed to study the problems of wheat production in the study area. Weed infestation, lack of fertilizers, and insect pests were found to be three major problems.

## 1. INTRODUCTION

Wheat (*Triticum spp.*) is a cereal grain, originated from the Southwest Asia, but now cultivated worldwide. It has been described as the “King of Cereal” (Agam *et al.*, 2017). It can be grown from below sea level to 5000 m altitude and in areas where rainfall ranges between 300-1130 mm (Kumar *et al.*, 2014). Wheat (*Triticum aestivum*) belongs to the family Gramineae (grass family). Wheat is one of the most important food grains of Nepal and is the staple food of Nepalese people. It is cultivated in 21% area of land and accounts for 17% of total cereal production in Nepal (Sharma, 2018). Wheat contributed 7.1 % in agricultural Gross Domestic Product (GDP) following rice (20.575%) as well as vegetable (9.69%) and followed by maize (6.88%) during the fiscal year 2017/18 (Sharma, 2018). Nepal so far has forty two (42) wheat varieties (SQCC, 2023). Wheat was cultivated in 703,992 ha of land in fiscal year 2018/19 and the production was 2,005,665 metric tons with the productivity of 2.849 mt/ha (MoALD, 2020). In most of the urban areas in the country, the use of baked leavened bread, cakes, and biscuits are increasing at faster rate creating higher demand of wheat flour and the higher deficit of flour in the country (Sharma and Chauhan, 2003).

Ministry of Agricultural and Livestock Development (MoALD) has launched the 10 years Prime Minister Agriculture Modernization Project (PMAMP) in 2016/17. The MoALD has envisaged pockets, blocks, zones and super zone for agriculture commodity to address the fragmentation of arable land, which is considered to be a major barrier for agriculture commercialization and mechanization in the country (PMAMP, 2016/17). As per PMAMP, at least 10 ha of land is needed to identify it as a ‘pocket area’; 100 ha to be considered a ‘block’; 500 ha to be termed as a ‘zone’, while a ‘super zone’ would make up of 1,000 ha of area. Kailali is one of the super zones established in 2017 A.D for specialization and commercialization of wheat in that region. Wheat superzone not only assists in mechanization and commercialization, but it also provides various technical assistances to the farmers through its technicians and specialists (Devkota *et al.*, 2020). It also aims to enhance the livelihood and economy of the region through a transformation in wheat cultivation and production technology (Wheat Super zone, 2018). But little is known about the perception of farmers towards the activities of super zone and its impact at the farm level. It is, therefore, necessary to know how

far PMAMP, Project Implementation Unit (Wheat Super zone) has been able to promote the adoption of improved wheat cultivation practices in the area. Farmers were facing high cost of inputs, adulteration of seeds, shortage of chemical fertilizers, insecticides & pesticides (Kumar S. , 2001). Thus, this study was deemed necessary and was undertaken to determine the profitability and resource use efficiency of the beneficiaries of super zone compared to non-beneficiaries.

## 2. MATERIALS AND METHODS

### 2.1. Site of study

Kailali district was selected as the research area. The Kailali district lies between 28022'N to 81030'E and elevation ranging from 109 and 1950 meters above sea level. The climate varies from tropical to subtropical. Kailali district has been selected as a wheat production super zone to be established under the Prime Minister Agricultural Modernization Project (PMAMP).

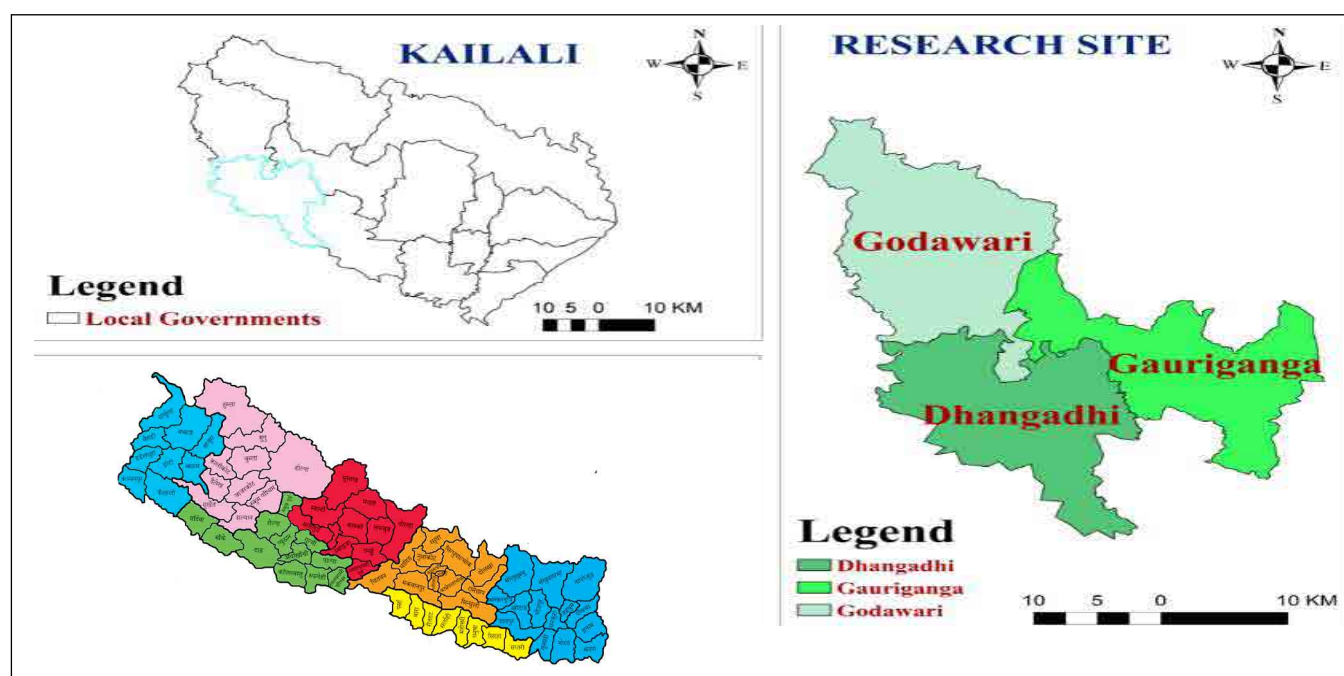


**Figure 1.** Map of Nepal showing research sites (GIS, Extract)

### 2.2. Sample size

The size of the sample, and amount of variation, usually affect the quantity and quality of information obtained from the survey. Using appropriate sampling methods, both factors can be controlled (Scheaffer, 1979). Casley and Kumar (1988) and Kinnear and Tayler (1987) suggested that a good survey sample should have both a small sampling error and minimum standard error. A sample size of 60 is generally regarded as the minimum requirement for larger population that yields a sufficient level of certainty for decision-making (Poate and Daplyn, 1993). Selection of wheat growers was from different rural municipalities. Altogether 200 wheat growers were selected randomly for the survey taking 50 growers each from Kailari Rural Municipality, Gauriganga Municipality, Godawari Municipality and Dhangadhi Sub-Metro Politian. A total of 100 samples

were taken from household who were member of PMAMP (Kailari and Rajipur villages) while remaining samples were from non-member households.

### 2.3. Sample selection procedure

The best way to avoid bias in the sample selection process is use of simple random sampling in which each unit of the population has an equal chance for selection (Scheaffer, 1979). Thus by using sampling frame, a simple random sampling procedure was used to collect necessary information from wheat growers. The procedure was comprehensive and representative of the whole population.

### 2.4. Methods of data collection

Various sources and technique were used for the collection of necessary data. In this study, both the

primary and secondary data were collected. The methodologies consisted of field survey, review of previous studies, and interviews with key informants.

### 2.5. Techniques of data collection

Primary data were collected through interview schedule. Information on various aspects of wheat production was collected. Information regarding the farm and household characteristics, production and management aspects was collected through face to face interview.

### 2.6. Pre-testing of interview schedule

Organizing fieldwork plan, testing the validity of the questionnaire and estimating the various cost components such as financial costs, travel time, interview time etc. before main survey were taken into consideration. The interview schedule was pre-tested in nearby villages with 10 farmers from Mahara village, prior to administering to the actual respondents which is common in the pre-test of the questionnaire (Perneger *et al.*, 2015). Necessary modifications were made and the schedule was revised and put in the final form.

### 2.7. Methods and techniques of data analysis

After collection of necessary data, they were coded and entered into the computer for analysis. Data was fed and analysis was done by using statistical packages for social sciences (SPSS) and Stata-16.

### 2.8. Quantitative data analysis

Quantitative data were analyzed by using both descriptive and analytical statistics. Socioeconomic and farm characteristics of the respondents like family size, age, gender, occupational pattern, land holding size, economically active population etc. were described using simple descriptive statistics like frequency count, percentage, mean, standard deviation etc.

### 2.9. Benefit-cost ratio

Benefit cost analysis is done by the calculation of total variable cost and gross return from wheat cultivation. Cost of production was calculated by summing the variable cost items incurred in the production process. Benefit cost ratio is the quick and easiest method to determine the economic performance of a business (Dhakal *et al.*, 2015). For calculating gross return, income from product sale was accounted. So, the benefit cost analysis was carried out by using formula:

$$\frac{B}{C} = \frac{\text{Gross return}}{\text{Total variable cost}}$$

### 2.10. Cobb-douglas production function

Cobb-Douglas production function is one of the most commonly used function type especially in agricultural economic studies (Kip and Isyar, 1976). Cobb-Douglas production function is employed to estimate the coefficient of variables analyzed and Marginal Physical Product (MPP), Minimum Viable Product (MVP) and allocate efficiency index, which is also used to estimate the efficiency of resources use (Osti *et al.*, 2017). Cobb-Douglas production function was used to assess the factors determining production of wheat in 0.0038 ha of land in the study area. The choice of the functional form was based on its theoretical fitness to agriculture and its computational manageability. Further most production studies in agricultural sector have used this function. The model specified and used was represented by:

$$Y = AK^{b_1}L^{b_2}M^{b_3}N^{b_4}O^{b_5}P^{b_6}Q^{b_7}R^{b_8}$$

Where Y was dependent variable and K, L, M, N, O, P, Q and R were the explanatory variables. 'A' was the constant and  $b_1, b_2, b_3, b_4, b_5, b_6, b_7$  and  $b_8$  were the production coefficients. The production function was converted into logarithmic form so that it could be solved by least square method i.e.

$$\log Y = \log A + b_1 \log K + b_2 \log L + b_3 \log M + b_4 \log N + b_5 \log O + b_6 \log P + b_7 \log Q + b_8 \log R + E$$

Where,

Y = Production in 0.0038 ha (kg)

K = Seed (NRs)

L = Fertilizers (NRs.)

M = Manure (NRs.)

N = Pesticides (NRs)

O = Land preparation (NRs)

P = Irrigation (NRs)

Q = Harvesting (NRs)

R = Threshing (NRs)

E = Error Term

## 2.11 Efficiency ratio estimation through cobb-douglas production function

The efficiency of a resource used was established by the proportion of the Marginal Value Product (MVP) of variable input and the Marginal Factor Cost (MFC) for the input. However, the denominator always sets its value as one, and consequently, the ratio will be equal to their respective MVP (Majumder *et al.*, 2009).

The efficiency of resource use was calculated as:

$$r = \frac{MVP}{MFC} \text{ (Goni et al., 2007)}$$

Where,

r: Efficiency ratio

MVP: Marginal value product of a variable input

MFC: Marginal factor cost

$MVP = dy/dx$

$MVP_i = b_i \cdot Y/X_i$

Where,

$b_i$ : Estimated regression coefficients

Y and  $X_i$  are the values from the geometric mean

Decision criteria as given by Effiong (2005):

$r = 1$  indicates the efficient or optimum use of resource

$r > 1$  indicates under-use of resource

$r < 1$  indicates over-use of resource

The relative percentage change in MVP of each resource was estimated as:

$$D = \left( \frac{MFC}{1-MVP} \right) \times 100$$

$$\text{or, } D = \left( 1 - \frac{11}{rr} \right) \times 100$$

Where,

D: absolute value of percentage change in MVP of each resource

r: efficiency ratio

## 2.12. Return to scale analysis (RTS)

Return to scale provides the technical property of production which examines changes in output consequent to the relative change in all inputs. It is

obtained by summing up of coefficients (Bajracharya and Sapkota, 2017). For the calculation of return to scale on wheat production, coefficients from Cobb Douglas production function was used and calculated using the formula:

$$\text{Return to scale (RTS)} = \sum b_i$$

Where,  $b_i$  = Coefficient of  $i$ th explanatory variables

## 2.13. Indexing

Qualitative data was analyzed using indexing. Index of severity was constructed for various problems faced by wheat growers in super zone and non-super zone area using three point scale. The scale value assigned for three scales of problems viz. high, little bit and no problem were 2, 1 and 0 respectively. The problems were ultimately ranked on the basis of index values. The formula given below was used to find the index for intensity of production and marketing problem faced by producers and traders respectively.

$$I_{\text{prob}} = \sum \left( \frac{S_{if_i}}{N} \right)$$

Where,

$I_{\text{prob}}$  = Index value for intensity of problem

$S_i$  = Scale value of  $i$ th intensity

$f_i$  = Frequency of  $i$ th response

N = Total number of respondents

## 3. RESULTS AND DISCUSSION

### 3.1. Socio-economic and demographic characteristics

Descriptive analysis of various socioeconomic, demographic, and institutional variables are presented in Tables 1 and 2. The average age of household heads indicated that farmers had sufficient skills and experience in the production process. The average household size was higher than her national average of 4.88 (CBS, 2012). This data is higher than the national proportion of the population engaged in agriculture (65.6), as suggested by CBS (2012).

**Table 1.** Description of socio-economic, demographic and institutional variables

|                                  | Total (n=2000) | Super zone (n=100) | Non-super zone (n=100) | Chi-square value |
|----------------------------------|----------------|--------------------|------------------------|------------------|
| Occupation                       |                |                    |                        |                  |
| Agriculture                      | 182 (91)       | 89(89)             | 93 ( 93)               | 0.977            |
| Teaching                         | 7 (3.5)        | 4(4)               | 3(3)                   |                  |
| Business                         | 4 (2)          | 2(2)               | 2(2)                   |                  |
| Study                            | 2 (1)          | 1(1)               | 1(1)                   |                  |
| Government or Private sector Job | 4 (2)          | 4(1)               | 1(1)                   |                  |
| Ethnicity                        |                |                    |                        |                  |
| Brahmin                          | 58 (29)        | 37(37)             | 21(21)                 | 13.569***        |
| Chhetri                          | 30 (15)        | 23(23)             | 7(7)                   |                  |
| Janajati                         | 106 (53)       | 40(40)             | 66(66)                 |                  |
| Dalit                            | 6 (3)          | 0(0)               | 6(6)                   |                  |
| Total                            | 200 (100)      | 100(100)           | 100(100)               |                  |

Source: Field Survey, 2021

Figures in parentheses indicate percent

**Table 2.** Description of socio-economic, demographic and institutional variables

| Variables                               | Total (n=200) | Super zone (n=100) | Non-super zone (n=100) | Mean Differences | T-value   |
|-----------------------------------------|---------------|--------------------|------------------------|------------------|-----------|
| Age of the respondents (year)           | 43.56         | 42.94              | 44.19                  | 1.250            | 0.700     |
| Year of schooling of respondents (year) | 5.83          | 7.57               | 4.10                   | -3.470           | -4.761*** |
| Age of household head (year)            | 46.745        | 47.59              | 45.90                  | -1.690           | -1.050    |
| Landholding(hectare)                    |               |                    |                        |                  |           |
| Lowland own                             | 0.64          | 0.53               | 0.76                   | 0.23             | 3.127***  |
| Lowland rented in                       | 0.17          | 0.24               | 0.11                   | -0.13            | -2.123**  |
| Lowland rented out                      | 0.10          | 0.13               | 0.06                   | -0.068           | -1.665**  |
| Upland own                              | 0.075         | 0.081              | 0.069                  | -0.012           | -1.626*   |
| Total own                               | 0.72          | 0.611              | 0.832                  | 0.22             | 2.851***  |
| Total rented in                         | 0.17          | 0.24               | 0.11                   | -0.13            | -2.123**  |
| Total rented out                        | 0.109         | 0.14               | 0.06                   | -0.081           | -1.912**  |

Source: Field Survey, 2021

\*\*\* indicates the significant at 1% level of significant

### 3.2. Wheat production

Table 3 shows that area, production and productivity of wheat in the study area. The average land area under wheat cultivation was found to be 0.59 hectare. The

average wheat production was found to be 1814.5 kg per household while the productivity was 3.3 mt/ha.

**Table 3.** Wheat production status in study area

| Wheat production | Total (n=200) | Super zone (n=100) | Non-super zone (n=100) | Mean Differences | T-value |
|------------------|---------------|--------------------|------------------------|------------------|---------|
| Area             | 0.566         | 0.59               | 0.575                  | -0.015           | 0.331   |
| Wheat production | 54435         | 54675              | 54195                  | 480              | 0.778   |
| Productivity     | 3.3           | 3.55               | 3.04                   | 0.51             | 0.9     |

Source: Field Survey, 2021

### 3.2.1 Purpose of wheat production

Majority of respondents (55 percent) cultivated wheat for home consumption. In super zone area, 50 percent farmers cultivate wheat for home consumption while

remaining 50 percent cultivate to sell in the market. Likewise, in non-super zone area 60 percent farmers were found cultivating wheat for home consumption while 40 percent were cultivating for sale. The details are presented in Figure 2.

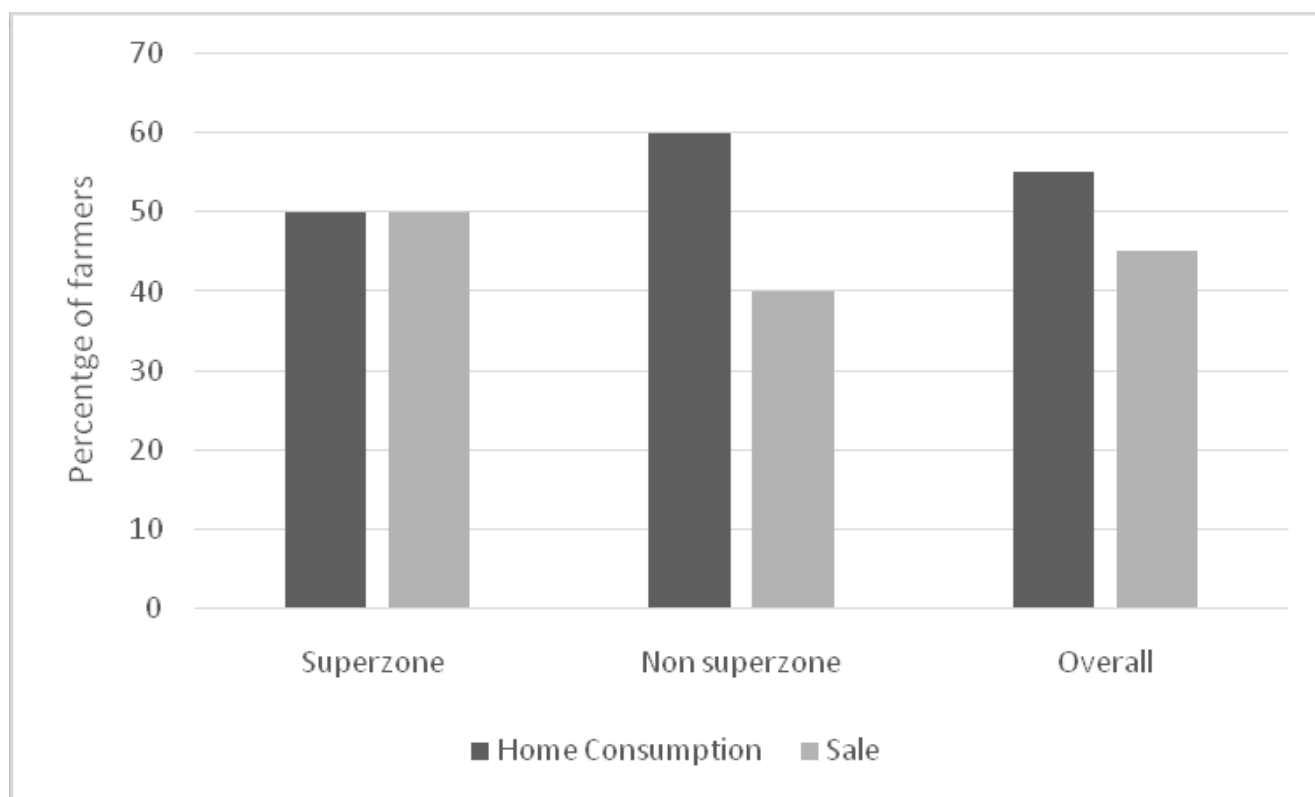


**Figure 2.** Bar diagram showing the purpose of cultivation of wheat in the study area

### 3.2.2 Production economics of wheat

#### 3.2.2.1 Benefit and cost from wheat production in the study area

The benefit cost ratio from wheat enterprise in the non-super zone and super zone area were found to be 1.487 and 1.497 respectively. The difference was found to be statistically non-significant but the ratio was higher in super zone area than in the non-super zone area. The average B:C ratio was found to be 1.492 as shown in Table 4.

**Table 4.** Average cost, benefit and B:C ratio from wheat production in the study area

| Description                               | Overall | Super zone | Non-super zone |
|-------------------------------------------|---------|------------|----------------|
| Average cost of production(NRs./ hectare) | 65430   | 63330      | 67560          |
| Average benefit(NRs/ hectare)             | 91410   | 90000      | 92850          |
| B:C ratio                                 | 1.492   | 1.497      | 1.487          |

Source: Field Survey, 2021

#### 3.2.3 Factors affecting the production of wheat

The different factors affecting wheat production is shown in Table 5. A Cobb-Douglas production function was estimated to determine the effect of different inputs (NRs.) on total wheat production (mt/ha). The Cobb-Douglas function was transformed into linear form by using the natural logarithm of both dependent and independent variables. After transformation, the Cobb-Douglas production function was estimated using Ordinary Least Square (OLS) method. The obtained coefficient represents the elasticity of individual inputs. The elasticity associates with all inputs were less than one. So, for inputs less than one, a unit increase in the respective input would result in less than a unit increase in wheat production. The overall F value was 23.64 and it was statistically highly significant at 1 percent level of significance. This indicates that the explanatory variables includes in model were important for the explanation of variation on wheat production. The R<sup>2</sup> value was 0.49 which implies that about 49 percent of variation on wheat production was explained by the explanatory variables.

There were five variables found significant at the 1 percent, 5 percent, and 10 percent levels. Regarding

fertilizer, we found that a 1% increase in fertilizer cost, keeping the other factor constant, resulted in a 0.06% decrease in wheat production, which was found to be highly significant at the 1% significance level. . Similarly, a 1% increase in pesticide costs leads to a 0.01% increase in wheat production, while the other factors remain constant and is statistically significant at the 10% significance level. We find that a 1% increase in land preparation costs reduces wheat production by 0.15% while other factors remain constant, which is statistically significant at the 5% significance level. . A 1% increase in irrigation costs was found to reduce wheat yield by 0.083%, which was found to be statistically significant at the 5% significance level. We also found that a 1% increase in threshing costs resulted in a 0.60% increase in wheat production, which was found to be statistically significant at the 1% significance level, suggesting that mechanization achieves efficiency.

The return to scale was calculated by summing up the elasticity of respective individual inputs which was estimated using Cobb-Douglas production function and found 0.261 which implies that there was decreasing returns to scale of wheat production.

**Table 5.** Factors affecting the production of wheat in the study area

| Variables               | Coefficient | Std. Err. | T     | p>t   |
|-------------------------|-------------|-----------|-------|-------|
| Log seed                | -0.009      | 0.049     | -0.18 | 0.854 |
| Log manure              | 0.0037      | 0.003     | 1.16  | 0.248 |
| Log fertilizer          | -0.061***   | 0.015     | -3.87 | 0.000 |
| Log pesticides          | 0.010*      | 0.006     | 1.79  | 0.075 |
| Log land preparation    | -0.154**    | 0.070     | -2.20 | 0.029 |
| Log irrigation          | -0.083**    | 0.035     | -2.36 | 0.019 |
| Log harvesting          | -0.054      | 0.045     | -1.20 | 0.233 |
| Log threshing           | 0.604***    | 0.049     | 12.18 | 0.000 |
| Summary Statistics      |             |           |       |       |
| Number of observation   | 200         |           |       |       |
| F                       | 23.64       |           |       |       |
| Prob>F                  | 0.000       |           |       |       |
| R square                | 0.497       |           |       |       |
| Adjusted R <sup>2</sup> | 0.476       |           |       |       |

Source: Field Survey, 2021

\*\*\*, \*\* and \* indicate 1%, 5% and 10% levels of significance, respectively.

#### 3.2.4 Extension services in the study area

The percentage of respondents who were in personal contact with the extension workers was found very

low (17 percent). The figure reached to 28 percent in the super zone while it was down to 6 percent in the non-super zone area. Chi squared value showed that the difference was significant at 1 percent level of significance. The percentage of respondents who were involved in trainings related to wheat cultivation was only 9 percent. The percentage of respondents involved in training was higher in super zone area but the difference was found to be insignificant.

A total of 36 percent of respondents from super zone

area were taking regular advice from technicians while the percentage doing so was significantly lower (11 percent) in non-super zone area. Overall, 23.5 percent of farmers were taking advice from technicians. Likewise, about 42 percent of total respondents were involved in different types of farmers' group. The detail about the status of extension worker is represented in Table 6. Devkota *et al.*, (2020) reported that when a farmer has contact with extension agent, yield increases by 0.419 mt/ha keeping other factors constant.

**Table 6.** Status of extension services in the study area

| Extension Services           | Total (n=200) | Super Zone (n=100) | Non-super zone (n=100) | Chi-square value |
|------------------------------|---------------|--------------------|------------------------|------------------|
| Personal contact (Yes)       | 34 (17)       | 28 (28)            | 6 (6)                  | 17.151***        |
| Training (Yes)               | 18 (9)        | 12 (12)            | 6 (6)                  | 2.198            |
| Advice from technician (Yes) | 47 (23.5)     | 36 (36)            | 11 (11)                | 17.383***        |
| Involve in group (Yes)       | 84 (42)       | 46 (46)            | 38 (38)                | 1.314            |

**Notes:** Figures in parentheses indicate percent. \*\*\* indicates significant different at 1% level

### 3.2.5 Problems in wheat production

Table No. 7 represents the index of severity regarding the problems in wheat production. Index of severity was constructed to study the problems in wheat production in the study area. The four major problems in non- super zone area were lack of fertilizers, weeds, insect problems and labor scarcity in descending order. Similarly, the four major problems in wheat production

in super zone area were weeds infestation, lack of fertilizers, insect problems and nutrient management in descending order.

Due to high temperature and high rainfall, most of the farmers were sowing poor quality seeds, which might be one of the main causes of low productivity of wheat in Nepal (Thapa, 2005).

**Table 7.** Index of severity regarding problems in production of wheat in super zone and non-super zone areas

| In super zone       | Index value | Rank | In non-super zone    | Index value | Rank |
|---------------------|-------------|------|----------------------|-------------|------|
| Weeds               | 0.85        | I    | Lack of fertilizers  | 0.97        | I    |
| Lack of fertilizers | 0.83        | II   | Weeds                | 0.88        | II   |
| Insect problems     | 0.78        | III  | Insect problems      | 0.86        | III  |
| Nutrient management | 0.77        | IV   | Labor scarcity       | 0.81        | IV   |
| Diseases            | 0.77        | V    | Climatic variability | 0.79        | V    |

Source: Field survey, 2021

### 3.3 Cost, return and profitability of wheat

The average production cost of wheat in the study area was NRs. 65430 per hectare. An average cost was slightly higher in non-superzone than in superzone. Similarly, average benefit from a 0.0038 ha of wheat cultivation was NRs. 3095 and NRs. 3000 for non super-zone can be explained by the fact that the non super-zone

areas are nearer to market thus fetching higher prices in comparison to super-zone. . The B:C ratio was found to be 1.492 which is higher than the national figure. The average benefit cost ratio of irrigated wheat in terai and hill district were about 1.39 and 1.53, respectively and Kailali district has the benefit cost ratio of 1.27 (MoAD, 2017). The higher B:C ratio can be simply explained as the survey site is the pocket region for wheat cultivation

and it is expected to have higher profitability than the national average. To support our finding, study done in Bangladesh in wheat showed B:C ratio of wheat to be 1.42 (Rahman & Hasan, 2019). The difference in B:C ratio was not statistically significant.

The average profit from 1/30 ha of land from wheat cultivation was NRs. 949.61 with B/C ratio of 1.43 (Yadav et al., 2022). BCR ration can be increased by RUE, found the overall undiscounted benefit cost ratio considering total variable cost to be 1.79 in neighboring district Kanchanpur (Bist et al., 2017). Bhujel et al., (2009) reported a benefit-cost ratio of 1.77 in a traditional method and 2.19 in machine used RCT in wheat production in the eastern terai region of Nepal. Ghimire, (2013) and Dhital, (2017) found BCR of 1.38 and 1.22 respectively in wheat production in Nepal. Similarly, Gairhe et al. (2017) also reported BCR in wheat production under irrigated and rainfed conditions as 1.2 and 1.3 respectively.

### 3.4 Factors contributing to wheat production

Cobb- Douglas production function is a kind of differentiable function type applied in industry and economy and has a bilateral logarithmic pattern (Tanriover and Genc, 2005; Gujarati, 2009). The regression estimates showed negative and significant contribution of fertilizers, land preparation, and irrigation costs on production of wheat. This showed that those resources were being overused and to obtain efficiency spending on these factors had to be decreased. Similarly, threshing showed positive and significant results. This showed that using thresher properly for longer period of time increases the production by decreasing the loss during threshing. The return to scale was found to be decreasing which means increase of factor proportion by a certain scale would result increase in output by a smaller scale.

The results of the efficiency estimation revealed that land was 6.63, fertilizer was 1.76 and seed was 10.84 were underutilized and labor was 0.000036 and chemical were being highly over utilized in the study area (Osti et al., 2017). The study examine resource use efficiency on yam production in Delta and Kogi States of Nigeria show that there were under utilization of land, labor and planting materials (seed yam) as the ratio of the

value of marginal product to marginal fixed cost were greater than one in both state (Ekunwe et al., 2008). Marginal value product to the factor cost ratio of human labor, seed, fertilizer and irrigation were negative and less than one that means there was excess used of these inputs, hence, there should be reduction in the use of these inputs for efficient wheat production (Mane et al., 2015). The study to examine resource use efficiency and the constraints faced in production of wheat crop in Betul district of Madhya Pradesh by the farmers to achieve the potential yield of wheat in 2013-2014, reveals that human labor was found significant for large (0.419) and overall (0.156) farm, while it non-significant for small and medium farms. Machine labor was found significant for all size of farm i.e. small (0.551) medium (0.526) large (0.370) and overall (0.687) farm size. Seed and fertilizer was found significant for small and overall farm. In case of marginal value productivity, it was found that in small size farm the factor of production i.e. machine labor, seed, fertilizer and irrigation had been found underutilized, whereas human labor implying overutilization under the study area (Gautam et al., 2017).

## 4. CONCLUSION

The benefit cost ratio of wheat in the study area was 1.492 which indicates wheat is a profitable crop in the area and thus wheat cultivation needs to be promoted. Chemical Fertilizers and land preparation showed negative contribution to production which shows the need to decrease fertilizers use and decrease the number of tillage, opening a door for conservation agriculture. Increase in thresher use showed positive effect on production which hints towards achieving efficiency through mechanization. The major problems of wheat cultivation in the study were lack of balanced dose of fertilizers, weed infestation and insect pests which shows the need of input suppliers, government organizations and agriculture experts.

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# ANALYSIS OF SUPPLY CHAIN AND FISH MARKETING IN NAWALPARASI (EAST), NEPAL

Mina Mahatara<sup>1,\*</sup> and Punam G.C.<sup>2</sup>

<sup>1</sup> Prime Minister Agriculture Modernization Project, PIU, Jajarkot

<sup>2</sup> Central Department of Zoology, Tribhuvan University

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\*Correspondence:

mahataramina72@gmail.com

Tel: +977-9845817816

## ABSTRACT

Study was conducted during October 2021 to November 2021, to investigate the supply chain, present market situation, marketing constraints, marketing margins, and the consumer's behavior and purchasing pattern towards the fish in Nawalparasi (East) district of Nepal. Fish producers, traders and consumers were surveyed through semi-structure questionnaire. Five fish Producers, eight wholesalers, twelve retailers and twenty consumers were randomly surveyed. Among 20 traders majority of the traders belonging to the Madhesi and Tharu community. Finding of the study revealed that fish trading almost dominated by male (84%). All the respondents involved in fish trading more than 30 years old. there was no specific marketing channel used by the farmers, 55.65% of total fish produce is sold to the wholesalers, 32.49% to the retailers and 11.86% is sold to the customers directly by the fish farmers. The marketing margin of Nepalese live fish Rohu and Common Carp was higher than other species of fish in the Nawalparasi (East). Nepali fish Rohu and common carp fetch highest price Rs 425/kg and Pangasius fetch lowest price Rs. 280/kg. Nepalese fish were mostly packed in plastic crates and transported by pick up van. Indian average fish sizes were 2.91 kg and Nepali average fish sizes were 1.91 kg. Among the major carp species; the most preferred one by the consumers was Rohu followed by Bhakur.

## 1. INTRODUCTION

Aquaculture is one of the fastest growing activity in the world that plays a significant role in the economy. The current total national fish production is 104,623 Mt of which 20 % is contributed by capture fisheries while 80% is from aquaculture (CFPCC, 2022). Fisheries sector contributes about 1.83% in Agricultural Gross Domestic Production and 0.44% in Gross Domestic Production (CFPCC, 2022).

The actual development of commercial fish farming and organized fish marketing in Nepal began with the implementation of Aquaculture Development Project under the support of Asian Development Bank (ADB) and United Nations Development Program (UNDP) after 1980 (Budhathoki and Sapkota, 2018). The value of fish as a supply of high quality protein has emphasized its important role in food security of the country. The fish marketing system of Nepal is self-regulating with increasing production and demand

among the consumers. The growth of fish production as well as development of fishery sector in terms of economy and infrastructure is highly dependent on an efficient fish marketing system (Chourey *et al.*, 2014).

Nawalparasi (East) is one of the leading fish farming districts of Nepal with increasing numbers of farmers involved in commercial fish farming. According to the data of 2021, there was total of 828 fish ponds with the production of 903 Mt annually (MoALD, 2021). Fish farm occupies 1123.17 hectares of land within the district. Nawalparasi has huge fish production potentiality with annual productivity of 4934 kg per hectare (MoALD, 2021). Despite the abundance of fisheries resources and relatively high consumption of fish in Nepal, the level of productivity seems far below the carrying capacity of the ponds (Koirala *et al.*, 2021). The domestic output still falls short of demand. With the aim of import replacement and export promotion

government body had provided special programs for farmers but they are not being able to take proper advantage of it, however farmers are attracted towards fish farming day by day. Since domestic fish production alone is not sufficient to supply the fish demand of the country, significant amount of fish being imported from other countries (Ranjan, 2019). Due to open border, it is not possible to have record from government level. Along with this, lack of systematic fish market has also played a significant role in unavailability of exact export import data (Ranjan, 2019). Absence of cold storage facilities, insulated vehicles for holding the harvest and regulate supply are some of the fish marketing problems in Nepal causing spoilage loss during sales (Husen, 2019). This study is designed to find out the supply chain and marketing of fish in Nawalparasi (East) which help to identify the actual situation in fish production, supply and marketing system.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was carried out in the Danda and kawasoti bazar of kawasoti -16, Nawalparasi (East) district, Gandaki Province, Nepal. Nawalparasi (East) District is one of the high fish production district and the fish produced here are supplied most of the cities of the country.

### 2.2 Methods

Primary data were collected through interview with the help of semi-structural questionnaire. A set of questionnaires was prepared for the collection of primary data. The major variables included in questionnaires were trader's socio-demographic, gender role on trading business, source of fish, method of storage, packaging, and transportation, price of different species of fish, problem ranking, and volume of imported and exported fishes. The pretesting of the questionnaire was carried out with the traders at Kawasoti city and correction was done accordingly. Four places-connected with the road corridors- were selected purposively. Traders and Consumers were selected by using simple random sampling.

The field survey was conducted from October 2021 to November 2021. Commercial fish farmers, traders of kawasoti Municipality were considered the survey population and thus were included in sampling. Five fish Producers, eight wholesalers, twelve retailers and twenty consumers were randomly surveyed through semi structure questionnaire. The respondents were

interviewed through visiting their homes and fish shop. The secondary information was collected by reviewing different publication from national level organizations such Central Fisheries Promotion and Conservation Center (CFPCC), literatures and journals.

### 2.3 Data processing and analysis

Data collected through respondents during survey was coded, compiled and entered in MS Excel. Statistical analysis of the mean for frequency, percentage, etc. was carried out using descriptive statistical tools and MS-Excel. As per the necessity; pictures, graphs, diagrams, narrative analysis, and other inferential statements was used that sufficiently extrapolate the prevailing status of marketing of fish, market constraints, market share of imported and domestic fish.

## 3. RESULTS AND DISCUSSION

### 3.1 Socio-economic characteristics of the respondents

#### Gender of respondents

From the study, it was found that most of the fish farmers were male (84.00%) and number of females was quite low. Female members helped in some of the activities like feeding, marketing but were not fully involved in fish farming.

**Table 1.** Gender of fish trader by trading category

| Categories of trader | Gender     |            | Total       |
|----------------------|------------|------------|-------------|
|                      | Male (%)   | Female (%) |             |
| Producer             | 4 (80.00)  | 1 (20.00)  | 5           |
| Wholesaler           | 7 (87.5)   | 1 (12.5)   | 8           |
| Retailer             | 10 (83.33) | 2 (16.66)  | 12          |
| Total                | 21 (84.00) | 4 (16.00)  | 25 (100.00) |

Figures in parentheses indicate percentage

#### Age of respondents

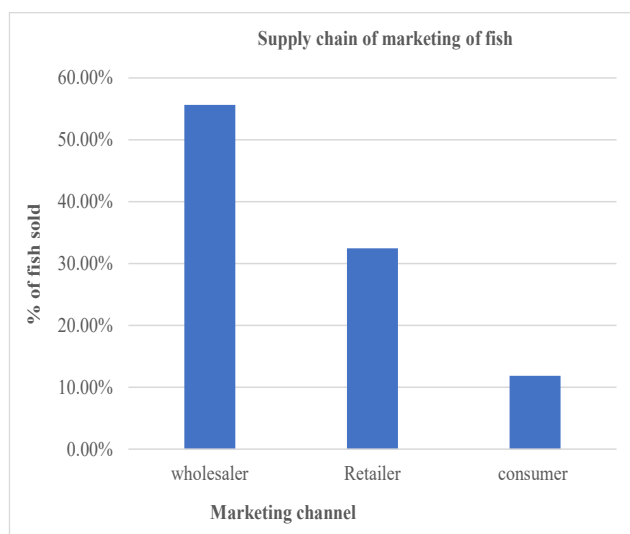
It was observed that almost all the traders involved in fish trading business were more than 30 years of age. The mean age of producer, wholesaler and retailer involved in trading business were 39 years, 34 years and 46 years respectively. Generally, the retailer was older than the wholesaler (Table 2).

**Table 2.** Age of respondents by trading category

| Categories of trader | Age (Mean± SD) |
|----------------------|----------------|
| Producer             | 39±9.61        |
| Wholesaler           | 34.5±8.1       |
| Retailer             | 46.8±13.43     |

### 3.2 Supply chain of marketing of fish

The findings suggest that there was no specific marketing channel used by the farmers. The farmers in the study area sell fish in daily or periodical local market. Most of the harvest was sold in local market (hatiya) and within the district to different wholesalers, retailers or directly to the consumers following different marketing channels. Certain amount of harvest is also sold to distant market like Chitwan, Butwal, and Kathmandu. 55.65% of total fish produce is sold to the wholesalers, 32.49% to the retailers and 11.86% is sold to the customers directly by the fish farmers. It was found that the farmers get good market on some days while some times it is hard to sell their produce. Unsystematic marketing channel is a serious problem. Similar result was found which stated that Market links and channels are not feasible to small and medium scale farmers which make them unable to sell their products (Shrestha, 2072).

**Figure 1.** Supply chain of marketing of fish

### 3.3 Marketing margin of Nepalese fish

As shown in Table 3, the Rohu and common carp fish had greater marketing margins of Rs.125/kg. The marketing margin of Naini, Silver and Bighead had Rs.100/kg followed by Chhadi, Mangur and Pangasius Rs. 70/kg, Rs.50/kg, Rs.50/kg respectively. Pangasius and Magur had least marketing margin in Nawalparasi (East) district.. The cost of live fish was found relatively higher than the fresh fish

**Table 3.** Marketing margin of Nepali fish

| Species of fish | Producer average price (Rs./kg) | Retailer average price (Rs./kg) | Marketing margin (Rs./kg) |
|-----------------|---------------------------------|---------------------------------|---------------------------|
| Chadi           | 230                             | 300                             | 70                        |
| Rohu            | 300                             | 425                             | 125                       |
| Naini           | 250                             | 350                             | 100                       |
| Bighead         | 250                             | 350                             | 100                       |
| Silver          | 250                             | 350                             | 100                       |
| Common          | 300                             | 425                             | 125                       |
| Pangasius       | 250                             | 300                             | 50                        |
| Mangur          | 230                             | 280                             | 50                        |

### 3.4 Market price of Nepali and Indian fish in Nawalparasi (East)

Fish is considered to be relatively cheaper food product compared to mutton and chicken. Price of fish may vary due to a variety of factors such as season, location of sale, size, and species of fish and inflow of fish from India (Koirala et.al., 2021). As shown in Table, study shows that Nepalese fish fetch higher price than Indian fish because fish bringing fish from India in ice packs taking long time, influence the quality of the product, which consumers not prefer Nepali fish Rohu and common carp fetch highest price Rs 425/kg and Pangasius fetch lowest price Rs. 280/kg.

**Table 4.** Market price of Nepali fish

| Species of fish    | Farmgate average price (Rs./kg) | Wholesaler average price (Rs./kg) | Retailer average price (Rs./kg) |
|--------------------|---------------------------------|-----------------------------------|---------------------------------|
| <b>Nepali Fish</b> |                                 |                                   |                                 |
| Chadi              | 230                             | 250                               | 300                             |
| Rohu               | 300                             | 355                               | 425                             |
| Naini              | 250                             | 278.89                            | 350                             |
| Silver             | 250                             | 281.11                            | 350                             |
| Bighead            | 250                             | 281.11                            | 350                             |
| Common             | 300                             | 367.78                            | 425                             |
| Pangasius          | 250                             | 280                               | 300                             |
| Mangur             | 230                             | 250                               | 280                             |
| <b>Indian Fish</b> |                                 |                                   |                                 |
| Rohu               |                                 | 310                               | 380                             |
| Pangasius          |                                 | 240                               | 280                             |

### 3.5 Marketing size of Nepali and Indian fish

The fish imported from India were bigger in size and the average weight was 2.91 kg ranging from 0.48 kg to 5.34 kg whereas the fish produced in Nepal were smaller in size; the average weight was 1.91 kg ranging from 0.03 kg to 3.79 kg.

**Table 5.** Marketing sizes of Nepali and Indian fish

| Parameter              | Source of fish |       |
|------------------------|----------------|-------|
|                        | Nepal          | India |
| Max. size of fish (kg) | 3.79           | 5.34  |
| Min. size of fish (kg) | 0.03           | 0.48  |
| Mean size of fish (kg) | 1.91           | 2.91  |

### 3.6 Fish transportation

As presented in the table below, most of the wholesaler (25%) used Motorcycle as a means of transportation, 62.5% used pick up while 12.5% used buses for the transportation of fish. On the other hand, retailer used

pickup (41%) as a means of transportation followed by motorcycle & cycle (25%) and buses (8.33%) for the transportation of fish. In total, 50% traders used pick van as a means of transportation. Only 10% traders used buses as a means of transportation medium to transport the fish.

**Table 6.** Use of transportation means by traders

| Categories of trader | Transportation Medium |           |            |          | Total |
|----------------------|-----------------------|-----------|------------|----------|-------|
|                      | Bus                   | Pickup    | Motorcycle | Cycle    |       |
| Wholesaler           | 1(12.5)               | 5(62.5)   | 2(25.00)   | 0(0.00)  | 8     |
| Retailer             | 1(8.33)               | 5(41.66)  | 3(25.00)   | 3(25.00) | 12    |
| Total                | 2(10.00)              | 10(50.00) | 5(25.00)   | 3(15.00) | 20    |

Figures in parentheses indicate percentage

### 3.7 Packaging container

It is evident that majority of traders (65%) use Styrofoam crates packed with crushed ice for packaging the fish and 25% use bamboo baskets and hundies for packaging the fish. Whereas, 10% of the traders transported live fish by providing aeration as consumers prefer live fish. Out of the total 20 traders, 13 used Styrofoam crates, 5 used bamboo baskets and hundies and 2 used aeration boxes for fish packaging.

**Table 7.** Use of packaging container by traders

| Traders category | Modes of Packaging    |                                 |          | Total |
|------------------|-----------------------|---------------------------------|----------|-------|
|                  | Bamboo basket/hundies | Styrofoam crates aeration boxes |          |       |
| Wholesaler       | 1(12.5)               | 5(62.5)                         | 2(25.00) | 8     |
| Retailer         | 4(33.33)              | 8(66.66)                        | 0(00.00) | 12    |
| Total            | 5(25.00)              | 13(65.00)                       | 2(10.00) | 20    |

Figures in parentheses indicate percentage

### 3.8 Fish purchasing pattern

Purchasing time or pattern means how many times consumer buy the product. During the study period, consumer purchasing pattern was classified as once, twice, thrice and more than three times in a month. The figure shows that higher number of consumers purchase the fish twice in a month (75%). Only 7.50% of the total respondent purchased the fish thrice in a month and 17.50% of the total respondents purchased once in a month.

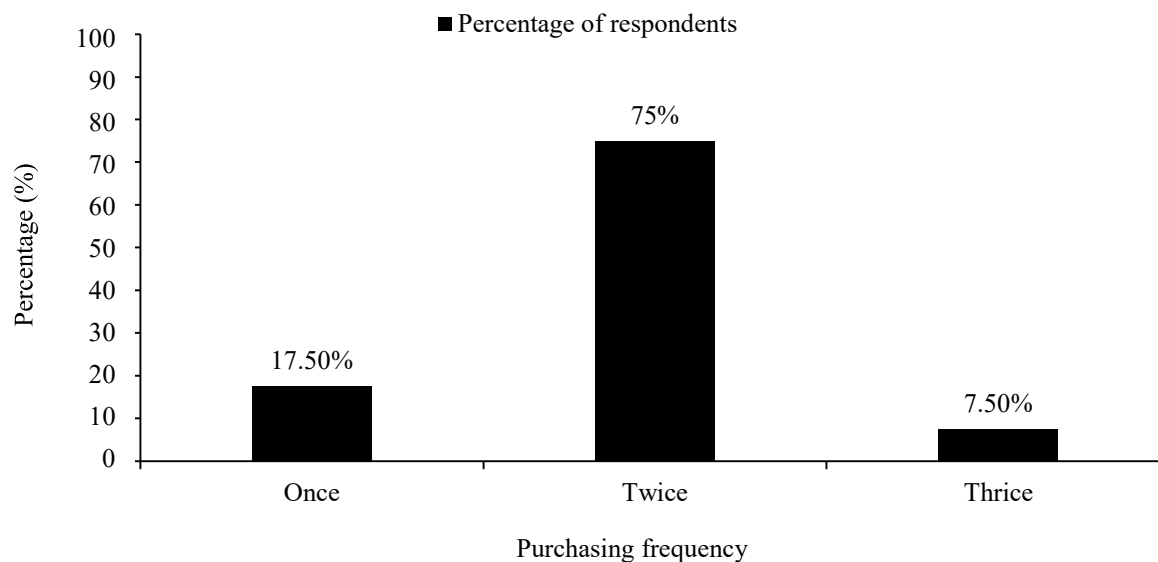


**Figure 2.** Consumer purchasing frequency (in a month)

### 3.9 Marketing situation

The Study shows most of the consumers (50%) said that the fish marketing of Nawalparasi (East) is considerable or average, 15% of the consumer said that marketing of fish is good and 35% of consumers said that the fish marketing need to be improved.

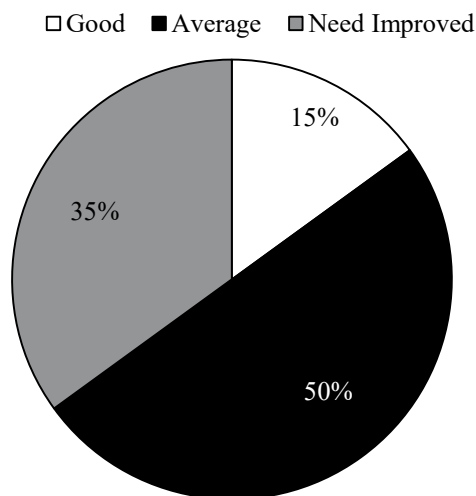


**Figure 3.** Consumer opinion towards marketing of fish

### 3.10 Consumer preferences on different fish species

Among the major carp species; the most preferred one by the consumers was Rohu followed by Bhakur similar result was found by Koiralaa et.,al. (2021) . It

was due to the good taste of those fish species despite high price of Bhakur. Bighead carp and common carp were average preferred fish by the consumers within the district. Similarly, Chhadi fish of Naini was found to be preferred by consumers as it was produced in high quantity and was comparatively cheaper. Likewise Silver carp and Grass carp were found to be less preferred by the consumers.

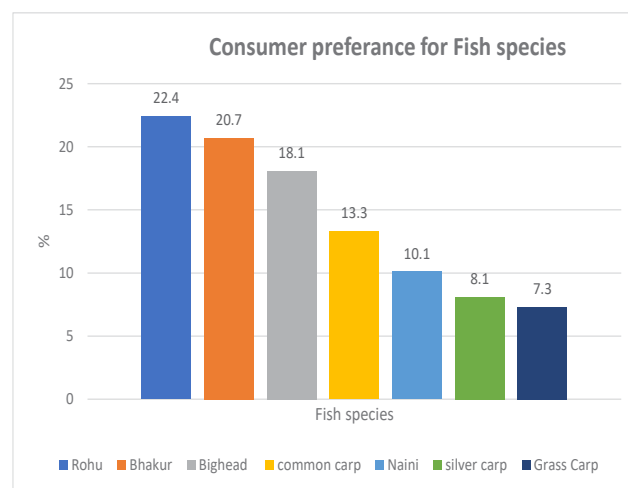


**Figure 4.** Consumer preferences on different fish species

## 4. CONCLUSION

From the study, it was found that most of the fish farmers were male, female members helped in some of the activities like feeding, marketing but were not fully involved in fish farming. The farmers in the study area

sell fish in daily or periodical local market. Most of the harvest was sold in local market (hatiya) and within the district to different wholesalers, retailers or directly to the consumers following different marketing channels. Rohu and common carp fish had greater marketing margins of Rs.125/kg. The fish imported from India were bigger in size and whereas the fish produced in Nepal were smaller in size. Nepalese fish fetch higher price than Indian fish. Due to open boarder conditions of district large number of fish are imported from India which can directly affected on Nepalese fish market. Local fish market management is the key factor for the enhancement of Nepalese fisheries sector. Fish farming along with fish marketing is one of the growing branch of agriculture in Danda and Kawasoti Bazar of Nawalparasi (East) district by providing employment oppurtunities to the households. With the proper management of fish preservation facilities, establishment of cold storage, cold vans for fish transportation, improvement of existing fish marketing chains ensure healthy fish marketing chain from producers to the consumers. Hygienic fish

marketing supply provide nutritional and food security along with post-harvest loss and quality declination of fish. Strict rules and policies should be implemented to check the quality of fish import from the neighboring country India. Similarly, effective management system policies should be made in local government level for controlling overexploitation in riverine fisheries resources. The involved human resources in the fish farming and marketing channels should be well trained with fish preservation, fish handling techniques to elevate quality of marketing system in Nawalparasi(East) district.

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## APPRAISAL OF FARM MECHANIZATION AND FARMER'S FIELD PRACTICE OF MAIZE FARMING AT LAMAHI MUNICIPALITY, DANG, NEPAL

Prabhat Jha <sup>1,2,\*</sup> and Santosh Marahatta <sup>1</sup>

<sup>1</sup> Agriculture and Forestry University, Chitwan, Nepal

<sup>2</sup> Nepal Agricultural Market Development Programme (NAMDP), Morang, Nepal

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#### \*Correspondence:

afuprabhat@gmail.com  
Tel: +977-9849578200

### ABSTRACT

*The study compares farm mechanization and farmer's field practice to document the agronomic and economic feasibility of mechanized maize farming. The research was carried out in randomized complete block design with 2 treatments of mechanized practice and farmers practice and 3 replications as location for the winter maize using 10v10 hybrid variety. In agronomic aspect, seed rate and urea application in farmers practice (17.05 kg ha<sup>-1</sup> and 186.41 kg ha<sup>-1</sup> respectively) were found lower to mechanized practice (30.00 kg ha<sup>-1</sup> and 348.00 kg ha<sup>-1</sup> respectively). Consequently, lower yield was found in farmers practice (7.56 t ha<sup>-1</sup>) to mechanized practice (10.43 t ha<sup>-1</sup>) which was attributed by lower plant population, higher sterility, and smaller cob diameter in farmers' practice. The benefit cost ratio was higher in mechanized practice (3.76) to farmer's practice (2.44). Labour shortage can be mitigated by mechanization through the custom hiring center even in small holder maize growers.*

### 1. INTRODUCTION

Maize (*Zea mays* L.) contributes 28.27% of total cereal's area and 26.96% of total cereal's production in Nepal (MoALD, 2021). In addition to being used as food, there is a growing demand for maize for livestock and poultry feed, as well as for biofuel and starch production (Bhujel & Thakur, 2015). The domestic annual production of the maize is 3.00 million tons (MoALD, 2021) whereas the estimated domestic requirement of maize by accounting for both feed (1.5 million tons) and human consumption (2.9 million tons) is 4.4 million tons (Timsina *et al.*, 2016; KC *et al.*, 2015). As a result, Nepal imports a significant amount of maize from other countries. To address the demand, winter maize is being promoted under the rice-wheat system in Nepal, with a focus on increasing quality seed, recommended application of farm inputs, farm mechanization, technology adaptation, and improved post-harvest practices. Winter maize produces both qualitative and quantitative grain yield due to low incidence of diseases and pests, and higher carbohydrate production per day per unit land in winter (KC *et al.*, 2015). Although many farmers in the Dang region practices winter maize farming, the inadequate input

application and labour-intensive production practices compounded with labour shortage and high wage rates has increased the cost of cultivation and thereby, affecting the profitability of maize farming (Dhital, 2017; Bhandari *et al.*, 2015; Dawadi and Sah, 2012). The Prime Minister Agriculture Modernization Project (PMAMP) aims to promote commercialization and mechanization of maize farming in Dang, by making Dang region a center for maize production and linking it to industrialization with potential end markets for maize and maize products (MOAD, 2016). One of its interventions is through promoting the mechanization practices in the region. Mechanization can increase the efficiency of farm inputs, alleviate labor shortages, increase productivity and production of maize, and narrow the yield gap and demand deficit (Rijk, 1989). Documenting the agronomic and economic feasibility of mechanized farming will encourage farmers to appreciate the benefits of farm mechanization and motivate them to participate in Custom Hiring Center's activities.

## 2. MATERIALS AND METHODS

### 2.1 Selection of study area

The site of the research was Dang district which lies in Lumbini province of Nepal. The selected locations were Satbariya, Sonpur and Keruniya of the Lamahi municipality. The reason for selecting these places is the existence of PMAMP field office in the Lamahi municipality and the collaboration of the farmer's groups with PMAMP. The research was commenced on 31<sup>st</sup> October 2017 and concluded on 29<sup>th</sup> May 2018 on 10v10 Indian hybrid maize variety.

### 2.2 Sample and sampling technique

The research was carried out in randomized complete block design with Two treatments viz: mechanized practice and farmers' field practice and with Three replications as location (Satbariya, Sonpur and Keruniya). The mechanized practice involved implementing all PMAMP recommended agronomic management practice using machines, except for harvesting due to unavailability of maize harvesting machine in the study area. On the other hand, the prevailing farmer's practice represents the blend of different agronomic management practices employed by different farmers in the study area, with limited mechanization particularly during land preparation.

Since, uniform agronomic management practice was trialed in the mechanized practice location while, there were heterogeneity in the same for prevailing farmer's practice, only Three samples for mechanized practice and Thirteen samples for prevailing farmer's practice in regard to agronomic management practices and the cost of cultivation data were taken through pre-tested interview schedule. The Three samples were collected from Three different locations for the mechanized practice, while Four samples each from Satbariya and Sonpur, and Five samples from Keruniya were taken for the prevailing farmer's practice.

To collect yield attribute data, the Three mechanized practice field samples were outlined into a total of Thirteen harvest plots. Among these, Four harvest plots were outlined each in Satbariya and Sonpur while, Five harvest plots were in Keruniya, from where 10 dehusked maize were selected from each individual plot (10 m<sup>2</sup>). Likewise, each of the Thirteen prevailing farmer's practice field samples were further outlined into Three harvest plots of 10 m<sup>2</sup> each. A total of 10 dehusked maize were selected from combined Three harvest plots. Thus, 130 cob samples from each two set of treatments were taken.

### 2.3 Types and time of data collection

Agronomic practices and the cost of cultivation data for land preparation, seed and sowing, intercultural operations including irrigation, fertilizer, pesticide and harvesting; its frequencies, time duration and cost per unit were recorded at respective periods.

Yield attribute data on plant population, cob bearing plant population, total cob number, total cob weight, selected individual cob weight, cob length, grain length, mid-diameter of cob, row-line, number of grains, weight of grains and their moisture percentage were recorded at the time of harvest.

### 2.4 Statistical Analysis

The data gathered were coded, analyzed, and tabulated through Excel (version 15.0) and SPSS (version 20.0). Graphs, tables, and charts were used to interpret the results.

## 3. RESULTS AND DISCUSSION

### 3.1 Agronomic management practice

As shown in Table 1, the seed rate of mechanized practice was higher (30 kg ha<sup>-1</sup>) than farmers practice (17.05 kg ha<sup>-1</sup>) and was found significantly different at 0.1% level of significance. This was chiefly due to the difference in seed sowing practice. The farmers mostly sow the seed by using wooden plough or by using rope and hoe to carve the line where seed is sown by using hand, which results in lower application of seed compared to tractor operated eight row seed cum fertilizer drill. The date of weeding of mechanized practice was 33.33 days after sowing (DAS) which was higher than farmers practice (27.33 DAS) but was found statistically non-significant. The date of thinning of mechanized practice was higher (30.00 DAS) than farmers practice (21.25 DAS) and was found statistically non-significant. Earthing up was practiced only in mechanized practice at 33.33 DAS because farmers in the region did not perceive it to be beneficial. The total number of irrigations was higher in farmers practice (3.92) than mechanized practice (3) but was found statistically non-significant. Similarly, among the fertilizer application, only urea rate was found significantly different at 5% level of significance with mechanized practice applying higher (348.00 kg ha<sup>-1</sup>) than farmers practice (186.41 kg ha<sup>-1</sup>). While DAP and MOP both were found higher in mechanized practice i.e., 130.50 kg ha<sup>-1</sup> and 66.00 kg ha<sup>-1</sup> respectively than farmers practice i.e., 112.25 kg

ha<sup>-1</sup> and 25.61 kg ha<sup>-1</sup> respectively but was found statistically non-significant in both cases. The low dosage of fertilizer in farmers practice was due to inadequate knowledge to farmers about recommended dose and lack of availability of sufficient fertilizer to the farmers. The number of nitrogen split was higher in

mechanized practice (3.00) than farmers practice (2.25) but was statistically non-significant. The physiological maturity of mechanized practice was lower (172.66 DAS) than farmers practice (177.33 DAS) and was statistically non-significant.

**Table 1.** Agronomic management practices of sample plots of maize Superzone area of Lamahi, Dang, 2018

| SN | Agronomic management practices   | Farmers practice | Mechanized practice | t value |
|----|----------------------------------|------------------|---------------------|---------|
| 1  | Seed rate (kg ha <sup>-1</sup> ) | 17.05±1.37       | 30±0.00             | 4.59*** |
| 2  | Weeding (DAS)                    | 27.33±2.84       | 33.33±3.33          | 0.99    |
| 3  | Thinning (DAS)                   | 21.25±3.15       | 30.00±8.66          | 1.07    |
| 4  | Earthing up (DAS)                | N/A              | 33.33±3.33          |         |
| 5  | Total number of irrigations      | 3.92±0.45        | 3.00±0.00           | -0.99   |
| 6  | Fertilizer application           |                  |                     |         |
|    | Urea rate (kg ha <sup>-1</sup> ) | 186.41±31.12     | 348.00±0.00         | 2.52*   |
|    | DAP (kg ha <sup>-1</sup> )       | 112.25±21.13     | 130.50±0.00         | 0.42    |
|    | MOP (kg ha <sup>-1</sup> )       | 25.61±9.99       | 66.00±0.00          | 1.96    |
|    | Number of Nitrogen split         | 2.25±0.22        | 3.00±0.00           | 1.67    |
| 7  | Physiological maturity (DAS)     | 177.33±4.23      | 172.66±4.66         | -0.52   |

\*\*\* indicates significance difference at 0.1 percent level; \* indicates significance difference at 5 percent level, N/A indicates non-availability, and value after ± indicates standard error of mean

The fertilizer dose comparison is also illustrated in Figure 1.

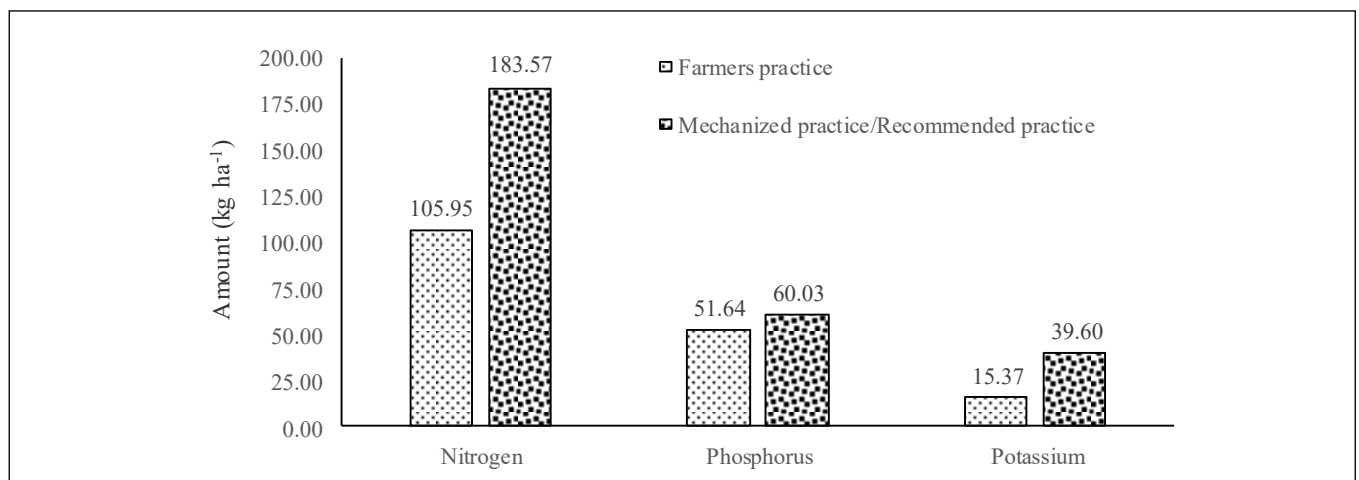


**Figure 1.** NPK comparison of mechanized and farmers' practice

The application of nitrogen, phosphorus and potassium in farmers practice was 105.95 kg ha<sup>-1</sup>, 51.64 kg ha<sup>-1</sup> and 15.37 kg ha<sup>-1</sup> respectively while in mechanized practice was 183.57 kg ha<sup>-1</sup>, 60.03 kg ha<sup>-1</sup> and 39.60 kg ha<sup>-1</sup> respectively. The fertilizer application in mechanized practice is also the recommended practice. Thus, above figure 1 shows comparable difference in nitrogen and potassium application between these two practices.

### 3.2 Yield Attributes

As shown in Table 2, the average plant population per individual plot (10 m<sup>2</sup>) was higher in mechanized practice (70.18) than farmers practice (53.70) and was found significantly different at 5% level of significance. This was due to the difference in seed rate and plant spacing of farmers and mechanized practice. Though the average number of cob bearing plants per 10 m<sup>2</sup> was higher in mechanized (65.53) than farmers practice (50.85) but it was statistically non-significant. The average cob length was higher in mechanized (16.32 cm) than farmers practice (16.16 cm) and was statistically non-significant. Similarly, average sterility was found higher in farmers (6.79%) than mechanized practice (3.83%) and was found significantly different at 5% level of significance. One of the possibility of higher sterility in farmers practice was due to lower application of Nitrogen to the maize (Marahatta, 2020). The average cob diameter was found higher

in mechanized (4.78 cm) than farmer practice (4.66 cm) and was found significantly different at 5% level of significance. This was due to the difference in urea application of farmers practice and mechanized practice. The average number of kernel row per cob was found higher in mechanized (15.64) than farmers practice (14.94) but was statistically non-significant. The average number of kernels per kernel row was higher in mechanized (28.52) than farmers practice (27.44) and was found statistically non-significant. The average thousand kernels weight was found higher in mechanized practice (388.83) than farmers practice (370.25) but was statistically non-significant. Lastly the average yield was found higher in mechanized (10427.62 kg ha<sup>-1</sup>) than farmers practice (7563.75 kg ha<sup>-1</sup>) resulting in yield difference of 27.46% and was found significantly different at 1% level of significance. The difference in yield was due to variance in yield attribute which was in turn affected by agronomic practices especially seed rate and urea application which were found significantly different in between farmers practice and mechanized practice. A similar study in winter maize cv rampur composite reveals the similar effect of cropping density and nitrogen at 55,555 ha<sup>-1</sup> with 100 Kg N ha<sup>-1</sup> and 66.666 ha<sup>-1</sup> with 200 kg N ha<sup>-1</sup> on yield is increment by 33.98% in later (Shrestha, 2014). A similar study on hybrid maize cv Dijamant 6 reveals the effect of crop density at 51,020 ha<sup>-1</sup> and 71,429 ha<sup>-1</sup> on yield is increment by 11.35% in later (Mandic *et al.*, 2015).

**Table 2.** Average yield Attributes and yield influenced of sample plots in maize Superzone area of Lamahi, Dang, 2018

| SN | Average yield attributes and yield                 | Farmers practice | Mechanized practice | t value |
|----|----------------------------------------------------|------------------|---------------------|---------|
| 1  | Plant population per 10 m <sup>2</sup>             | 53.70±4.65       | 70.18±5.09          | 2.39*   |
| 2  | Number of cob bearing plants per 10 m <sup>2</sup> | 50.85±5.36       | 65.53±5.74          | 1.869   |
| 3  | Cob length (cm)                                    | 16.16±0.05       | 16.32±0.49          | 0.318   |
| 4  | Sterility (%)                                      | 6.79±0.57        | 3.83±0.88           | -2.831* |
| 5  | Cob diameter (cm)                                  | 4.66±0.04        | 4.78±0.03           | 2.651*  |
| 6  | Number of Kernel row per cob                       | 14.94±0.28       | 15.64±0.72          | 0.897   |
| 7  | Number of kernels per kernel row                   | 27.44±0.12       | 28.52±0.65          | 1.621   |
| 8  | Thousand kernels weight (g)                        | 370.25±5.33      | 388.83±22.66        | 0.798   |
| 9  | Yield (kg ha <sup>-1</sup> )                       | 7563.75±436.73   | 10427.62±537.57     | 3.6**   |

\*\* indicates significance difference at 1 percent level; \* indicates significance difference at 5 percent level, and value after ± indicates standard error of mean

### 3.3 Cost of Inputs

For each ha of land as shown in Table 3, the cost of rotavator was higher in farmers practice (NPR 5,310.00) than mechanized practice (NPR 3,391.67) but was statistically non-significant. Similarly, cultivator cost was more in farmers practice (NPR 4,347.43) than mechanized practice (NPR 2,366.67) and was statistically non-significant. The price dissimilarity was due to frequency of tillage by machine. In farmers practice who used machine, majority used either rotavator or cultivator and when used alone, they tilled the soil twice therefore individual cost of operating rotavator and cultivator were higher while in mechanized practice, one till of rotavator was followed by single till by cultivator. The use of power tiller and draft during land preparation was limited to only farmers practice with the cost of NPR 10,183.73 and NPR 15,000.00 respectively. Thus, the overall land preparation cost for ha of land was found out to be higher in farmers practice (NPR 9,677.57) than mechanized practice (NPR 5,758.33) but was statistically non-significant. The cost of seed of mechanized practice was found higher (NPR 14,000.00) than farmers practice (NPR 8,365.16) and was found significantly different at 1% level of significance. This was due to a higher seed rate

in mechanized practice (30.00 kg ha<sup>-1</sup>) than farmers practice (17.05 kg ha<sup>-1</sup>). For seeding equipment aspect, the mechanized practice used Seed cum fertilizer drill which cost NPR 2,173.34 during seed sowing operation while almost all did not use any machine, Jab planter was found in one case of farmers practice cost NPR 100.00. During intercultural operation, the mechanized practice used mini tiller which cost NPR 1,833.34 while in the farmers practice no machinery cost was incurred. During irrigation, cost was found to be higher in farmers practice (NPR 4,071.75) than mechanized practice (NPR 3,150.00) but was statistically non-significant. Similarly, among the fertilizer, urea cost in mechanized practice was found higher (NPR 5,939.20) than farmers practice (NPR 3,284.42) and was found significantly different at 5% level of significance. While DAP and MOP cost was found to be higher in mechanized practice i.e., NPR 6,194.40 and NPR 2,244.00 respectively than farmers practice i.e., NPR 5,283.98 and NPR 746.04 respectively but was statistically non-significant. The dissimilarity of cost of fertilizer was due to difference in fertilizer dose between these practices. Also, the pesticide cost in mechanized practice was higher (NPR 862.50) than farmers practice (NPR 266.67) because of difference in product.

**Table 3.** Cost of inputs (NPR Per ha) of sample plots in maize production in Superzone area of Lamahi, Dang, 2018

| SN | Inputs                    | Farmers practice | Mechanized practice | t value |
|----|---------------------------|------------------|---------------------|---------|
| 1  | Land Preparation          | 9677.57±1398.62  | 5758.33±1025.14     | -1.35   |
|    | Rotavator                 | 5310.00±763.39   | 3391.67±559.08      | -1.532  |
|    | Cultivator                | 4347.43±1784.15  | 2366.67±491.03      | -1.07   |
|    | Power Tiller              | 10183.73±2458.84 | N/A                 |         |
|    | Draft                     | 15000.00±0.00    | N/A                 |         |
| 2  | Seed                      | 8365.16±948.89   | 14000.00±1000.00    | 2.817** |
| 3  | Seed equipment            | 100.00±0.00      | 2173.34±261.73      |         |
|    | Seed drill cum fertilizer | N/A              | 2173.34±261.73      |         |
|    | Jab Planter               | 100.00±0.00      | N/A                 |         |
| 4  | Intercultural             |                  |                     |         |
|    | mini tiller               | N/A              | 1833.34±440.96      |         |
| 5  | Irrigation                | 4071.75±900.43   | 3150.00±1441.35     | -0.471  |
| 6  | Fertilizer                | 9314.46±1453.17  | 14377.60±112.91     | 1.69    |
|    | Urea                      | 3284.42±523.75   | 5939.20±23.20       | 2.46*   |
|    | Diammonium phosphate      | 5283.98±985.54   | 6194.40±96.88       | 0.45    |
|    | Murate of Potash          | 746.06±351.34    | 2244.00±0.00        | 2.07    |
| 7  | Pesticide                 | 266.67±68.87     | 862.50±337.50       | 2.94*   |
|    | Total Cost of Inputs      | 31570.62±2366.80 | 41867.60±1366.24    |         |

\*\* indicates significance difference at 1 percent level; \* indicates significance difference at 5 percent level, N/A indicates non-availability, and value after ± indicates standard error of mean

### 3.4 Cost of Labour

For each ha of land as shown in Table 4, the cost of labour during seed sowing in farmers practice was NPR 5,371.28 compared to sample plot in mechanized practice where no labour was used financially, because seed cum fertilizer drill where labour wage of operators are inclusive in hiring charge with significant difference at 0.1% level of significance. Also, the cost of labour during intercultural operation in farmers practice was higher (NPR 11,461.27) than mechanized practice (NPR 433.33) and was found significantly different at 1% level of significance. This is because in farmers practice, manual hoe was used for weeding while in

mechanized mini tiller was used whose hourly charge is inclusive of labour wage of operator. However, the cost attributed in mechanized field was due to manual thinning operation. The cost of labour during irrigation aspect was more in farmers practice (NPR 4,493.05) than mechanized practice (NPR 3,600.00) but was statistically non-significant. While in the pesticide aspect the cost of labour were equal i.e., NPR 800.00 attributing to same practice employed in both practices. The cost of labour for harvesting aspect was higher in mechanized practice (NPR 9,200.00) than farmers practice (NPR 8,819.85) and was statistically non-significant.

**Table 4.** Cost of labour (NPR per ha) of sample plots in maize production in Superzone area of Lamahi, Dang, 2018

| SN | Inputs               | Farmers practice | Mechanized practice | t value  |
|----|----------------------|------------------|---------------------|----------|
| 1  | Seed sowing aspect   | 5371.28±641.96   | 0.00±0.00           | -4.07*** |
| 2  | Intercultural aspect | 11461.27±1386.17 | 433.33±16.67        | -3.87**  |
|    | Thinning             | N/A              | 433.33±16.67        |          |
|    | Weeding              | 11461.27±1386.17 | N/A                 |          |
| 3  | Irrigation aspect    | 4493.05±676.90   | 3600.00±0.00        | -0.64    |
| 4  | Pesticide aspect     | 800.00±0.00      | 800.00±0.00         |          |
| 5  | Harvesting aspect    | 8819.85±674.62   | 9200.00±200.00      | 0.273    |
|    | Total cost of labour | 30878.80±1411.43 | 14566.67±420.65     |          |

\*\*\* indicates significance difference at 0.1 percent level; \*\* indicates significance difference at 1 percent level, N/A indicates non-availability, and value after ± indicates standard error of mean

### 3.5 Economic Analysis

The economics of crop production shown in Table 5, the cost of cultivation of farmer practice was higher (NPR 62,449.42) than mechanized practice (NPR 56,434.27) which were not found significantly different. And the gross return of mechanized practice was higher (NPR 211,137.40) than farmer practice (NPR 151,274.95) but the difference was not statistically significant. But the Net return of mechanized practice was higher (NPR 154,703.13) than farmer practice (NPR 88,825.53) at the current market price of NPR 20 kg<sup>-1</sup> maize grain and was found significantly different at 5% level of significance. Similarly, the benefit-cost ratio (B:C ratio) of mechanized practice was higher (3.76) than farmer practice (2.44) and was found significantly different at 5% level of significance. Cost of cultivation was not found significant due to fact that in mechanized practice, absence of harvesting machine led to increase cost of cultivation through use of labour instead and in farmer practice, low amount of seed and urea by 43.17% and

46.4% respectively lead to low cost of cultivation in farmer practice of seed aspect by 40.2% and urea by 44.7%. This, however, in farmer practice led to a yield gap of 27.46%. This resulted in a significant difference in net return and B:C ratio.

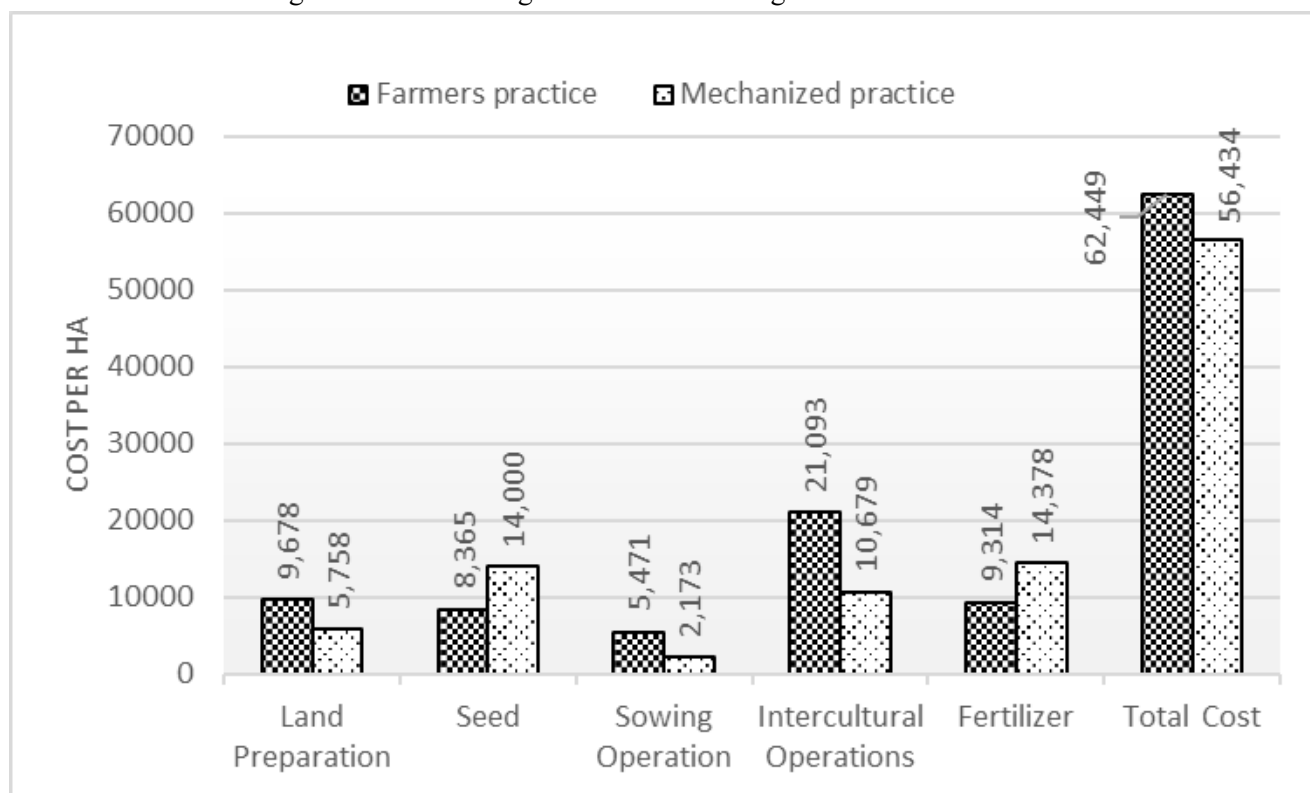
A study conducted in Pakistan determined that in mechanized practice, the total production cost for maize was PKR 16,805.0 ha<sup>-1</sup> (NPR 17,813.3 ha<sup>-1</sup>) which gave the gross income of PKR 31,025.0 ha<sup>-1</sup> (NPR 32,886.5 ha<sup>-1</sup>) and yielded net profit of PKR 14,220.0 ha<sup>-1</sup> (NPR 15,073.2 ha<sup>-1</sup>) with B:C ratio of 1.84. For the traditional farms, cost of production was PKR 16,450.0 ha<sup>-1</sup> (NPR 17,537.0 ha<sup>-1</sup>) which gave the gross income of PKR 24,555.0 ha<sup>-1</sup> (NPR 26,028.3 ha<sup>-1</sup>) and yielded net profit of PKR 8,105.0 ha<sup>-1</sup> (NPR 8,591.3 ha<sup>-1</sup>) with B:C ratio of 1.49 (Aurangzeb, Nigar, & Shah, 2007).

**Table 5.** Economics of crop production per hector of sample plots in maize production in Superzone area of Lamahi, Dang, 2018

| SN | Economic variables                          | Farmers practice   | Mechanized practice | t value |
|----|---------------------------------------------|--------------------|---------------------|---------|
| 1  | Cost of cultivation (NPR ha <sup>-1</sup> ) | 62449.42±2970.32   | 56434.27±1424.44    | -0.98   |
| 2  | Gross return (NPR ha <sup>-1</sup> )        | 151274.95±14776.92 | 211137.40±17465.10  | 1.91    |
| 3  | Net return (NPR ha <sup>-1</sup> )          | 88825.53±14093.52  | 154703.13±18884.44  | 2.19*   |
| 4  | B:C ratio                                   | 2.44±0.24          | 3.76±0.40           | 2.58*   |

\* indicates significance difference at 5 percent level, and value after ± indicates standard error of mean

The cost of maize farming across various stages is illustrated in Figure 2.

**Figure 2.** Comparison of cost of maize farming across mechanized and farmers' practice

Aside from the cost of inputs which is seed and fertilizer, which has been underused in farmer's practice, the cost related to operations can be clearly seen under the scope of reducing in farmer's practice. During land preparation, instead of using machinery such as power tiller or standalone rotavator and cultivator, concurrent use of rotavator and cultivator has the scope of reducing the cost. Likewise, during sowing operation, the use of tractor operated seed cum fertilizer drill is strongly advised to reduce the cost in the study area. Mini tiller, instead of manual labour, would reduce cost by more than half during the intercultural operation. The use of recommended machinery and the agronomic practice is

an ideal way to reduce costs as well as increase yield in the study area.

### 3.6 Socio-economic aspects of mechanization

There can be inference regarding socioeconomic demerits of mechanization like in short run due to farm labour replacement, but it is crucial to recognize that mechanization also creates new employment opportunities in the form of skilled labour, machineries operation, maintenance, repair, and technology support. As agriculture becomes more mechanized, there is scope of skilled labour to operate and maintain complex machinery. This presents an opportunity for

skill development programmes and vocational training initiatives. Likewise, adoption of farm mechanization leads to higher agricultural output and surplus production. This surplus can be directed towards value-added processing, agribusiness ventures, and export markets, stimulating economic growth and creating opportunities for all associated agribusiness actors. The revenue can be redirected towards the local economy to support essential services. Similarly, by reducing physical labour and opportunity cost of spending less time on field, mechanization has potential to improve the living conditions of the farmers by allowing them to focus on other important activities such as crop management, marketing, and planning. Also, in the context of higher out-migration of men and increased participation of women in farm activities, mechanization reduces physical demand of farming tasks thereby, contributing towards gender inclusivity and empowerment in agriculture. Thus, by investing in skill development, promoting economic diversification, and improving living conditions, the adoption of mechanization can contribute to overall socioeconomic progress. However, it is crucial to ensure that appropriate policies and support mechanisms are in place to mitigate the potential negative effects and facilitate a smooth transition for workers and communities.

#### 4. CONCLUSION

The study shows that not only the yield was found to be lower in the farmer's sample field but also the cost of cultivation was found to be higher too. This concludes PMAMP should disseminate information on recommended seed rate and fertilizer application practices to farmers for increasing the yield. The cost of cultivation can be reduced by minimizing the cost of labour through adopting recommended mechanization. Thus, the problem of labor unavailability and higher wage can be mitigated through mechanization in maize cultivation along with effectively reducing the time spent on field. Thus, it will be beneficial for farmers to hire the machines for cultivation through Custom hiring center (CHC) for higher economic return even in small holder maize growers.

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# ASSESSMENT OF LATE SOWN WHEAT (*Triticum aestivum* L.) GENOTYPES UNDER HIGH TEMPERATURE STRESS CONDITIONS

Suman Bohara<sup>1\*</sup>, Basistha Acharya<sup>2</sup>, Surakshya Bohora<sup>3</sup> and Jharana Upadhyaya<sup>1</sup>

<sup>1</sup> Directorate of Agricultural Research, Lumbini Province, Khajura, Banke, Nepal.

<sup>2</sup> National Plant Pathology Research Centre, Khumaltar, Lalitpur, Nepal.

<sup>3</sup> South China Agricultural University, Guangzhou, Guangdong, China.

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\*Correspondence:  
suman.bohara44@gmail.com;  
Tel.: +977-9847099644

## ABSTRACT

In recent years, climate change has led to an increase in extreme weather conditions, including high-temperature stress. High-temperature stress is the term for an increase in temperature ( $> 30^{\circ}\text{C}$ ) following anthesis during grain development. Wheat is particularly vulnerable to high temperature stress, and its productivity is severely affected. With the objective of identifying wheat genotypes tolerant to heat stress in terms of grain yield, a total of fifty wheat genotypes were evaluated under heat stress conditions at Directorate of Agricultural Research, Lumbini Province, Khajura, Banke in 2021/22. There was highly significant difference ( $<0.001$ ) among genotypes for yield. The association between the grain yield (GY) and the biomass yield (BM), thousand grain weight (TGW), and spike number per meter square (SPMS), was highly significant and positive. The highest harvest index value of 0.59 and the highest grain yield were achieved from 20HTWYT#36 (4133 kg/ha), followed by Bandganga (3992 kg/ha), 20HTWYT#23 (3978 kg/ha), 20HTWYT#11 (3943 kg/ha), and 20HTWYT#05 (3758 kg/ha) exhibiting higher tolerance to high temperature stress and indicating the potential for this genotypes to be used as domain specific varieties suitable for heat stress conditions of Banke district and for breeding climate resilient varieties in the future.

## 1. INTRODUCTION

Wheat is the 3rd most important cereal crop of Nepal, in terms of area and production. At national level, wheat is grown in 711,067 hectares of land to produce 2,127,276 metric tonnes of grain (MoALD, 2022). The country's wheat production has increased significantly in recent years, especially after technological advancements and their application at the farm level. Wheat area and production have greatly risen since 1960, increasing by more than 7 and 17 times, respectively (Basnet, R, 2023).

Wheat is one of the most important staple crops in the world, providing a vital source of food for billions of people. Meanwhile, the production and quality of wheat are often threatened by various abiotic stresses, including high temperature stress. High temperature stress is a condition in which wheat crops are exposed to high temperature ( $>30^{\circ}\text{C}$ ), specially during the reproductive stage, mainly from heading to maturity stage (Nesar *et al.*, 2022). High temperature stress can negatively affect various aspects of wheat growth and

development, such as photosynthesis, respiration, and nutrient uptake, ultimately leading to reduced yield and poor grain quality (Prasad *et al.*, 2011).

The primary agricultural system in Nepal is the rice-wheat pattern, in which the timing of wheat planting is governed by the timing of rice harvest. In lowland Terai area, farmers choose to cultivate medium to late ripening rice types, which is harvested from late November to early December. Wheat has a brief window of opportunity for growth and maturation following its planting in November and December (Sah, 2022). Hot air movement during the maturation period results in early maturation without physiological maturity, which severely reduces wheat production (NARC, 2020). Agricultural productivity itself is largely influenced by a range of abiotic stresses, including high temperature stress. Wheat, one of the most important staple crops in the world, is particularly vulnerable to high temperature stress, which can have a significant impact on grain yield and yield components. Late sown wheat, in particular,

is at greater risk of exposure to high temperature stress ( $>30^{\circ}\text{C}$ ).

The research is conducted considering identifications of wheat genotypes with greater tolerance to heat stress for further breeding as specific objective to increase productivity of late sown wheat with high yielding, terminal heat stress tolerant parameters for western terai.

### 1.1. Theoretical framework

With present trend of population growth rate and food demand, it is anticipated that by 2050, demand for wheat will have increased by 50% from current levels (CIMMYT, 2023). However, the crop is at high risk of new biotic and abiotic stresses. Of these stresses, high temperature is one of the major abiotic stresses that affect crop growth and yield, especially in the arid and semi-arid regions. Although having the biggest total harvested area of all the cereals, including rice and maize, wheat nonetheless has the lowest overall production. Abiotic stresses including drought, salt, and high temperatures account for the majority of production losses in wheat (Abhinandan, *et al.*, 2018). Thus, for wheat development initiatives of Nepal that have mostly relied on the genetic variants contained in the wheat genome through traditional breeding, knowing the consequences of these stresses becomes essential.

In the case of wheat, high temperature stress during the reproductive stage can reduce the grain yield and yield components such as spike length, number of grains per spike, and thousand grain weight (Farooq *et al.* 2011). Late sowing of wheat is also a common practice in lowlands, which exposes the crop to high temperature stress during the critical growth stages. Therefore, it is essential to understand the effect of high temperature stress on grain yield and yield components of late sown wheat. Several studies have reported the negative impact of high temperature stress on wheat yield and yield components (Prasad *et al.* 2008; Dhakal *et al.* 2019). A study by Pandey *et al.* (2020) revealed that exposure to high temperature stress during the reproductive stage led to a decrease in grain yield and a decline in yield components of wheat.

Despite the increasing number of studies on the effect of high temperature stress on wheat yield, there is a paucity of research on the effect of high temperature stress on late sown wheat. There is limited information on the effect of high temperature stress induced by hot

and dry air wave in late March and early April that coincides with the reproductive stage of the late sown wheat. Global temperatures continue to rise due to climate change; it is becoming increasingly important to develop wheat cultivars with improved tolerance to high temperature stress. This can be achieved through the identification of genotypes that exhibit greater resilience to high temperature stress, as well as a deeper understanding of the underlying physiological and molecular mechanisms involved in heat stress tolerance. Overall, this study has the potential to provide valuable insights into the development of more resilient wheat cultivars that can withstand the challenges of high temperature stress, and ultimately contribute to local, as well as provincial food security.

## 2. MATERIALS AND METHODS

### 2.1 Experimental site:

An open field experiment was conducted in the year 2021/22 at Directorate of Agricultural Research (DoAR), Lumbini Province, Khajura, Banke. The geographical location of the experimental site is  $81^{\circ} 37''$  East longitudes and  $28^{\circ} 06''$  North latitude, 181 meter above mean sea level. The site has sub-tropical climate and receives an average annual rainfall of 1000-1500mm. It has sandy to silty loam soil and pH ranges from 7.2 to 7.5 (DoAR Khajura, 2022).

### 2.2 Planting materials:

Forty-nine early maturing wheat genotypes were obtained from CIMMYT, Mexico via National Plant Breeding and Genetic Research Centre (NPBGRC), Khumaltar, Lalitpur, Nepal and Bandganga as a standard check variety were kept for testing (Table 1).

### 3.3 Experimental design:

The experimental was set up in Alpha Lattice with two replications, five blocks with ten plots in each block. The plot size was maintained  $4.5 \text{ m}^2$  (6 rows of 3m length) and the planting geometry was maintained as 25cm row to row spacing and continuous sowing. The plot-to-plot distance was maintained at 50 cm likewise a gap of 50cm was given between the blocks and 75 cm between the replications.

### 2.4 Irrigation and fertilizer:

A pre-sowing irrigation was applied prior tillage. First irrigation was given at CRI Stage (21 DAS) and second irrigation at booting stages.

The fertilizer was applied at the rate of 68:40:40 kg N: P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O per hectare through Urea, Di-ammonium phosphate (DAP), and Murate of Potash (MOP). Full dose of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O was applied as a basal dose at the time of final land preparation, before sowing. Remaining half dose of nitrogen was applied in two splits; one after a week of the first irrigation (28 DAS) and other after a week of second irrigation at late booting stage.

### 2.5 Land preparation and sowing:

One disc harrowing and two crisscross ploughing using rotavator (rotary tiller) was done to bring soil in good sowing condition. Sowing was done on 23rd December, 2021 which is about a month late than the normal recommended date. Late sowing was done to synchronize the reproductive stage of the crop with the weather of late march to early April. Banke district receives hot air waves at the end of March to mid-April, that causes heat stress to the crops, thus helps in screening resilient genotypes.

Pendimethalin 30% EC was applied @2ml/l water as pre-emergence herbicide, 48 hours after sowing to control weed competition at the early crop stage.

### 2.6 Agro-meteorological information:

Day to day agro-meteorological data on maximum-minimum temperature, rainfall and relative humidity was retrieved from the weather station installed at DoAR, Khajura. Relative humidity (RH%) was calculated by using formula followed by Huang *et al.* in 2013.

$$RH\% = \frac{e - A * P * \Delta t}{E}$$

Where,  $\Delta t$  = The difference between the dry-bulb temperature (Td) and the wet-bulb temperature (Tw)

Ew = The saturation vapour pressure in the wet-bulb thermometer, calculated by using Buck formula as:

$$E_w = 6.112 * e^{\{(17.502 * T_w) / (240.97 + T_w)\}}$$

Ed = The saturation vapour pressure in the dry-bulb temperature, calculated as:

$$E_d = 6.112 * e^{\{(17.502 * T_d) / (240.97 + T_d)\}}$$

A = The measuring humidity coefficient, calculated as:

$$A = 0.00066 * (1 + 0.00115 * T_w)$$

P = The mean atmospheric pressure (assumed to be 1013.25024 mb)

### 2.7 Data collection and analysis:

Phenological data were monitored and meticulously gathered, including days to heading (DH), days to maturity (DM), plant height (PH), spike length (SL), spikes/m<sup>2</sup> (SPMS), biomass yield (BM), and grain yield (GY). DH was noted when ear heads began to emerge from flag leaf sheaths in 50% of the plant population. Similar to this, DM was identified when more than 80% of spikes started to turn yellow. When the plants become brittle due to drying after maturity, middle four rows were harvested and threshed to measure grain yield per plot which was later converted to kg/ha after adjusting moisture to 13% by using following formula:

$$\text{Grain yield} \left( \frac{\text{Kg}}{\text{ha}} \right) \text{ at 13\% moisture level} = \frac{(100 - M\%) * \text{Plot yield (kg)} * 10000 \text{m}^2}{(100 - 13) * \text{Net Plot Area in m}^2}$$

Likewise, Harvest index (HI) was computed for each genotype by using following formula:

$$HI = \frac{\text{Economic yield or Grain yield}}{\text{Total Biomass Yield above ground i.e straw+grain yield}}$$

Where, M% = moisture percentage of sample grain.

Microsoft Excel 2013 was used to carry out simple mathematical calculations, tabulation, and graphical representation of the result. ADEL-R developed by CIMMYT was used to perform analysis of variance and IBM SPSS Statistics 20 was used to do correlation and cluster analysis.

## 3. RESULTS AND DISCUSSION

### 3.1 Agro-meteorological information:

The weather condition of the experimental site during the crop period is depicted in the Figure 1. This information on agro-meteorological condition was obtained from the weather station of DoAR, Khajura. The graph shows a gradual downfall of the maximum and minimum temperature until February and there was a gradual increment of both temperatures till April. Relative humidity (RH%) also decreased steadily after February. As crops reached the ripening stages in April, maximum (> 30°C) and minimum temperature (~ 20°C) were at the highest of the entire crop duration with lowest RH%. The ideal temperature for wheat throughout the ripening process is between 21°C and 25°C (Farooq *et al.*, 2011). From this, we can ensure that the crops were at high temperature stress during grain filling stage.

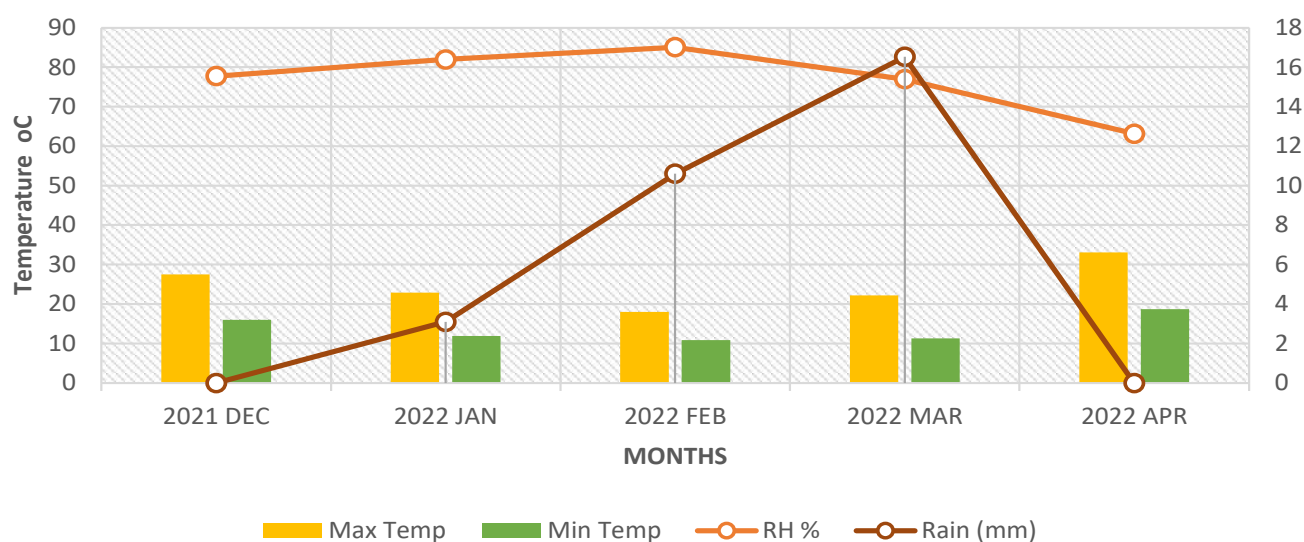


**Figure 1.** Distribution of maximum-minimum temperature, rainfall and relative humidity in the experimental site during crop period.

### 3.2 Days to heading and days to maturity:

Highly significant difference ( $P < 0.001$ ) were observed among the tested wheat genotypes for days to maturity and days to heading (Table 2), which is in agreement with the findings of Upadhyaya & Bhandari (2022). Genotypes 20HTWYT#42 and 20HTWYT#47 showed earliest heading (70 days) while 20HTWYT#06 showed earliest physiological maturity of 104 days followed by 20HTWYT#42, 20HTWYT#47, 20HTWYT#02, 20HTWYT#03, 20HTWYT#38 and 20HTWYT#07 with 105 days to maturity (Table 3).

### 3.3 Plant height and spike length:

Tested genotypes showed significant different ( $p < 0.05$ ) for the plant height. Similar result was obtained by Bohara *et al* (2023) while testing late sown wheat genotypes in drought stress environments. Final height was maximum for 20HTWYT#17 (98 cm), followed by 20HTWYT#47 and 20HTWYT#48 with 96cm height, whereas, shortest plant height was observed in 20HTWYT#25 (84 cm), followed by 20HTWYT#34 (86 cm), as presented in Table 3.

### 3.4 Grains per spike and 1000 grain weight:

Highly significant ( $p < 0.001$ ) and significant ( $P < 0.05$ ) differences were found for 1000 grain weight and no of grains per spike respectively among the tested genotypes. Number of grains per spike was obtained maximum for check variety: Bandganga (332), followed by 20HTWYT#30 (299), 20HTWYT#23

(281), 20HTWYT#03 (278), and 20HTWYT#05 (277). Likewise, genotypes 20HTWYT#18 (41.3 gm), 20HTWYT#31 (41.0 gm), 20HTWYT#14 (40.7 gm), 20HTWYT#04 (40.4 gm), and 20HTWYT#47 (40.4 gm) had higher thousand grain weight correspondingly. With late sown wheat, it is common to find sunken and shrivelled seeds, which have low germination rates thus unfit for seed production (Tahir *et al.*, 2009). Due to the inferior quality of the grains, late-sown wheat sells for cheap prices (Aalam *et al.*, 2018).

### 3.5 Grain yield and harvest index:

Grain yield was recorded maximum from 20HTWYT#36 (4133 kg/ha) with highest harvest index value of 0.59, followed by Bandganga (3992 kg/ha), 20HTWYT#23 (3978 kg/ha) 20HTWYT#11 (3943 kg/ha), and 20HTWYT#05 (3758 kg/ha). There were highly significant differences ( $< 0.001$ ) for yield among the tested genotypes, which is similar with the finding of Ahmad *et al.*, (2006), Khamssi and Najaphy (2012) and Poudel *et al.*, (2021). Lowest yield was found in 20HTWYT#13 (2063 kg) with minimum harvest index (0.31). Harvest Index was supreme in 20HTWYT#36, followed by 20HTWYT#05 (0.44), 20HTWYT#04, 20HTWYT#11, 20HTWYT#23, and 20HTWYT#34 with 0.43 HI.

### 3.6 Correlation analysis:

To establish the selection criteria for various features during varietal selection programs, correlation analyses of wheat grain yield and its related component are essential. Grain yield (GY) showed positive association

with days to maturity while, negative association with heading days, which coincide with the finding of Upadhyaya *et al.* (2022). Grain yield had highly significant and positive correlation with spike number per meter square (SPMS), thousand grain weight (TGW), and biomass yield (BM). Similarly, harvest index (HI) had highly significant and positive correlation with GY (Table 4).

### 3.7 Cluster analysis:

Fifty wheat genotypes were clustered into five clusters based on mean values of DH, DM, PH, SL, GY, TGW, SPMS, BM and HI (Figure 2). Cluster 1 is the largest cluster with twenty-seven members including Bandganga (Table 5), indicating close relationship among each other based on the phenological traits and yield components. Similarly, cluster 2, cluster 3, cluster 4 and cluster 5 had fourteen, four, three and two genotypes respectively.

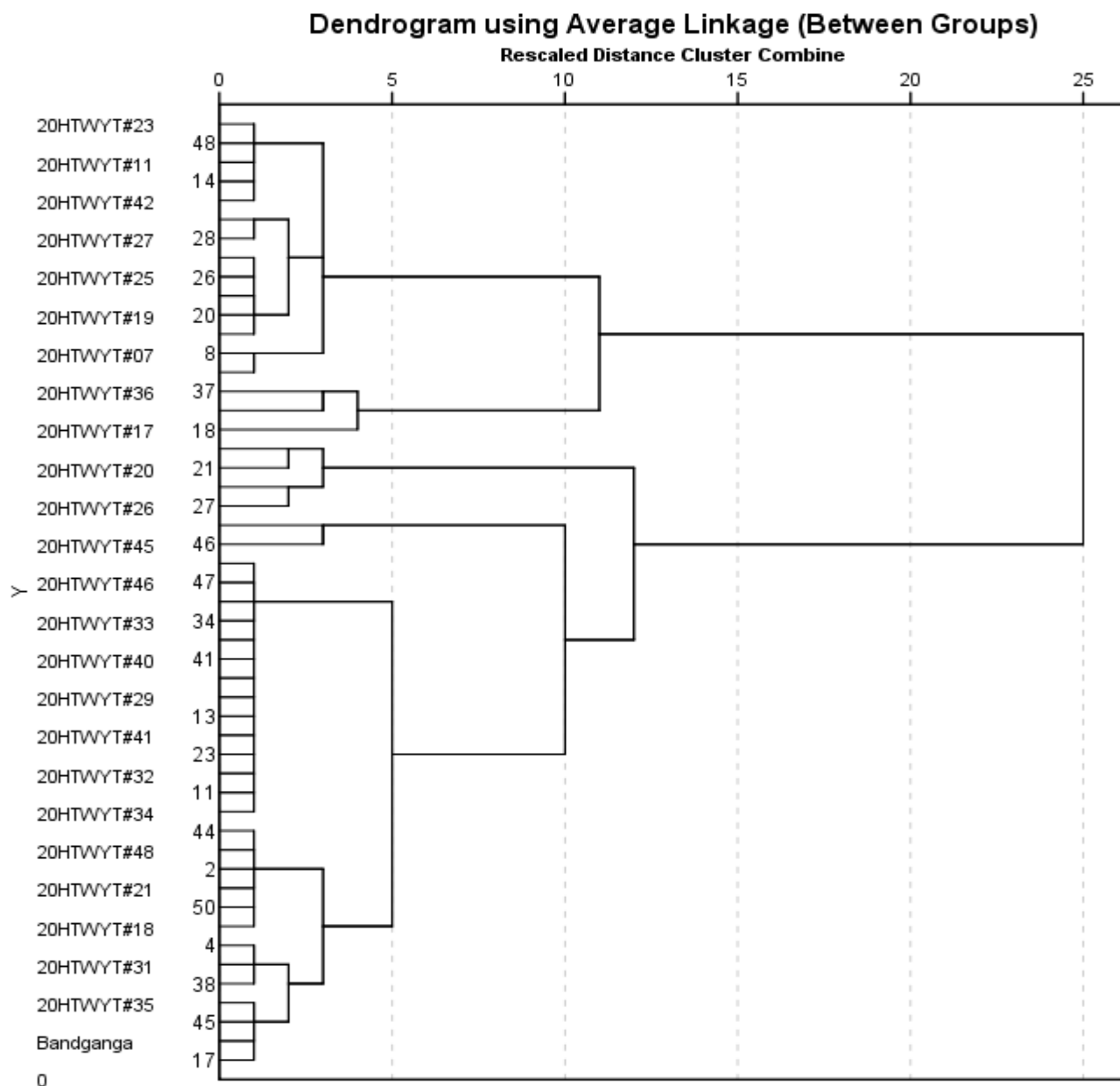


**Figure 2.** Clustering of wheat genotypes based on yield and yield components

## 4. CONCLUSION

Based on the study, it is concluded that the tested genotypes differed significantly at heat stress conditions for most of the important yield components. Some genotypes gave higher grain yield and thousand grain weight exhibiting higher tolerance to high temperature stress. Highest grain yield of 4133 kg/ha with highest HI value was obtained from 20HTWYT#36, followed by Bandganga (3992 kg/ha), 20HTWYT#23 (3978 kg/ha), 20HTWYT#11 (3943 kg/ha), and 20HTWYT#05 (3758 kg/ha) indicating the potential for this genotype to be used in breeding programs to develop heat-tolerant wheat varieties. Under high temperature stress conditions, grain yield of wheat was significantly dependent upon thousand grain weight, number of effective tillers per unit area, and biomass yield. As this research had focused on grain yield and only

a few phenological parameters, it has a restricted ability to describe overall genotypic performance. Thus, further studies are needed to explore response against economically important disease and find the molecular basis of heat tolerance for more efficient screening of wheat with high tolerance to heat stress.

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## ANNEX

**Table 1.** List of genotypes used in the experiment with their pedigree and origin.

| SN | Genotype   | Pedigree                                                                                               | Origin           |
|----|------------|--------------------------------------------------------------------------------------------------------|------------------|
| 1  | Bandganga  | n.d.                                                                                                   | NWRP, Bhairahawa |
| 2  | 20HTWYT#01 | NADI #1                                                                                                | CIMMYT, Mexico   |
| 3  | 20HTWYT#02 | WADER #1                                                                                               | CIMMYT, Mexico   |
| 4  | 20HTWYT#03 | BORL14*2//BECARD/QUAIU #1                                                                              | CIMMYT, Mexico   |
| 5  | 20HTWYT#04 | ND643/2*WBLL1//VILLA JUAREZ F2009/3/KACHU//KIRITATI/...                                                | CIMMYT, Mexico   |
| 6  | 20HTWYT#05 | KAKURU/BORL14                                                                                          | CIMMYT, Mexico   |
| 7  | 20HTWYT#06 | KAKURU//SUP152/BAJ #1                                                                                  | CIMMYT, Mexico   |
| 8  | 20HTWYT#07 | KACHU*2//SUP152/3/WBLL1*2//BRAMBLING*2//BAVIS                                                          | CIMMYT, Mexico   |
| 9  | 20HTWYT#08 | FRANCOLIN #1//NELOKI/3/PRL/2* PASTOR//KACHU                                                            | CIMMYT, Mexico   |
| 10 | 20HTWYT#09 | KACHU/4//WHEAR/SHAMA/3/C80.1/3*BATAVIA//2*WBLL1/5/...                                                  | CIMMYT, Mexico   |
| 11 | 20HTWYT#10 | TOH #1//KFA/2*KACHU                                                                                    | CIMMYT, Mexico   |
| 12 | 20HTWYT#11 | HARTOG SUMAI3 (LINE B)/2* NAVJ07/4/MUTUS//KIRITATI/...                                                 | CIMMYT, Mexico   |
| 13 | 20HTWYT#12 | BECARD//FRNCLN//BAJ #1//TECUE #1                                                                       | CIMMYT, Mexico   |
| 14 | 20HTWYT#13 | BORL14*2/3/WBLL1*2//BRAMBLING*2//BAVIS                                                                 | CIMMYT, Mexico   |
| 15 | 20HTWYT#14 | KACHU/DANPHE*2//MUTUS*2//HARIL #1                                                                      | CIMMYT, Mexico   |
| 16 | 20HTWYT#15 | BAJ #1/3/KIRITATI//HUW234+LR34//PRINIA/4/KIRITATI/...                                                  | CIMMYT, Mexico   |
| 17 | 20HTWYT#16 | BAJ #1/3/KIRITATI//HUW234+LR34//PRINIA/4/KIRITATI/...                                                  | CIMMYT, Mexico   |
| 18 | 20HTWYT#17 | BAJ #1/3/KIRITATI//HUW234+LR34//PRINIA/4/KIRITATI/...                                                  | CIMMYT, Mexico   |
| 19 | 20HTWYT#18 | BAJ #1/3/KIRITATI//HUW234+LR34//PRINIA/4/KIRITATI/...                                                  | CIMMYT, Mexico   |
| 20 | 20HTWYT#19 | WBLL1*2//BRAMBLING*2//BAVIS*2/3//SUP152/BAJ #1                                                         | CIMMYT, Mexico   |
| 21 | 20HTWYT#20 | PFAU/MILAN/3/BABAX/LR42//BABAX/11/CROC_1/...                                                           | CIMMYT, Mexico   |
| 22 | 20HTWYT#21 | WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1*2/4//NIINI #1/5/...                                            | CIMMYT, Mexico   |
| 23 | 20HTWYT#22 | PFAU//WEAVER*2//TRANSFER#12,P88.272.2/3//WHEAR//2*PRL/...                                              | CIMMYT, Mexico   |
| 24 | 20HTWYT#23 | SUP152/BAJ #1//KIDEA                                                                                   | CIMMYT, Mexico   |
| 25 | 20HTWYT#24 | SUP152/KENYA SUNBIRD/3/KACHU//KIRITATI/2*TRCH                                                          | CIMMYT, Mexico   |
| 26 | 20HTWYT#25 | SOKOLL/3/PASTOR//HXL7573/2*BAU/4/SOKOLL/WBLL1/5/MUCUY                                                  | CIMMYT, Mexico   |
| 27 | 20HTWYT#26 | KISKADEE #1/5//KAUZ*2//MNV//KAUZ/3/MILAN/4/BAV92/6//WHEAR/ MX12                                        | CIMMYT, Mexico   |
| 28 | 20HTWYT#27 | DANPHE/3//ROLF07/YANAC//TACUPETO F2001//BRAMBLING/4/...                                                | CIMMYT, Mexico   |
| 29 | 20HTWYT#28 | TOH #1//MUTUS*2//TECUE #1                                                                              | CIMMYT, Mexico   |
| 30 | 20HTWYT#29 | PFAU/MILAN/3/BABAX/LR42//BABAX*2/4//NIINI #1/7/W15.92/4/...                                            | CIMMYT, Mexico   |
| 31 | 20HTWYT#30 | HD 2967/3//SWSR22T.B./2*BLOUK #1//WBLL1*2//KURUKU                                                      | CIMMYT, Mexico   |
| 32 | 20HTWYT#31 | KACHU/BECARD//WBLL1*2//BRAMBLING*2/3//ABLEU                                                            | CIMMYT, Mexico   |
| 33 | 20HTWYT#32 | WBLL1*2//KIRITATI//FRNCLN/3//BECARD/4/2*KACHU/DANPHE                                                   | CIMMYT, Mexico   |
| 34 | 20HTWYT#33 | WBLL1*2//KIRITATI//FRNCLN/3//BECARD/4/2*KACHU/DANPHE                                                   | CIMMYT, Mexico   |
| 35 | 20HTWYT#34 | WBLL1*2//KIRITATI//FRNCLN/3//BECARD/4/2*KACHU/DANPHE                                                   | CIMMYT, Mexico   |
| 36 | 20HTWYT#35 | TAM200/PASTOR//TOBA97/3//HEILO/4//PAURAQ/5//BRBT1*2/...                                                | CIMMYT, Mexico   |
| 37 | 20HTWYT#36 | BAJ #1//KISKADEE #1/3//WBLL1*2//BRAMBLING*2//BAVIS/4/...                                               | CIMMYT, Mexico   |
| 38 | 20HTWYT#37 | BAJ #1//KISKADEE #1/3//WBLL1*2//BRAMBLING*2//BAVIS/4/...                                               | CIMMYT, Mexico   |
| 39 | 20HTWYT#38 | PRL/2*PASTOR/4//CHOIX/STAR/3//HE1/3//CNO79//2*SERI/5/...                                               | CIMMYT, Mexico   |
| 40 | 20HTWYT#39 | SHORTENED SR26 TRANSLOCATION//2*WBLL1*2//KKTS/3//BECARD/... MX12                                       | CIMMYT, Mexico   |
| 41 | 20HTWYT#40 | SHORTENED SR26 TRANSLOCATION//2*WBLL1*2//KKTS/3//BECARD/... MX120                                      | CIMMYT, Mexico   |
| 42 | 20HTWYT#41 | MUTUS//KIRITATI/2*TRCH/3//WHEAR/KRONSTAD F2004/4/...                                                   | CIMMYT, Mexico   |
| 43 | 20HTWYT#42 | MUNAL*2//CHONTE*2/3//SWSR22T.B./2*BLOUK #1//WBLL1*2/...                                                | CIMMYT, Mexico   |
| 44 | 20HTWYT#43 | MUNAL #1*2/4//HUW234+LR34//PRINIA//PBW343*2//KUKUNA/3/... SS14B01663T-099TOPY-099M-099NJ-099NJ-5Y-0WGY | CIMMYT, Mexico   |
| 45 | 20HTWYT#44 | WBLL1*2/4//YACO/PBW65/3//KAUZ*2//TRAP//KAUZ/5//KACHU #1*2/.. MX120-2                                   | CIMMYT, Mexico   |
| 46 | 20HTWYT#45 | ORION/5/2*FRNCLN/4//WHEAR/KUKUNA/3/C80.1/3* BATAVIA/...                                                | CIMMYT, Mexico   |
| 47 | 20HTWYT#46 | ESTOC/7/2*KISKADEE #1/5//KAUZ*2//MNV//KAUZ/3//MILAN/4/...                                              | CIMMYT, Mexico   |
| 48 | 20HTWYT#47 | CROC_1/AE.SQUARROSA (205)//BORL95/3//PRL/SARA//TSI/...                                                 | CIMMYT, Mexico   |
| 49 | 20HTWYT#48 | ADI#2/MUCUY                                                                                            | CIMMYT, Mexico   |
| 50 | 20HTWYT#49 | MUNAL #1//SUJATA//CHIPAK                                                                               | CIMMYT, Mexico   |

**Table 2.** Analysis of variance of phenological parameters, grain yield and yield attributing traits

|         | DH     | DM     | PH<br>(cm) | SL(cm) | SPMS    | GY<br>(kg/ha) | 1000<br>grain wt.<br>(gm) | BM<br>(kg/ha) | Grains/<br>spike (GPS) | HI     |
|---------|--------|--------|------------|--------|---------|---------------|---------------------------|---------------|------------------------|--------|
| Mean    | 74.73  | 108.51 | 90.71      | 10.28  | 257.93  | 3132.85       | 32.692                    | 8157.05       | 43.9                   | 0.387  |
| CV (%)  | 1.065  | 1.453  | 3.926      | 10.164 | 19.715  | 11.764        | 6.607                     | 16.035        | 13.793                 | 12.672 |
| Std MSE | 0.796  | 1.576  | 3.561      | 1.045  | 50.851  | 368.543       | 2.16                      | 1308.023      | 6.055                  | 0.049  |
| LSD0.05 | 1.608  | 3.184  | 7.191      | 2.11   | 102.695 | 744.287       | 4.362                     | 2641.606      | 12.228                 | 0.099  |
| P-value | <0.001 | <0.001 | 0.005      | 0.371  | 0.605   | <0.001        | <0.001                    | 0.331         | 0.038                  | 0.015  |

**Table 3.** Means of different phenological traits, grain yield, yield attributing traits and harvest index of the wheat genotypes.

| S.N | Genotypes  | Days<br>to 50%<br>heading | Days<br>to 80%<br>maturity | Plant<br>height<br>(cm) | Spike<br>length<br>(cm) | No. of<br>spike<br>per m <sup>2</sup> | Grain<br>yield<br>(kg/ha) | 1000<br>grain<br>wt.<br>(gm) | Biomass<br>yield<br>(kg/ha) | Grains/<br>spike | Harvest<br>Index |
|-----|------------|---------------------------|----------------------------|-------------------------|-------------------------|---------------------------------------|---------------------------|------------------------------|-----------------------------|------------------|------------------|
| 1   | Bandganga  | 72                        | 110                        | 89                      | 12                      | 332                                   | 3992                      | 39.1                         | 10400                       | 43               | 0.38             |
| 2   | 20HTWYT#01 | 75                        | 109                        | 91                      | 10                      | 266                                   | 3439                      | 36.6                         | 8683                        | 40               | 0.4              |
| 3   | 20HTWYT#02 | 72                        | 105                        | 93                      | 9                       | 272                                   | 3094                      | 26.5                         | 7994                        | 56               | 0.39             |
| 4   | 20HTWYT#03 | 73                        | 105                        | 88                      | 10                      | 278                                   | 2446                      | 26.9                         | 6911                        | 43               | 0.35             |
| 5   | 20HTWYT#04 | 73                        | 107                        | 92                      | 10                      | 258                                   | 3749                      | 40.4                         | 8806                        | 37               | 0.43             |
| 6   | 20HTWYT#05 | 74                        | 107                        | 95                      | 10                      | 277                                   | 3758                      | 32.1                         | 8756                        | 56               | 0.44             |
| 7   | 20HTWYT#06 | 72                        | 104                        | 85                      | 9                       | 246                                   | 2878                      | 25.4                         | 7961                        | 49               | 0.36             |
| 8   | 20HTWYT#07 | 74                        | 105                        | 88                      | 11                      | 244                                   | 2615                      | 27.6                         | 7421                        | 47               | 0.35             |
| 9   | 20HTWYT#08 | 76                        | 109                        | 89                      | 10                      | 230                                   | 2477                      | 30.8                         | 6133                        | 42               | 0.4              |
| 10  | 20HTWYT#09 | 76                        | 108                        | 93                      | 12                      | 276                                   | 3420                      | 31.6                         | 9439                        | 53               | 0.36             |
| 11  | 20HTWYT#10 | 76                        | 110                        | 90                      | 11                      | 242                                   | 3865                      | 39                           | 9822                        | 42               | 0.39             |
| 12  | 20HTWYT#11 | 75                        | 107                        | 91                      | 10                      | 440                                   | 3943                      | 38.7                         | 9311                        | 48               | 0.43             |
| 13  | 20HTWYT#12 | 76                        | 107                        | 89                      | 11                      | 272                                   | 2880                      | 30.9                         | 7778                        | 45               | 0.37             |
| 14  | 20HTWYT#13 | 76                        | 108                        | 85                      | 11                      | 210                                   | 2063                      | 33.1                         | 6717                        | 46               | 0.31             |
| 15  | 20HTWYT#14 | 76                        | 111                        | 95                      | 11                      | 238                                   | 3741                      | 40.7                         | 8978                        | 40               | 0.42             |
| 16  | 20HTWYT#15 | 72                        | 107                        | 95                      | 11                      | 247                                   | 3101                      | 31.4                         | 8917                        | 43               | 0.35             |
| 17  | 20HTWYT#16 | 74                        | 107                        | 92                      | 10                      | 253                                   | 2670                      | 37.1                         | 7461                        | 38               | 0.36             |
| 18  | 20HTWYT#17 | 73                        | 109                        | 98                      | 11                      | 220                                   | 3023                      | 33.5                         | 8539                        | 46               | 0.35             |
| 19  | 20HTWYT#18 | 73                        | 108                        | 92                      | 10                      | 250                                   | 3390                      | 41.3                         | 10611                       | 40               | 0.33             |
| 20  | 20HTWYT#19 | 75                        | 109                        | 87                      | 10                      | 203                                   | 2776                      | 29.4                         | 6544                        | 42               | 0.42             |
| 21  | 20HTWYT#20 | 81                        | 115                        | 90                      | 10                      | 251                                   | 3118                      | 30.6                         | 8561                        | 47               | 0.37             |

| S.N        | Genotypes  | Days to 50% heading | Days to 80% maturity | Plant height (cm) | Spike length (cm) | No. of spike per m <sup>2</sup> | Grain yield (kg/ha) | 1000 grain wt. (gm) | Biomass yield (kg/ha) | Grains/ spike | Harvest Index |
|------------|------------|---------------------|----------------------|-------------------|-------------------|---------------------------------|---------------------|---------------------|-----------------------|---------------|---------------|
| 22         | 20HTWYT#21 | 75                  | 110                  | 94                | 9                 | 262                             | 3561                | 28.8                | 9094                  | 52            | 0.39          |
| 23         | 20HTWYT#22 | 73                  | 107                  | 95                | 11                | 234                             | 2725                | 33.4                | 7383                  | 44            | 0.37          |
| 24         | 20HTWYT#23 | 76                  | 109                  | 93                | 11                | 281                             | 3978                | 32.8                | 9244                  | 49            | 0.43          |
| 25         | 20HTWYT#24 | 74                  | 110                  | 88                | 10                | 238                             | 3330                | 36.9                | 7872                  | 43            | 0.42          |
| 26         | 20HTWYT#25 | 79                  | 111                  | 77                | 12                | 257                             | 2645                | 30.1                | 8017                  | 36            | 0.33          |
| 27         | 20HTWYT#26 | 77                  | 109                  | 84                | 9                 | 235                             | 2814                | 26.3                | 7517                  | 35            | 0.37          |
| 28         | 20HTWYT#27 | 78                  | 109                  | 94                | 11                | 243                             | 2609                | 30.2                | 7856                  | 38            | 0.33          |
| 29         | 20HTWYT#28 | 74                  | 109                  | 94                | 11                | 233                             | 2784                | 32.1                | 7472                  | 44            | 0.38          |
| 30         | 20HTWYT#29 | 77                  | 109                  | 89                | 11                | 263                             | 2930                | 33                  | 7339                  | 37            | 0.41          |
| 31         | 20HTWYT#30 | 80                  | 111                  | 93                | 12                | 299                             | 3610                | 24.8                | 7300                  | 49            | 0.56          |
| 32         | 20HTWYT#31 | 72                  | 106                  | 93                | 10                | 268                             | 3122                | 41                  | 9206                  | 40            | 0.34          |
| 33         | 20HTWYT#32 | 76                  | 113                  | 92                | 10                | 259                             | 3407                | 28.7                | 8700                  | 45            | 0.39          |
| 34         | 20HTWYT#33 | 76                  | 113                  | 92                | 10                | 279                             | 3351                | 34                  | 8356                  | 44            | 0.4           |
| 35         | 20HTWYT#34 | 76                  | 111                  | 86                | 11                | 247                             | 3514                | 33.4                | 8267                  | 51            | 0.43          |
| 36         | 20HTWYT#35 | 75                  | 108                  | 90                | 10                | 270                             | 3146                | 32.4                | 8317                  | 40            | 0.38          |
| 37         | 20HTWYT#36 | 76                  | 109                  | 87                | 10                | 260                             | 4133                | 36.8                | 6944                  | 37            | 0.59          |
| 38         | 20HTWYT#37 | 76                  | 110                  | 89                | 11                | 214                             | 2638                | 28                  | 7406                  | 46            | 0.36          |
| 39         | 20HTWYT#38 | 73                  | 105                  | 88                | 8                 | 276                             | 2514                | 27.3                | 7167                  | 33            | 0.35          |
| 40         | 20HTWYT#39 | 77                  | 109                  | 87                | 10                | 268                             | 2723                | 33                  | 7644                  | 38            | 0.36          |
| 41         | 20HTWYT#40 | 72                  | 107                  | 93                | 11                | 261                             | 2884                | 30.5                | 7561                  | 34            | 0.38          |
| 42         | 20HTWYT#41 | 76                  | 109                  | 94                | 10                | 269                             | 2663                | 33.1                | 7422                  | 45            | 0.36          |
| 43         | 20HTWYT#42 | 70                  | 105                  | 87                | 10                | 248                             | 3318                | 32.5                | 8150                  | 49            | 0.41          |
| 44         | 20HTWYT#43 | 76                  | 112                  | 95                | 11                | 265                             | 2403                | 33                  | 7389                  | 51            | 0.33          |
| 45         | 20HTWYT#44 | 76                  | 110                  | 95                | 10                | 244                             | 2653                | 31.4                | 7333                  | 47            | 0.36          |
| 46         | 20HTWYT#45 | 76                  | 109                  | 88                | 10                | 260                             | 2801                | 30.8                | 7200                  | 46            | 0.39          |
| 47         | 20HTWYT#46 | 72                  | 106                  | 90                | 9                 | 239                             | 3513                | 33.4                | 9317                  | 50            | 0.38          |
| 48         | 20HTWYT#47 | 70                  | 105                  | 96                | 10                | 274                             | 3657                | 40.2                | 9400                  | 40            | 0.39          |
| 49         | 20HTWYT#48 | 75                  | 109                  | 96                | 11                | 228                             | 3638                | 34.5                | 9317                  | 48            | 0.39          |
| 50         | 20HTWYT#49 | 76                  | 109                  | 91                | 10                | 221                             | 3136                | 29.6                | 7922                  | 47            | 0.4           |
| Grand Mean |            | 74.73               | 108.51               | 90.71             | 10.28             | 257.93                          | 3132.85             | 32.692              | 8157.05               | 43.9          | 0.387         |

**Table 4.** Pearson's correlation coefficients(r) of grain yield and associated components.

|      | DH | DM     | PH    | SL    | SPMS  | GY     | TGW    | BM     | GPS   | HI     |
|------|----|--------|-------|-------|-------|--------|--------|--------|-------|--------|
| DH   | 1  | .739** | -.228 | .299* | -.030 | -.113  | -.263  | -.261  | -.024 | .172   |
| DM   |    | 1      | .018  | .328* | -.088 | .122   | .036   | .036   | .026  | .155   |
| PH   |    |        | 1     | .079  | .063  | .246   | .259   | .320*  | .271  | .024   |
| SL   |    |        |       | 1     | .014  | .070   | .138   | .113   | .055  | .028   |
| SPMS |    |        |       |       | 1     | .412** | .206   | .332*  | .093  | .229   |
| GY   |    |        |       |       |       | 1      | .493** | .713** | .185  | .632** |
| TGW  |    |        |       |       |       |        | 1      | .557** | -.254 | .069   |
| BM   |    |        |       |       |       |        |        | 1      | .170  | -.068  |
| GPS  |    |        |       |       |       |        |        |        | 1     | .082   |
| HI   |    |        |       |       |       |        |        |        |       | 1      |

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

**Table 5.** Cluster Membership

| SN | Cluster   | 1          | 2          | 3          | 4          | 5          |
|----|-----------|------------|------------|------------|------------|------------|
| 1  | Genotypes | Bandganga  | 20HTWYT#02 | 20HTWYT#04 | 20HTWYT#17 | 20HTWYT#28 |
| 2  |           | 20HTWYT#01 | 20HTWYT#07 | 20HTWYT#20 | 20HTWYT#36 | 20HTWYT#45 |
| 3  |           | 20HTWYT#03 | 20HTWYT#08 | 20HTWYT#24 | 20HTWYT#39 |            |
| 4  |           | 20HTWYT#05 | 20HTWYT#09 | 20HTWYT#26 |            |            |
| 5  |           | 20HTWYT#06 | 20HTWYT#11 |            |            |            |
| 6  |           | 20HTWYT#10 | 20HTWYT#13 |            |            |            |
| 7  |           | 20HTWYT#12 | 20HTWYT#15 |            |            |            |
| 8  |           | 20HTWYT#14 | 20HTWYT#19 |            |            |            |
| 9  |           | 20HTWYT#16 | 20HTWYT#23 |            |            |            |
| 10 |           | 20HTWYT#18 | 20HTWYT#25 |            |            |            |
| 11 |           | 20HTWYT#21 | 20HTWYT#27 |            |            |            |
| 12 |           | 20HTWYT#22 | 20HTWYT#38 |            |            |            |
| 13 |           | 20HTWYT#29 | 20HTWYT#42 |            |            |            |
| 14 |           | 20HTWYT#30 | 20HTWYT#47 |            |            |            |
| 15 |           | 20HTWYT#31 |            |            |            |            |
| 16 |           | 20HTWYT#32 |            |            |            |            |
| 17 |           | 20HTWYT#33 |            |            |            |            |
| 18 |           | 20HTWYT#34 |            |            |            |            |
| 19 |           | 20HTWYT#35 |            |            |            |            |
| 20 |           | 20HTWYT#37 |            |            |            |            |
| 21 |           | 20HTWYT#40 |            |            |            |            |
| 22 |           | 20HTWYT#41 |            |            |            |            |
| 23 |           | 20HTWYT#43 |            |            |            |            |
| 24 |           | 20HTWYT#44 |            |            |            |            |
| 25 |           | 20HTWYT#46 |            |            |            |            |
| 26 |           | 20HTWYT#48 |            |            |            |            |
| 27 |           | 20HTWYT#49 |            |            |            |            |



# COMPARATIVE ANALYSIS OF THE EFFECTIVENESS OF ORGANIC AND INORGANIC NITROGEN SOURCES ON POTATO YIELD AND SOIL FERTILITY

Suraj Singh Karkee<sup>1,\*</sup> and Subas Bishwokarma<sup>2</sup>

<sup>1</sup> Department of Agronomy, Faculty of Agriculture, Agriculture and Forestry University (AFU), Rampur, Chitwan

<sup>2</sup> Shree Bhanubhakta Secondary School, Chujathati, Pyuthan

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\*Correspondence:  
sskarki@afu.edu.np  
Tel: +977-9848627115

## ABSTRACT

Potato is a vital food source for the high hills of Nepal. The crop requires the right amount of nutrient at the appropriate time for optimal growth and production. However, the low availability, high cost, and inappropriate timing of applying nitrogenous fertilizers like urea have made it challenging for farmers to use the correct amount in their fields. Therefore, affordable and renewable sources of plant nutrients should be used to supplement chemical fertilizers. In addition to this, using organic sources can improve soil properties. To determine the effects of nitrogen supplied through different sources, an experiment was conducted in a randomized block design. There were eight treatments, each with a different combination of nitrogen supplied through urea and various organic sources as N1(100% rec N via Urea), N2 (50 % rec N via FYM + 50 % rec N via Urea), N3 (50 % rec N via PM + 50 % rec N via Urea), N4(50 % rec N via GM + 50 % rec N via Urea) , N5 (75 % rec N via FYM + 25 % rec N via Urea), N6 (75 % rec N via PM + 25 % rec N via Urea), N7 (75 % rec N via GM + 25 % rec N via Urea), N8 (Farmer practices (FYM 10 t ha<sup>-1</sup>)). The yield of potato tubers was significantly higher when recommended nitrogen was applied 50% through organic manure like poultry, FYM, and goat, and 50% through urea than other treatments, including farmer practice of using 10 t ha<sup>-1</sup> FYM. However, the yield was statistically at par with nitrogen via sole urea. Additionally, the application of organic manures led to an increase in soil properties such as organic manure content, nitrogen, phosphorous, and potassium content. In conclusion, application of recommended nitrogen as 50% via organic manures like poultry, FYM, and goat, and 50% N via urea can enhance potato yield while also positively affecting soil properties.

## 1. INTRODUCTION

Potato is a crucial food source in the high hills of Nepal and are highly versatile in the kitchen as they complement various vegetables. It is the fifth most important staple food after rice, wheat, maize, and millet. The Central Bureau of Statistics (CBS), 2019 had reported that the average yearly per capita potato consumption in Nepal is approximately 29.9 kilograms and contribute 2.17% to the national GDP and 6.57% to the AGDP. However, the national productivity of potato is very low as compared to other neighboring countries (Upadhyay and Timilsina, 2020). One possible reason for this is the inadequate and haphazard application of chemical fertilizers (Subedi *et al.*, 2019).

Production of an average yield per hectare of potato tuber requires a large amount of nutrients. The yield of a fully grown potato crop, which is typically between 25-30 t ha<sup>-1</sup>, results in the removal of approximately 110 kg of nitrogen, 50 kg of P<sub>2</sub>O<sub>5</sub>, and 225 kg of K<sub>2</sub>O per hectare (Choudhary, 1990). Among this primary nutrient nitrogen is most important and is crucial for increasing plant height, leaf area index, shoot dry matter, and tuber yield (Najm, *et al.*, 2010). However, many Nepalese farmers cannot afford to purchase huge quantity of nitrogenous fertilizer like urea, which is often in short supply and expensive. Furthermore, the unbalanced and excessive use of nitrogenous fertilizer

has degraded the soil, increasing soil acidification, impairing soil physical condition, reducing soil organic matter, creating micronutrient deficiencies, decreasing soil microbial population, and causing environmental pollution.

Fortunately, organic manures such as farmyard manure (FYM), poultry manure, and goat manure are affordable and renewable options for supplementing chemical fertilizers. Farmers in Nepal often integrate their crop husbandry with the rearing of cattle, poultry, goats, and other domestic animals, and the waste from these animals can be used as manure in potato fields. Organic manures supply essential nutrients to plants, including nitrogen, and improve soil quality by enhancing soil aggregation, increasing water and nutrient retention, regulating soil pH, and reducing disease incidence. Combining inorganic fertilizers and organic manure has been shown to have a significant effect on the yield of potatoes (Iqbal, *et al.*, 2019).

## 2. MATERIALS AND METHODS

An experiment was carried out in Pyuthan, a mid-hill region of Nepal, to investigate the effects of nitrogen supplied through inorganic and various organic sources on the growth and yield of Khumal rato-2 variety of potatoes. The soil at the site was sandy loam in texture, slightly basic in reaction (pH 7.7) with low organic matter content (1.63 %), and had total nitrogen, available phosphorus, and exchangeable potassium levels of 0.08%, 23.8 kg ha<sup>-1</sup>, and 370.8 kg ha<sup>-1</sup>, respectively. The experiment was designed as a Randomized Complete Block Design with three replications and eight treatments (table 1). The experiment consisted of 24 plots, each measuring 3.5 × 1 m<sup>2</sup>. The recommended dose of fertilizer (NPK) and FYM was 100:100:60 kg ha<sup>-1</sup> and 20 t ha<sup>-1</sup>, respectively. The required amount of organic manure as treatment were calculated based on nitrogen content of respective organic manure as in table 2. The recommended dose of FYM and organic manures as treatments were applied ten days before planting, and the full dose of phosphorous, potassium, and the initial dose of nitrogen were applied as a basal dose at the time of planting. Potato tubers were planted at a spacing of 75 cm × 25 cm. All the intercultural operations such as earthing up, weeding, and irrigation were performed during the growth stages of the potato. The biometric and morphological characteristics of ten tagged plants were recorded at different specified periods, and after the crop harvest, the data on yield and yield attributes were recorded. The data were

first tabulated in Microsoft Excel and analyzed using R-studio. The means were separated using Duncan's Multiple Range Test (DMRT) at the 1% and 5% levels of significance (Gomez and Gomez, 1984).

**Table 1.** Treatment details

| S.N. | Treatment      | Detail                               |
|------|----------------|--------------------------------------|
| 1    | N <sub>1</sub> | 100% rec N via Urea                  |
| 2    | N <sub>2</sub> | 50 % rec N via FYM + 50 % N via Urea |
| 3    | N <sub>3</sub> | 50 % rec N via PM+ 50 % via Urea     |
| 4    | N <sub>4</sub> | 50 % rec N via GM + 50 % via Urea    |
| 5    | N <sub>5</sub> | 75 % rec N via FYM + 25 % via Urea   |
| 6    | N <sub>6</sub> | 75 % rec N via PM + 25 % via Urea    |
| 7    | N <sub>7</sub> | 75 % rec N via GM + 25 % via Urea    |
| 8    | N <sub>8</sub> | Farmer practices (10 t FYM/ha)       |

**Table 2.** Nutrient status of various organic manures

| S.N. | Organic Manure         | N (%) | P <sub>2</sub> O <sub>5</sub> (%) | K <sub>2</sub> O (%) |
|------|------------------------|-------|-----------------------------------|----------------------|
| 1.   | Farm Yard Manure (FYM) | 0.79  | 0.37                              | 0.78                 |
| 2.   | Poultry Manure (PM)    | 3.3   | 2.1                               | 1.3                  |
| 3.   | Goat Manure (GM)       | 2.15  | 0.93                              | 1.8                  |

## 3. RESULTS AND DISCUSSION

### 3.1 Effect on height of plant

Height of plant was significantly ( $p < 0.05$ ) effected by source of nitrogen at 60 DAS, 75 DAS, and 90 DAS but was non-significant at 45 DAS (table 3). At 60 DAS significantly higher plants were observed when 100% recommended N via urea, 50 % recommended N via FYM + 50 % recommended N via Urea, 50 % recommended N via GM + 50 % recommended N via Urea than Farmer practices (10 t FYMha<sup>-1</sup>) and 50 % recommended N via PM + 50 % recommended N via Urea, 75 % recommended N via FYM + 25 % recommended N via Urea, 75 % recommended N via PM + 25 % recommended N via Urea and was statistically at par with highest one. Similarly, at 75 and 90 DAS significantly highest plant height was observed at 100 % recommended N via urea than Farmer practice its sole application of 10 t ha<sup>-1</sup>FYM.

Plant height was recorded significantly higher in sole application of urea for nitrogen or in combination with different organic manure i.e., Poultry Manure, FYM, and Goat Manure than Farmer's practice. Similar

result was observed by (Islam, *et al.*, 2021). This might be due to quick N through Urea in case of sole urea and in contrast lower yield in case of farmer practice might be slow mineralization of nutrient from organic source may not synchronize the nutrient demand of crop (Sharma, 2017). Similarly, above ground biomass was higher in above mentioned treatments that might have resulted in higher accumulation of photosynthates that might contributed in increased size and number of tubers (Congera, *et al.*, 2022).

**Table 3.** Effects of Nitrogen through organic and inorganic source on height of potato plant at Pyuthan

| Treatments                                      | Plant height (cm) |                     |                     |                    |
|-------------------------------------------------|-------------------|---------------------|---------------------|--------------------|
|                                                 | 45DAS             | 60DAS               | 75DAS               | 90DAS              |
| 100% rec N via Urea                             | 14.60             | 31.27 <sup>a</sup>  | 39.20 <sup>a</sup>  | 40.27 <sup>a</sup> |
| 50 % rec N via FYM +<br>50 % via Urea           | 12.47             | 28.47 <sup>a</sup>  | 31.83 <sup>ab</sup> | 31.83 <sup>a</sup> |
| 50 % rec N via PM +<br>50 % via Urea            | 12.92             | 24.23 <sup>ab</sup> | 36.90 <sup>ab</sup> | 39.80 <sup>a</sup> |
| 50 % rec N via GM +<br>50 % via Urea            | 11.73             | 26.80 <sup>a</sup>  | 33.10 <sup>ab</sup> | 35.37 <sup>a</sup> |
| 75 % rec N via FYM +<br>25 % via Urea           | 12.50             | 21.33 <sup>ab</sup> | 26.80 <sup>b</sup>  | 30.20 <sup>a</sup> |
| 75 % rec N via PM +<br>25 % via Urea            | 13.73             | 23.57 <sup>ab</sup> | 29.17 <sup>ab</sup> | 30.13 <sup>a</sup> |
| 75 % rec N via GM +<br>25 % via Urea            | 10.73             | 23.50 <sup>ab</sup> | 28.67 <sup>b</sup>  | 29.67 <sup>a</sup> |
| Farmer practices (10 t<br>FYMha <sup>-1</sup> ) | 8.87              | 14.40 <sup>b</sup>  | 15.67 <sup>c</sup>  | 16.93 <sup>b</sup> |
| <b>LSD</b>                                      | NS                | 9.158*              | 9.872**             | 10.546**           |
| <b>sem (±)</b>                                  | 0.630             | 1.793               | 2.547               | 2.603              |
| <b>C.V.</b>                                     | 20.43             | 21.61               | 18.70               | 18.95              |
| <b>Grand mean</b>                               | 12.19             | 24.20               | 30.17               | 31.78              |

Treatment means in columns followed by common letters are not significantly different from each other based on DMR at 5% level of significance

### 3.2 Effect on number of leaves

Number of leaves in potato plants were not significantly affected ( $p < 0.05$ ) by nitrogen from different sources at all stages of growth after sowing, including 45, 60, 75, and 90 days (table 4). The average number of leaves at 45 days after sowing was 29 and continued to increase until 75 days after sowing, where it reached 36, but then decreased to 35 at 90 days after sowing.

**Table 4.** Effects of Nitrogen through organic and inorganic source on leaf number of potato at Pyuthan

| Treatments                                      | Number of leaves |       |       |       |
|-------------------------------------------------|------------------|-------|-------|-------|
|                                                 | 45DAS            | 60DAS | 75DAS | 90DAS |
| 100% rec N via Urea                             | 29               | 33    | 41    | 40    |
| 50 % rec N via FYM +<br>50 % via Urea           | 28               | 31    | 36    | 35    |
| 50 % rec N via PM +<br>50 % via Urea            | 34               | 36    | 40    | 38    |
| 50 % rec N via GM +<br>50 % via Urea            | 25               | 33    | 38    | 37    |
| 75 % rec N via FYM +<br>25 % via Urea           | 35               | 29    | 36    | 34    |
| 75 % rec N via PM +<br>25 % via Urea            | 28               | 31    | 36    | 35    |
| 75 % rec N via GM +<br>25 % via Urea            | 33               | 30    | 35    | 32    |
| Farmer practices (10 t<br>FYMha <sup>-1</sup> ) | 16               | 23    | 25    | 25    |
| <b>LSD</b>                                      | NS               | NS    | NS    | NS    |
| <b>sem (±)</b>                                  | 2.13             | 1.29  | 1.70  | 1.63  |
| <b>C.V.</b>                                     | 24.00            | 17.34 | 15.76 | 17.49 |
| <b>Grand mean</b>                               | 29               | 31    | 36    | 35    |

Treatment means in columns followed by common letters are not significantly different from each other based on DMR at 5% level of significance

### 3.3 Effect on yield and yield attributes

The use of different nitrogen sources, both organic and inorganic, had a significant effect ( $p < 0.05$ ) on the average weight of tubers (figure 1). The highest tuber weight of 15.23 gm was recorded with 100% recommended N applied using Urea, surpassing other treatments. For instance, tubers weighed 11.79 gm with 50% recommended N via FYM + 50% recommended N via Urea, 12.01 gm with 75% recommended N via PM + 25% recommended N via Urea, 10.45 gm with 75% recommended N via FYM + 25% recommended N via Urea, 9.59 gm with 75% recommended N via GM + 25% recommended N via Urea, and 2.44 gm with sole FYM application. However, tubers from 50% recommended N via PM + 50% recommended N via Urea weighed 13.52 gm, and 50% recommended N via GM + 50% recommended N via Urea weighed 13.17 gm, which were statistically similar to the 15.23 gm weight obtained with 100% recommended N through Urea.

The number of tubers per plant was also significantly ( $p<0.05$ ) influenced by the application of nitrogen through organic and inorganic sources (figure 2). The highest number of tubers per plant was obtained from 100% recommended N via Urea (13 tubers/plant) compared to other treatments such as 75% recommended N via FYM + 25% N via Urea (9 tubers/plant), 75% recommended N via GM + 25% recommended N via Urea (9 tubers/plant), and solely through FYM (5 tubers/plant). Additionally, the remaining treatments, including 50% recommended N via PM+ 50% N via Urea (12 tubers/plant), 50% N via GM+ 50% recommended N via Urea (11 tubers/plant), and 50% recommended N via FYM + 50% recommended N via Urea (11 tubers/plant), as well as 75% recommended N via PM + 25% recommended N via Urea (10 tubers/plant), were statistically similar to 100% N via Urea (13 tubers/plant)).

The potato tuber yield was significantly ( $p<0.05$ ) affected by the application of nitrogen from different sources, both organic and inorganic, as shown in figure 3. The highest yield was obtained when 100% recommended nitrogen was applied via Urea ( $9.53 \text{ t ha}^{-1}$ ), and 50% recommended N Via PM + 50% N via Urea ( $9.50 \text{ t ha}^{-1}$ ), as compared to 75% recommended N Via PM + 25% recommended N Via Urea ( $6.77 \text{ t ha}^{-1}$ ), 75% recommended N Via FYM + 25% recommended N Via Urea ( $6.62 \text{ t ha}^{-1}$ ), 75% recommended N Via GM + 25% recommended N Via Urea ( $4.49 \text{ t ha}^{-1}$ ), and solely

through FYM ( $1.53 \text{ t ha}^{-1}$ ). Similarly, the yield obtained from the plots treated with 50% recommended N Via GM + 50% recommended N Via Urea ( $8.27 \text{ t ha}^{-1}$ ) and 50% recommended N Via FYM + 50% N Via Urea ( $8.20 \text{ t ha}^{-1}$ ) was statistically equivalent to that of 50% N Via PM+ 50% N Via Urea ( $9.50 \text{ t ha}^{-1}$ ). Additionally, the lowest tuber weight ( $1.53 \text{ t ha}^{-1}$ ) was observed from the farmer practice where  $10 \text{ t ha}^{-1}$  FYM was applied.

The application of nitrogen, whether through organic or inorganic sources, had a significant ( $p<0.05$ ) impact on the yield of biomass, as demonstrated in figure 4. The highest weight of potato biomass was obtained from 100% recommended Nitrogen applied via Urea ( $6.62 \text{ t ha}^{-1}$ ), which outperformed other treatments such as 75% recommended N Via FYM+ 25% recommended N via Urea ( $4.24 \text{ t ha}^{-1}$ ) and solely through FYM ( $2.76 \text{ t ha}^{-1}$ ). Moreover, the remaining treatments, including 50% recommended Nitrogen through Poultry Manure + 50% recommended Nitrogen through Urea ( $5.69 \text{ t ha}^{-1}$ ), 50% recommended Nitrogen through Goat Manure + 50% recommended Nitrogen through Urea ( $5.43 \text{ t ha}^{-1}$ ), 75% recommended Nitrogen through Poultry Manure + 25% recommended Nitrogen through Urea ( $4.86 \text{ t ha}^{-1}$ ), and 50% recommended Nitrogen through FYM + 50% recommended Nitrogen through Urea ( $4.80 \text{ t ha}^{-1}$ ), as well as 75% recommended nitrogen through GM +25% recommended nitrogen through Urea ( $4.76 \text{ t ha}^{-1}$ ), had similar statistical results as 100% recommended nitrogen applied through Urea ( $6.62 \text{ t ha}^{-1}$ ).

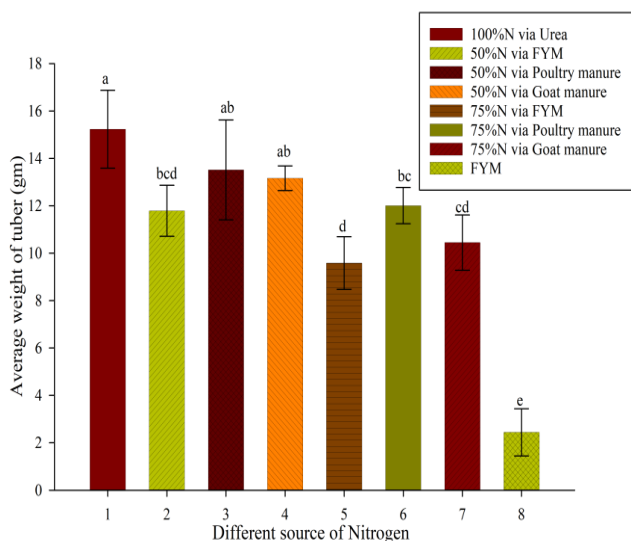


**Figure 1.** Average weight of a tuber as influenced by nitrogen from different sources

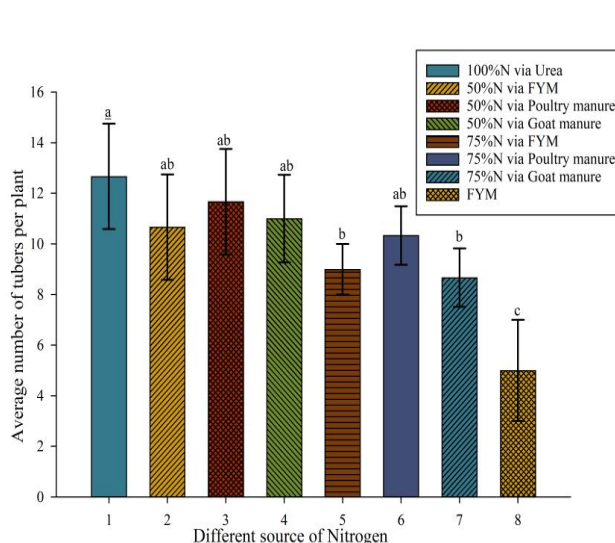


**Figure 2.** Number of a tubers as influenced by nitrogen from different sources

Means on bar graph not connected by same letters are significantly different by DMRT ( $p<0.05$ )

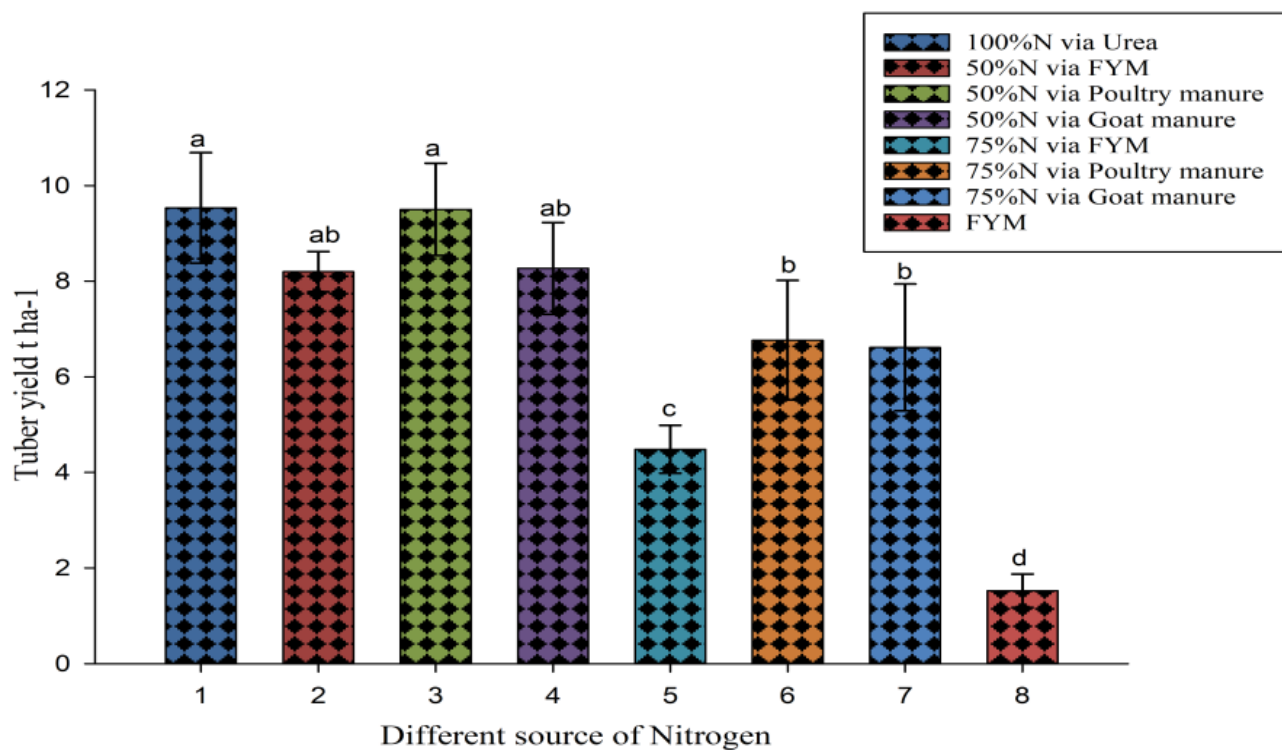


**Figure 3:** Effect of different source nitrogen on tuber yield of potato tuber  
Means on bar graph not connected by same letters are significantly different by DMRT ( $p < 0.05$ )

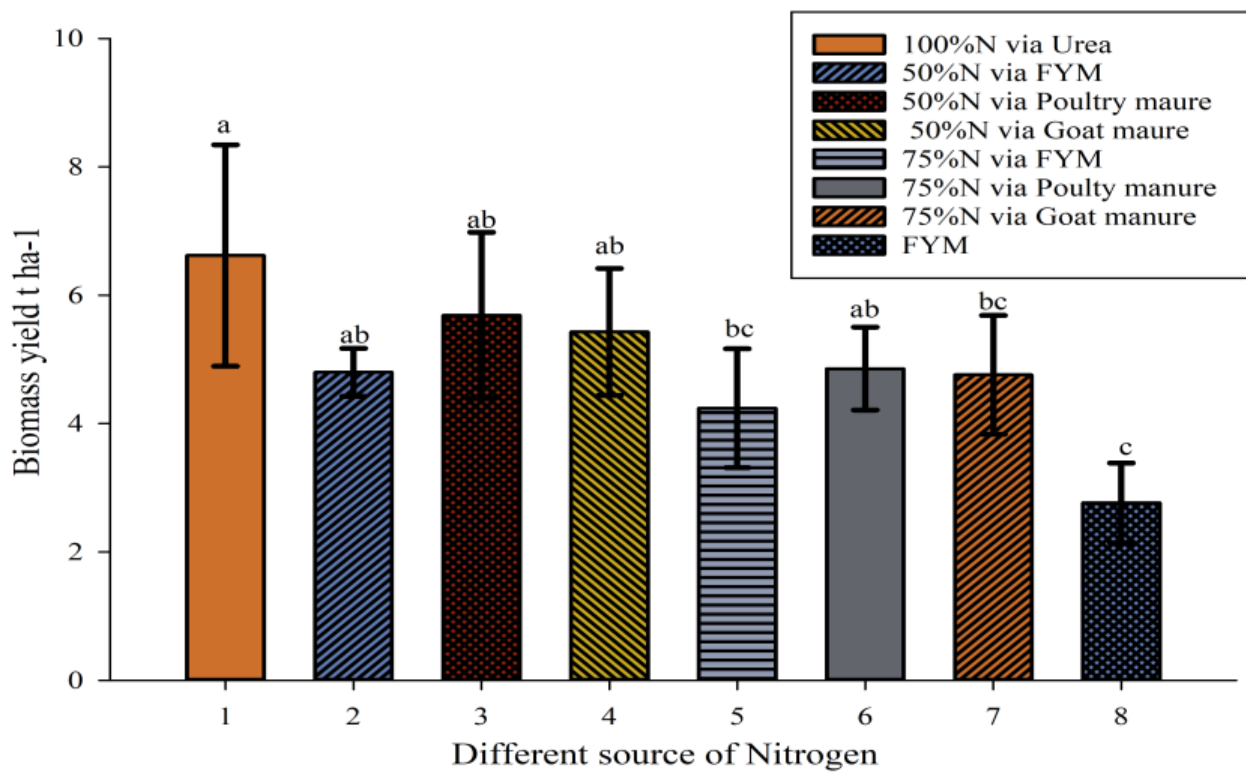


**Figure 4:** Effect of different source nitrogen on tuber yield of potato biomass yield (t/ha)  
Means on bar graph not connected by same letters are significantly different by DMRT ( $p < 0.05$ )

The recommended dose of nitrogen was applied solely through urea or with 50% urea and 50% recommended nitrogen from different organic manures like FYM, Poultry, and Goat, had significantly higher numbers of potato tubers and higher average potato tuber yield than those with only 25% recommended nitrogen from urea and 75% recommended nitrogen from organic manure like FYM, Goat, and the farmer's practice. Pandey *et al.* (2022) found that the potato yield was higher when N is applied in combination of both organic and inorganic source in case of poultry and goat manure. Similarly, in case of poultry manure Mohammed and Dawa, (2018) found higher yield of potato tubers when nutrients are applied via both organic and inorganic source. The reasons for this may be due to the balanced supply of nutrients from both sources, which can promote better plant growth, leading to more significant root development, and ultimately more tubers per plant. In addition to that there is good synergistic effect of the two fertilizers in 50-50 proportion as organic manures enhance the soil physical, chemical, and biological properties, leading to better nutrient availability and uptake by the plant. In contrast, synthetic fertilizers provide a quick supply of essential nutrients, which can boost plant growth and development. The yield of potato tuber was recorded significantly highest in sole application of inorganic nitrogen through urea or supplying 50% recommended N via Urea + 50% recommended N via organic manure like FYM, Poultry, Goat than supplying 50% recommended N via Urea + 75% recommended N via organic manures and farmer practices this might be due to contribution of larger average tuber weight and a greater number of tubers per plant.

### 3.4 Effect on soil fertility

Table 5 indicates that the use of nitrogen from both organic and inorganic sources had a positive impact on various soil properties, such as organic matter content, nitrogen, phosphorus, potassium, and pH. The application of farmyard manure (FYM) and urea in a ratio of 75:25 resulted in the highest organic matter content. This is because organic manures, such as FYM, are rich in organic matter. It is possible that a higher quantity of FYM was applied to meet the nitrogen dose required for the plant. Similarly, applying 50% of the recommended nitrogen through poultry manure (PM) and 50% recommended N through urea resulted in higher availability of major plant nutrients, such as nitrogen, phosphorus, and potassium. This is because poultry manure contains a higher amount of nutrients and mineralizes more quickly than other manures, which results in more nutrients being released in the soil. Similar findings were reported by Rayne and Lawrence (2020).

**Table 5.** Effects of nitrogen from organic and inorganic sources on soil fertility when applied in a potato at Pyuthan

| Treatment                                        | OM   | N    | P <sub>2</sub> O <sub>5</sub> | K <sub>2</sub> O | pH  |
|--------------------------------------------------|------|------|-------------------------------|------------------|-----|
| 100% rec N via Urea                              | 1.63 | 0.13 | 51.9                          | 394.8            | 7.7 |
| 50 % rec N via FYM +<br>50 % via Urea            | 2.96 | 0.11 | 62.1                          | 350              | 7.8 |
| 50 % rec N via PM +<br>50 % via Urea             | 2.12 | 0.16 | 92.4                          | 470.8            | 7.7 |
| 50 % rec N via GM +<br>50 % via Urea             | 2.61 | 0.11 | 41.9                          | 373.2            | 7.8 |
| 75 % rec N via FYM +<br>25 % via Urea            | 3.27 | 0.07 | 45.2                          | 350              | 7.8 |
| 75 % rec N via PM +<br>25 % via Urea             | 2.12 | 0.11 | 71.9                          | 373.2            | 7.7 |
| 75 % rec N via GM +<br>25 % via Urea             | 2.13 | 0.08 | 63.6                          | 347.2            | 7.8 |
| Farmer practices (10<br>FYM t ha <sup>-1</sup> ) | 2.94 | 0.15 | 73.3                          | 206.8            | 7.8 |
| Before Test                                      | 1.31 | 0.08 | 23.8                          | 370.8            | 7.7 |

## CONCLUSION

In this experiment, plant growth, tuber yield was significantly higher when nitrogen was applied in 50 % recommended N via urea and 50 % recommended N via organic manures like Poultry Manure, FYM, and Goat manure. Thus, it could be concluded that application of nitrogen in inorganic (urea) and organic source in 50-50 ratio for higher potato tuber yield at the same time enhance the soil fertility status of particular soil.

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## ECONOMICS OF POTATO PRODUCTION IN MUSTANG DISTRICT OF NEPAL

Aashish Karki<sup>1,\*</sup>, Nabin Bhusal<sup>2</sup>, Nabin Bhandari<sup>3</sup>, Bipin Bastakoti<sup>4</sup>, Kritim Shrestha<sup>1</sup> and Biraj Sharma<sup>1</sup>

<sup>1</sup> Agriculture and Forestry University, Chitwan, Nepal

<sup>2</sup> Department of Plant Breeding and Genetics, Agriculture and Forestry University, Chitwan, Nepal

<sup>3</sup> Central Fisheries Promotion and Conservation Center, Balaju, Kathmandu

<sup>4</sup> University of Louisiana at Lafayette

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\*Correspondence:  
asishkarki60@gmail.com  
Tel: +977-9860876511

### ABSTRACT

Potato possesses a huge potential for production in Nepal and is a major source to ensure food and nutritional security among people of mountainous region. The study was carried out in four rural municipalities of Mustang district to assess the economics of potato production. A pre-tested semi-structured questionnaire was administered randomly to 90 different farmers. The collected data was tabulated and analysed using STATA. Potato production was identified as a profitable farming business with an average productivity of 18.95 t/ha with benefit-cost ratio of 1.59. The average per hectare total cost of production of potato was found to be Rs 660774.8 on mustang district. The study reveals that expenditure on labour wage and organic manure contributed more than three-quarters of total production cost of potato. There were no any significant differences between the different rural municipalities in terms of the total cost of production but a significant difference (1% level of significance) was observed over the production of potato per hectare. An increment in 1% cost of seeds was found to result in increase in 0.27% of income from potato production, however a diminishing return to scale (0.232) was revealed in the study. Incidence of disease and pest and price fluctuation were identified as a major constraint for potato production and marketing respectively. Timely availability of inputs, mechanization to reduce labour cost, training on advanced technology, wide coverage of agriculture extension service, and adoption of climate smart farming technologies is recommended to ensure profitability of potato production in Mustang.

### 1. INTRODUCTION

Potato (*Solanum Tuberosum*), an herbaceous annual plant that belongs to the family Solanaceae of the flowering plants (Reddy *et al.*, 2018) is one of the important food crops of Nepal. In hilly and mountainous regions potato is regarded as a staple crop. Potato is the fifth most important crop grown in Nepal after rice, maize, wheat, and millet whereas it is the fourth major crop of the world (Kloos *et al.*, 1991; Reddy *et al.*, 2018). It ranks second in terms of production and ranks first in productivity in Nepal (Sapkota *et al.*, 2019). Potato possesses huge potential for production in Nepal and it can be cultivated from the lower belt of terai to higher elevations of 4400 metres above sea level (Kharel, 2022). The production of crops goes on

decreasing whereas the period required for maturation keeps on increasing with the increase in the altitude above sea level (MoALMC, 2020). Low productivity of cereal crops coupled with low access to food items due to physical and financial barriers results in food and nutrition insecurity in the high Himalayan region of Nepal (FAO, WFP, MoALD, 2019). In this scenario, potato can be grown as a major staple crop substituting rice, maize, wheat to ensure food and nutritional security. Potato is considered an important cash crop as well. The area under potato cultivation in the fiscal year 2020/21 was 1,98,788 ha whereas the national production of potato was 33,25,231 mt. (Figure 1) (MoALD, 2022).

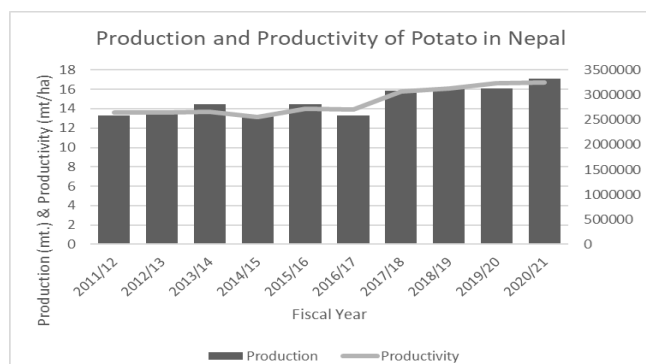


**Figure 1.** Production and Productivity of Potato in Nepal

Source: (MoALD, 2022)

Although the food security situation in Nepal has improved over the years, still 4.6 million people are food-insecure. Among this 20% of the households are moderately food-insecure and 10% are severely food insecure (Ministry of Health, Nepal; New ERA; and ICF, 2017). Households in rural communities and mountainous regions where food production is comparatively low and price of food items is high are more likely to be food insecure. Mustang district falls under the category of food deficit region of Gandaki province where the annual production is insufficient to meet the demand of food requirement (NPC and WFP, 2019). Mountainous topography, the cold climate at a higher altitude above sea level makes it challenging for the cultivation of cereals and other food crops. Potato is the only crop that can be grown at the altitude of 3000 m in a short duration of 4-5 months. It gives good production even up to the altitude of 4000 m. Hence, potato serves as an important substitute for any other staple crop in this region. Development Vision Nepal Pvt. Ltd. (DVN), 2018 has identified value chain development in potato and its linkage to the regional and global value chain as a source of better income for farmers of Gandaki Province. Kunjo, Lethe, Kujjo, Pangling, Falyak, Tukuche, Marpha, Jomsom, Kagbeni, Muktinath have been mentioned as pocket areas for potato cultivation in Mustang district under the Commercial Potato Cultivation Programme (National Potato Development Program, 2017).

In the Mustang district, potatoes are cultivated as a rainy season crop in the months of Falgun to Bhadra. In the fiscal year 2020/21, the total area under potato cultivation of Mustang district was 306 ha, total production is 4,795 mt. and total productivity was 15.67 mt. per ha. The average productivity of potato in Mustang district is above the average potato

productivity of Gandaki province (15.09), and below the national productivity of 16.73 mt. per ha (MoALD, 2022). Although potato is grown as a major staple crop, study on economics of potato production in Mustang district is limited. Thus, the study was undertaken to assess the economics of potato production in Mustang district with an aim to compute the cost associated with production, productivity and benefit-cost ratio and make a comparison among different rural municipalities and identify the problems associated with potato production. Moreover, the study aims to provide valuable insights into current state of potato production and will help to identify problems associated with production and marketing.

## 2. MATERIALS AND METHODS

### 2.1 Study area

Mustang district is one of the eleven districts of Gandaki Province and covers an area of 3,573 sq. km. It is located at the latitude of 29° 04' 60.00" N and longitude of 83° 54' 59.99" E. The district is surrounded by China in the North, Myagdi district in the South, Manang in the East, and Dolpa in the West. The total area of this district is 3,63,958 ha out of which 3,639 ha is suitable for agriculture. The selection of Mustang district for this study is based on its status as a food-insecure region within Gandaki Province, where potato serves as a crucial staple crop and a significant source of income. The study location is shown in the figure 2. The survey was conducted at different wards/villages of four rural municipalities namely Thasang, Gharapjong, Barhagaun Muktiokshetra and Dalome.

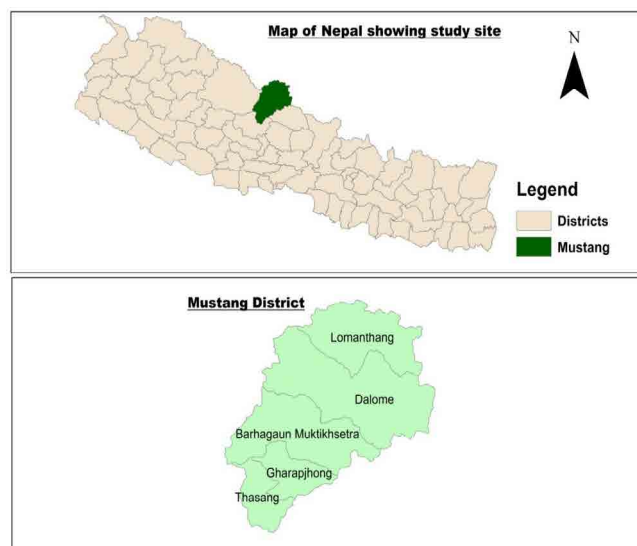


**Figure 2.** Map showing the study site

## 2.2 Sampling process

A total of 90 farmers were selected using simple random sampling technique. The sample size was determined by using Cochran's formula at 10% error (Cochran, 1977)

$$n = \frac{\frac{z^2 pq}{e^2}}{1 + \frac{\frac{z^2 pq}{e^2} - 1}{N}}$$

Where, n is the sample size, z is the selected critical value at desired confidence level (90% confidence level), p is the estimated proportion of an attribute that is present in the population (p=0.5), q=1-p and e is the desired level of precision (e=0.10), N is the population size (3,305 i.e., number of households in Mustang)

## 2.3 Data collection and analysis

The household survey was conducted using a pre-tested questionnaire to collect the necessary information from April to June 2021. Phone survey was also used for collection of data because of the second wave of Covid-19 and subsequent travel restrictions. Relevant secondary information was collected by assessing various secondary data sources, including annual report, publications, journals from National Potato Development Programme, Nepal Agricultural Research Council and government websites. The collected data was coded and entered into MS-Excel and the analysis was completed through the use of MS-Excel and STATA (version 16). Descriptive statistics (average, frequency, standard deviation) and analytical statistics (multiple regression model) were performed.

## 2.4 Benefit-cost ratio

The benefit-cost ratio was calculated by dividing the gross return by total variable cost as used by Bajracharya & Sapkota, 2017 using the formula below:

$$\text{Benefit-Cost Ratio} = \frac{\text{Gross Return}}{\text{Total Variable Cost}}$$

Total Variable Cost = Summation of all variable Costs (seeds, organic manure, chemical fertilizers, pesticides, labour)

## 2.5 Production Function and Return to Scale Analysis

Cobb–Douglas production function (CDPF) is widely used to represent the technological relationship between the various inputs used and output produced (Bajracharya & Sapkota, 2017). The following form of

CDP was used

$$Y = aX_1^{b_1}X_2^{b_2}X_3^{b_3}e^u$$

Where Y is the total income from potato production in hectares (NRs.),  $X_1$  is the cost of seeds,  $X_2$  is the expenditure on manure,  $X_3$  is the expenditure on manure, e error term and  $b_1$ ,  $b_2$ ,  $b_3$  coefficient to be estimated.

The CDPF in the form expressed above was linearized in logarithmic function for the ease in computation

$$\ln Y = \ln a + b_1 \ln X_1 + b_2 \ln X_2 + b_3 \ln X_3 + u$$

where  $\ln$ =natural logarithm, a=constant and u= random disturbance term

The summation of respective coefficients obtained from CDPF was used to calculate the value of return to scale (Ghimire & Dhakal, 2014).

$$\text{Return to Scale (RTS)} = \text{Summation of coefficients} \\ (b_1 + b_2 + b_3)$$

Decision rule:

Return to scale >1: Increasing return to scale

Return to scale =1: Constant return to scale

Return to scale <1: Decreasing return to scale

## 2.6 Ranking of problems

Based on a review of literature and discussion with farmers and key officials of the Agriculture Knowledge Centre (AKC), major problems associated with potato production and marketing in Mustang were identified and included in the questionnaire. The identified problems were ranked using force ranking technique as used by Subedi *et al.*, 2019. The formula given below was used to find the index of importance for problems where the value of score ranged from 0 to 1.

$$I_{\text{imp}} = \sum \frac{SiFi}{N}$$

Where,  $I_{\text{mp}}$  = Index of Importance

$\sum$  = Summation

$Si$  =  $i^{\text{th}}$  scale value

$Fi$  = frequency of  $i^{\text{th}}$  importance given by respondents

N = total number of respondents

### 3. RESULTS AND DISCUSSION

#### 3.1 Socio-demographic characteristics of the respondent:

The average age of the respondent was found to be 47.68 and within the range of 21 to 81 years. The majority of the respondents (76.67%) were male and the remaining 23.33% were female. The majority of the respondent (42.22%) were Thakali followed by Gurung (32.22%), Dalit (14.44%), Brahmin/Chhetri (6.67%), and Magar (4.44%) (Table 1).

**Table 1.** Gender and Ethnicity of the respondent in present survey

| Gender          | Frequency | Percentage |
|-----------------|-----------|------------|
| Male            | 69        | 76.67%     |
| Female          | 21        | 23.33%     |
| Ethnicity       |           |            |
| Thakali         | 38        | 42.22      |
| Gurung          | 29        | 32.22      |
| Dalit           | 13        | 14.44      |
| Brahmin/Chhetri | 6         | 6.67       |
| Magar           | 4         | 4.44       |

The total population of the study area of 90 households was 524 with an average family size of 5.82 members, which was higher than the national average (4.50) (CBS, 2017). The average household percentage of females was 49.24% and the participation of females in agriculture (both full-time and part-time) was more compared to male. Among the total respondents, 18.89% respondents were found to be illiterate and the remaining 81.11% were literate. The average household income of the respondents was Rs. 4,52,966.7 whereas the average household income from potato production was Rs. 95,788.89. From the survey, the average landholding of the respondent was found to be 0.667 hectares whereas the land used for potato cultivation

on an average was 0.1455 hectares. 42.22% of the respondents practiced sole-cropping of potato, 40% of them practiced mixed or intercropping with other crops, and the remaining 16% practiced both sole and mixed cropping. 16.67% of the farmers had attended training whereas 83.33% of the farmers hadn't participated in any sort of training. The majority (82.22%) of the respondents had cultivated local varieties of the potato (Local Rato and Local Seto). The majority (82.22%) of the farmers used organic manure (cattle, poultry, goat or sheep manure) as the only source of fertilizer in the potato field. The majority of farmers (82.22%) employed irrigation systems to cultivate potatoes, while 17.78% relied on rainfall as their source of irrigation. Late blight was considered the most important disease in the study area whereas white grub was the most important insect.

#### 3.2 Economics of potato production

##### 3.2.1 Cost of production of potato

The total cost of production associated with potato farming was found to be Rs. 660,774.8 per hectare for Mustang district. The major cost incurred comprised labor expenses of Rs 269,407.8 per hectare, cost of organic manure of Rs 249,172.2 per hectare and seed tuber expenses of Rs.132,825.4 per hectare (Table 2). A report on production cost of cereal, cash, vegetable and industrial crops in Nepal 2015/16 by Bhandari *et al.* (2016) calculated the average cost of potato production in Myagdi district as Rs 243,687.60 per hectare. The cost of potato production in Achham district was Rs 256,285.293 per hectare (Sapkota *et al.*, 2019) Rs. 197,186 per hectare in Baglung district (Bajracharya & Sapkota, 2017) and Rs. 172,292.2 per hectares in Nuwakot district (Dahal & Rijal, 2019). The cost of potato production in Mustang district was found to be much higher than the cost of production as suggested by the existing literature. This could have been because of remote and mountainous topography of the region.

**Table 2.** Variable Cost of Potato Production in Mustang district

| Items                    | Overall  | Thasang  | Gharapjong | Baragung-Muktikshetra | Dalome   |
|--------------------------|----------|----------|------------|-----------------------|----------|
| Seeds                    | 132825.4 | 125736.4 | 127813     | 136192.8              | 152385.2 |
| Organic Manure           | 249172.2 | 226270   | 243915.2   | 274249                | 218478.8 |
| Chemical Fertilizers     | 1630.6   | 660      | 1472.2     | 4242.8                | 0        |
| Pesticides               | 7738.8   | 9183.2   | 11589.6    | 4675.2                | 0        |
| Labor                    | 269407.8 | 237856.8 | 275142     | 270262.2              | 305714.6 |
| Total Cost of Production | 660774.8 | 599706.4 | 659931.8   | 689622                | 676578.6 |

The highest cost of production was associated with labour requirement which amounts to 41% of the total cost of production. Mainly labour was used in land preparation and planting, intercultural operations (earthing up, weeding), and harvesting of potato. Organic manure accounted the second highest cost, 38% of the total cost of production, followed by the cost of seed tuber attributing 20% of the total cost of production. The cost of chemical fertilizers and pesticides was minimal as farmers opted for organic manure (Figure 3). Bajracharya & Sapkota (2017) also identified the cost of farm-yard manure (FYM), seeds and labour as major costs incurred in the potato production. Similarly, Chauhan *et al.* (2022) reported cost of seeds, labour and FYM accountable for the majority of cost of production in Darchula district of Nepal.

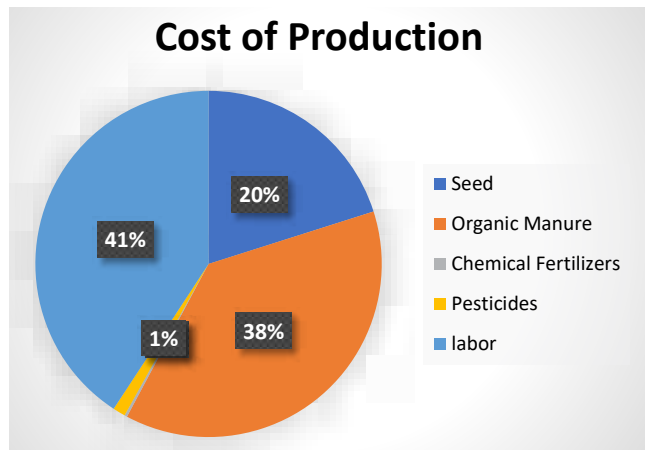


**Figure 3.** Cost of production of potato farming

There was no any significant difference among different rural municipalities with respect to the total cost of production as observed by the one-way ANOVA test (Table 3).

**Table 3.** Oneway ANOVA test among different rural municipalities with respect to the total cost of production

| Source        | SS         | df | MS         | F    | Prob > F |
|---------------|------------|----|------------|------|----------|
| Between group | 1.1013e+11 | 3  | 3.6712e+10 | 0.76 | 0.52     |
| Within group  | 4.1807e+12 | 86 | 4.8612e+10 |      |          |
| Total         | 4.2908e+12 | 89 | 4.8612e+10 |      |          |

### 3.2.2 Production, productivity and benefit-cost ratio

Potato was cultivated on an average area of 0.1455 hectares with an average production of 18.95 tonnes per hectare. Among the rural municipalities, Thasang had the lowest production per hectare at 14.13 tonnes, while Dalome had the highest at 26.9 tonnes. Gharapjong and Baragung-Muktikshetra recorded production rates of 19.16 and 18.26 tonnes per hectare, respectively (Table 4). The productivity (18.95) t/ha which is above the national productivity of 16.73 t/ha and the productivity of Mustang district reported in 2020/21 (15.67 ton/ha). However, the productivity was similar to the average productivity of Mustang district in 2019/20 i.e., 19.92 t/ha (MoALD, 2021). High altitude region with longer maturation time is more suitable for potato cultivation, especially for higher tuber yield and higher quality products (Islam *et al.*, 2020).

**Table 4.** Economic Indicators of potato farming in different rural municipalities of Mustang district

| Items                                | Potato grown area |          |            |                       |          |
|--------------------------------------|-------------------|----------|------------|-----------------------|----------|
|                                      | Overall           | Thasang  | Gharapjong | Baragung-Muktikshetra | Dalome   |
| Total Variable Cost (NRs. / hectare) | 660774.8          | 599706.4 | 659931.8   | 689622                | 676578.6 |
| Total Production (kg. / hectare)     | 18954.8           | 14139.6  | 19160      | 18269.6               | 26900    |
| Total Income (NRs. / hectare)        | 1052749.6         | 822783.2 | 997852.8   | 1021636               | 1601096  |
| Total Profit (NRs. /hectare)         | 391974.8          | 223076.8 | 337921     | 332014                | 924517.4 |
| Benefit-Cost Ratio (NRs. / hectares) | 1.59              | 1.37     | 1.51       | 1.48                  | 2.36     |

Despite the significantly higher cost of production, the benefit cost ratio was found to be comparable to other regions. This can be attributed to higher potato yield per unit area and higher selling price (Rs. 55.54 per kg) received by the farmers. Potato cultivation was found to be profitable with a B/C ratio of 1.59. The highest B/C ratio was observed at Dalome rural municipality with a value of 2.36, followed by Gharapjong (1.51), Baragung-Muktikshetra (1.48), and lastly Thasang (1.37) (Table 4). The Benefit-Cost ratio of potato production in Nuwakot was 1.71 (Dahal and Rijal, 2019), 1.23 in Okhaldhunga district (Phulara *et al.*, 2022), 1.47 in Achham (Sapkota *et al.*, 2019), 1.44 in Baglung (Bajracharya & Sapkota 2017), 1.62 in Darchula (Chauhan *et al.*, 2022). Higher production of potato per unit area along with the higher price received by the farmers for their produce (Rs. 55.54 per kg) could have been the reason for a higher B/C ratio in Mustang. The one-way ANOVA test revealed a significant difference at 1% level of significance in potato production per hectare across rural municipalities. Specifically, there was a significant difference at 5% level of significance in production per hectare between Dalome and Baragung-Muktichhetra, as well as between Dalome and Gharapjong. Additionally, the difference in production per hectare between Thasang and Dalome rural municipality was highly significant at a 1% level of significance as shown by the Bonferroni test (Table 5). The difference in productivity between rural municipalities maybe because of increasing incidence of diseases and pests and different environmental constraints such as erratic rainfall, hailstorm in in lower elevation of Mustang district. Bom *et al.* (2023) in his study has reported farmers' perception on an increase in temperature with warmers summers, decreased snowfall and variable rainfall pattern in Lower Mustang region. A study by Adhikari *et al.* (2021) also reported that agriculture has been adversely impacted by climate change with an increase in agricultural diseases, insects and invasive species in Muktinath region of Mustang district.

**Table 5.** One-way ANOVA test with Bonferroni test among different rural municipalities with respect to production per hectare in Mustang

| Source        | SS         | df | MS         | F    | Prob > F  |
|---------------|------------|----|------------|------|-----------|
| Between group | 1.4085e+09 | 3  | 469512136  | 8.04 | 0.0001*** |
| Within group  | 5.0207e+09 | 86 | 58380735.6 |      |           |
| Total         | 6.4293e+09 | 89 | 72239097.4 |      |           |

| Row Mean-Col-<br>umn Mean | Baragung-<br>Muktikshetra | Dalome              | Gharajong      |
|---------------------------|---------------------------|---------------------|----------------|
| Dalome                    | 8649.33<br>0.01**         |                     |                |
| Gharapjong                | 898.238<br>1.00           | -7751.09<br>0.01**  |                |
| Thasang                   | -4129.76<br>0.528         | -12779.1<br>0.00*** | -5028<br>0.106 |

\*\*indicates significant at 5% level

\*\*\* indicates significant at 1% level

### 3.2.3 Production function analysis

Table 6 represents the Cobb-Douglas production function analysis of potato production in the study area. It was found that the increase in cost of seeds by 1% would result in increase in total income from potato production by 0.27% and the increment was significant at 5% level of significance. A study by Bajracharya & Sapkota, 2017 also found a positive increment in income from potato production with increase in cost of seeds in Baglung district. In the present survey, it was revealed that labor and manure constitute over three-quarters of total cost of production which might explain the lack of significant relationship of manure and labor with total income from potato production. The mean variance inflation factor (VIF) was 1.05 and none of the variables had VIF higher than 2 indicating the absence of multicollinearity between independent variables.

**Table 6.** Production function analysis of potato production

| Explanatory Variable    | Coefficient | Standard Error | T value | P>  t |
|-------------------------|-------------|----------------|---------|-------|
| Seeds (NRs. / ha)       | 0.277**     | 0.133          | 2.08    | 0.040 |
| Manure (NRs. / ha)      | 0.000       | 0.000          | 0.10    | 0.919 |
| Labour (NRs. / ha)      | -0.045      | 0.107          | -0.42   | 0.675 |
| Constant                | 11.032      | 1.81           | 6.08    | 0.000 |
| R <sup>2</sup>          | 0.0493      |                |         |       |
| Adjusted R <sup>2</sup> | 0.0162      |                |         |       |
| F-value                 | 0.2237**    |                |         |       |
| Return to scale         | 0.232       |                |         |       |

\*\* indicate significance at 5% level of significance

### 3.2.4 Return to scale analysis

The sum of regression coefficients obtained from CDPF was 0.232 which indicated decreasing return to scale. This implies that increase in cost of variable inputs would return lesser amount of income from potato production. Amgai *et al.* (2011) in his study also reported a decreasing return to scale in apple production in Mustang district.

### 3.2.5 Problems associated with the production of potatoes

The incidence of disease and pests was identified as the primary concern in potato production, with the highest index value of 0.83. Lack of fertilizers and pesticides ranked second (0.76) followed by shortage of high-quality planting material (0.68) (Table 7). Erratic rainfall patterns and impacts of climate change were an emerging issue at Thasang rural municipality whereas lack of manpower was another additional problem at Dalome rural municipality, Upper Mustang. Upadhyay *et al.* (2020) also reported disease, pest problems as one of the most important problems in his study. Bajracharya & Sapkota (2017) also identified disease infestation in potatoes as a major reason behind low productivity in Baglung district. Similarly, Subedi *et al.* (2019) also reported lack of improved quality seeds and the incidence of disease and pest as major problems in potato production.

**Table 7.** Ranking of the problems associated with production of potato

| Problems                               | Index | Rank |
|----------------------------------------|-------|------|
| Incidence of disease and pest          | 0.83  | I    |
| Lack of fertilizers and pesticides     | 0.76  | II   |
| Lack of quality planting materials     | 0.68  | III  |
| Lack of training and extension service | 0.61  | IV   |
| Technical Constraints                  | 0.46  | V    |
| Lack of Irrigation                     | 0.42  | VI   |
| High Cost of Production                | 0.21  | VII  |

### 3.2.6 Marketing of the produce

The majority of the farmers (77.78%) were satisfied with the price received whereas 22.22% of the respondents were unsatisfied with the price. Fluctuation in the price of potato and delay in payment resulted in dissatisfaction in the farmers. The farmers received an average of Rs 55.54 per kg of potato produce with price varying from

Rs 36.67 to Rs. 90 per kg. The local cultivar of potato is sought out for their superior and excellent taste and has an excellent market demand which is why potatoes fetch such a high price in Mustang. The fluctuation of price can be attributed to several factors including, seasonal variation in production, involvement of marketing channel and intermediaries, weather and road conditions, lack of storage facilities. Traders collected the produced potatoes and were responsible for marketing of the major produce whereas only a small quantity of the produce was sold directly by farmers themselves.

### 3.2.7 Problems associated with marketing

Price fluctuation was identified as the most important problem with an index value of 0.90. Lack of transport was the second most important problem with an index value of 0.66 followed by high middleman margin with an index value of 0.65 (Table 8). Phulara *et.al.* (2022) also reported more middleman, lack of adequate market information and price fluctuation as major problems in marketing of potato at Okhaldhunga district.

**Table 8.** Ranking of the problems associated with marketing of potato

| Problems                      | Index | Ranking |
|-------------------------------|-------|---------|
| Price Fluctuation             | 0.90  | I       |
| Lack of transport facility    | 0.66  | II      |
| High middleman margin         | 0.65  | III     |
| Lack of storage               | 0.61  | IV      |
| Lack of grading and packaging | 0.45  | V       |

## 4. CONCLUSION

Potato is an important staple crop in food-insecure mountainous region of Nepal such as Mustang. According to the study, farmers in this region cultivated potato on an average area of 0.1455 hectares and the average household income from potato production was Rs. 95,788.89. The farmers grew local cultivars of potatoes and preferred organic manures over chemical fertilizers. The total cost of production associated with potato farming was found to be Rs. 660774.8 per hectare for Mustang district which was much higher compared to other regions primarily due to remote topography and lack of infrastructures resulting in high cost associated with labor, organic manure and seeds. However, potato farming was profitable with a B/C ratio of 1.59 because of higher productivity of 18.95 t/ha and higher per unit selling price. A significant difference was observed in production of potato per hectare

between rural municipalities which can be attributed to environmental constraints and increase in disease and pest in lower elevations as an impact of changing climate. It is recommended to prioritize development of local level policies and adaptation strategies aimed at mitigating adverse effects of climate change in agriculture. Production function analysis revealed that an increase in 1% cost of seeds, would increase the total income from potato production by 0.27%. The return to scale was found 0.232, indicating a decreasing return to scale. Incidence of disease and pest, lack of fertilizers and pesticides and lack of quality planting material were identified as major problems associated with potato production whereas price fluctuation and lack of transport were major constraints of marketing of potato. Moreover, a huge proportion of population had not received any training related to potato cultivation. Hence, strengthening agricultural extension activities

for dissemination of production technology along with timely availability of inputs is necessary to increase the profitability of potato production.

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## ECONOMIC ANALYSIS OF COFFEE SUBSECTOR IN NEPAL

Indra Hari Paudel<sup>1</sup>

<sup>1</sup> Ministry of Agriculture and Livestock Development, Singhadurbar, Kathmandu, Nepal

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\*Correspondence:  
indraharipaudel@gmail.com  
Tel.: +977-9849674744

### ABSTRACT

Coffee being one of the emerging crops with high potential to export to earn foreign currency, has been given due importance by the Government of Nepal. Among different agricultural goods produced in and exported from Nepal, the competitiveness of coffee has quickly increased in recent years, thus contributing to the improvement of rural livelihoods. This study was based on review of literature and analysis of secondary data/information. Statistical tools such as correlation analysis was also used to analyze data. According to the official records coffee production area has expanded from around 135.7 ha in fiscal year 1994/95 to 3052 ha in 2020/21 and the production has increased from 12.95 Mt. of dry cherry to 315 Mt. of green beans during the same period. Nepal's coffee subsector is characterized by low productivity, low production and poor quality. Inter alia, it was mainly due to incidence of insect pests, production done by smallholder farmers in a widely scattered area creating problems in quality control and collection leading to higher cost. The good price, high demand and overwhelmingly organic system of production are the encouraging factors but the formidable challenge remains on fighting with white stem borer and providing incentives for small holder farmers throughout the relatively long gestation period of coffee crop. Apart from low production level, organic certification and quarantine requirements pose serious challenges to export. Coffee produced in Nepal is Organic & Fair-trade and is readily accepted as a Specialty Coffee in specific international markets.

### 1. INTRODUCTION

Coffee is one of the most popular beverages in the world prepared from the roasted seeds of an evergreen plant of the genus *Coffea*. The two most important species of coffee are *Coffea arabica* (Arabica coffee) - which accounts for over 60 percent of world production - and *Coffea canephora* (Robusta coffee) (MoALD, 2014). Coffee plants are cultivated in more than 70 countries, mainly in equatorial Latin America, Southeast Asia, and Africa. Brewed Coffee has stimulating effect on humans because of its caffeine content. Coffee being one of the emerging crops with high potential to export and earn foreign currency, it has been given due importance by the Government of Nepal. The National Tea and Coffee Development Board is mainly responsible for the promotion of coffee from production to its end consumption in the country. Coffee is one of the highly traded commodities in the world. Among the

different agricultural goods produced in and exported from Nepal, the competitiveness of coffee has been quickly increased in recent years, thus contributing to the improvement of rural livelihoods. In Nepal, a saint, Hira Giri of Gulmi district bought some seeds from Myanmar and introduced them in Aapchaur of Gulmi in 1938 (MoALD, 2014). Gradually the plantation of coffee started spreading to adjoining districts viz, Palpa, Syangja, Kaski, Baglung and others. But the commercial cultivation started intensively since 1990s. Processing coffee began in 1983 with the establishment of Nepal Coffee Company (NeCCo) in Manigram, Rupandehi that processed dry cherries collected from farmers. By the time the national coffee policy was formulated in 2003, there were several international and national organizations working in coffee sector and by correctly identifying the prospects of coffee enterprise,

there are ever increasing numbers of actors involved, directly or indirectly, in its production, processing and trade. The farmers are mostly organized in producer groups and cooperatives, Nepal Coffee Producers' Association (NCPA) and Central Coffee Cooperative Union (CCCUL) being the apex farmers' organizations.

The self-pollinating Coffee Arabica is a highly acclaimed species of the coffee and entire coffee of Nepal belongs to this species. The agro-climate of mid-hills is highly suitable for the farming of this high-value plant thereby contributing for livelihoods, income generation and economic growth. The land that could be available for coffee farming lies between the altitudes of 800 meter and 1600 meter above mean sea level in Nepal and based on which around 1.19 million ha land area is potential for coffee production in the country. The productivity of coffee can be increased up to 1500 kg/ha through proper management, adequate shading and manuring practices. So, there is a huge opportunity for production of high grown, organic specialty coffee in Nepal. It creates employment not only in the farms but also in pulping centers, coffee industries and café houses. Additionally, it can enjoy duty free access for coffee beans under trade preferences for LDCs in all the major coffee importing countries. In the coffee value chain, the sector faces numerous challenges resulting in low productivity, low production and even poor quality. Presently, coffee cultivation is marked by sporadic production with farming done at small-sized lands that creates problems in quality control, coffee collection and prices. Farmers face a knowledge gap on improved and organic ways of plantation, orchard management, sanitation and harvesting. The incidence of disease and pests on the other hand, is believed to destroy around 20% of Nepal's coffee plants annually. Nepalese coffee is by default organic but not able to export all the product as organic because of the complicated certification process. Therefore, with this background this study is focused on the assessment of production and marketing aspects of coffee sub-sector in Nepal, with following specific objectives:

- assess the production status of coffee in Nepal; and
- appraise the export, import and business position of coffee in Nepal.

## 2. METHODOLOGY

This paper is primarily based on the review of literatures, secondary data published on different government

publications, statistical reports and journal articles. Specifically, the source of data is Trade and Export Promotion Center, Nepal, Department of Customs, Nepal, Economic Survey, Central Bureau of Statistics (CBS), Statistical Information on Nepalese Agriculture and other publications. The growth rates, percentage analysis, averages and qualitative methods have been used in statistical analysis. Most of the studies have used time series data to examine the relationship between different variables.

## 3. RESULTS AND DISCUSSION

### 3.1 Coffee production in Nepal

Among the different agricultural goods produced in and exported from Nepal, the competitiveness of coffee has quickly increased in recent years, thus contributing to the improvement of rural livelihoods. According to the official records, coffee production area has expanded from around 135.7 ha in fiscal year 1994/95 to 3052 ha in 2020/21 and the production has increased from 12.95 Mt. of dry cherry to 315 Mt. of green beans (equivalent to 394 Mt of dry parchment) during the same period of time (Figure-1). In 2001/02, coffee production reached a triple figure for the first time registering coffee output of 139.2 Mt. Its overall production was maximum (530 Mt. of green bean) for fiscal year 2018/19. However, the production has been found reduced to 297 Mt. of green beans in fiscal year 2019/20, a sign that does not indicate well to the coffee sector of Nepal. Notwithstanding the official figures of previous years, area under coffee has also shown a slight decrease for fiscal year 2019/20, presumably due to the deterrence of farmers from catastrophic insect, the white stem borer (*Xylotrechus quadripes*) among others (CBS, 2019). The existing 3052 hectare of land under coffee barely makes 0.26% of the area of the potential coffee growing areas. According to Commercial Coffee Survey 2018/19, 16% of the coffee farmers cultivate coffees at less than a ropani land area while 71% farm at farm area spread at 1-5 ropani land area (CBS. 2019). The production of 315 Mt. green beans would add approximately NRs. 412 million to the economy at a prevailing market rate, directly providing thousands of employments throughout the value chain. Gulmi, Palpa, Arghakhanchi, Lalitpur, Tanahu, Kavre, Sindhupalchok, Lamjung, Kaski, Gorkha, Syangja, Parbat and Baglung are the major coffee producing districts in the country. Among them, Syangja, is the leading producer in terms of plantation area (311 ha) and Sindhupalchok is the leading district in terms of volume of coffee production (19.6 Mt.) in FY 2020/21. More than 31,340 households are involved in

coffee farming in altogether 44 districts (MoALD, 2022). The prevalence of coffee farmer groups, associations and cooperatives have paved a way to assert themselves on policy lobbying, technology dissemination and collective marketing. The acreage of coffee per household is rather small and needs strong incentive to them to amplify their acreage. These developments led to the introduction of Nepal's first coffee policy in 2004. Organic certification

started a year later, and 'Nepal Coffee' logo was registered in the Department of Industry in 2010.

The coefficient of determination ( $R^2 = 0.968$ ) for the coffee plantation area in Nepal shows that there is a strong positive correlation between government's efforts, policies/programs on coffee promotion and area expansion in coffee subsector in Nepal (Figure 2).

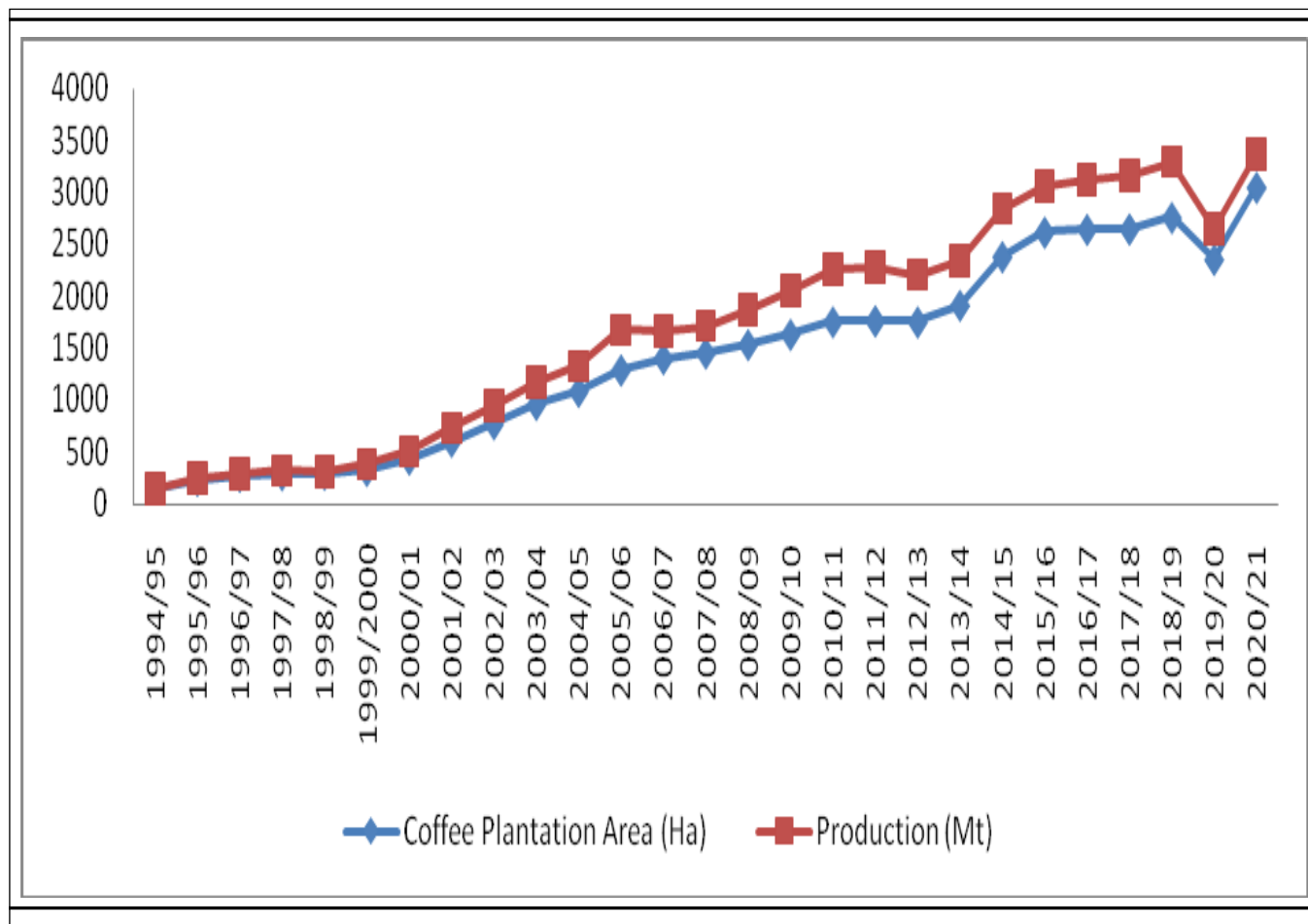


**Figure 1.** Plantation area and production trend of coffee in Nepal

*Source: Coffee Database in Nepal, MoALD, 2014*

However, the production of coffee shows the greater variability over the periods. There are several factors affecting national coffee production such as incidence of disease & pests, market price, brand value, certification, technical knowhow among farmers etc. In spite of these challenges, the annual average production of the coffee for the period of 27 years (1994/95 to 2020/21) is 286.16 Mt. which is good. The coefficient of determination ( $R^2 = 0.745$ ) for the coffee crop production in Nepal shows

that there is a positive correlation between agriculture policies/programs and national production of coffee in Nepal (Figure 2). Therefore, it can be interpreted that the government policies, programs and extension approaches that were adopted in the past for promotion of coffee subsector had positively contributed to increase the area, production and productivity of coffee in Nepal.

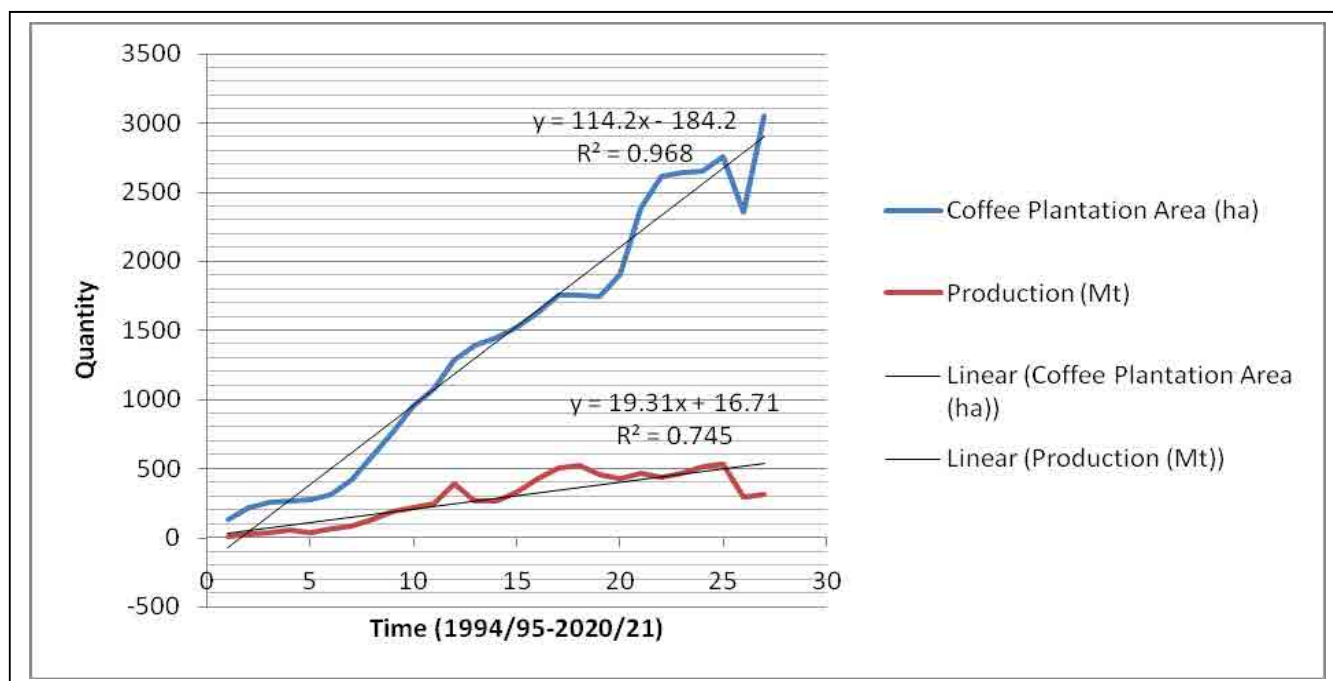


**Figure 2.** Coffee area and production in Nepal.

Statistical Information on Nepalese Agriculture 2013/14 to 2020/21 collection

In the coffee value chain, the sector faces numerous challenges resulting in low productivity, low production and even poor quality in Nepal. Specifically, coffee cultivation is marked by sporadic production with farming done at small-sized lands that creates problems in quality control, coffee collection and prices. Reason behind low production and productivity level is farmers' knowledge gap and reliance on traditional farming tools and techniques for crop production owing to sophisticated modern production and processing technology. In Nepal, selective hand picking of fully ripen cherries is done and pulped right after harvesting with mini hand pulper (wet processing) with necessary additional procedures. However, for domestic and home consumption dry processing method is also adopted where cherries are harvested and dried in sun at the farm level. Farmers face a knowledge gap on improved and organic ways of plantation, orchard management, sanitation and harvesting, which means that training farmers should sit at the top of coffee development strategy. For instance, farmers tend to sell immature beans without grading and standard postharvest practices. These bad practices at the farm level affect the final quality of the coffee. Unlike dominant producers of coffee in the world, Nepal's coffee sector is far below in terms of mechanization. Farmers still rely on traditional tools and techniques for grading, sorting

and processing. Farmers, including coffee processors, lack access to proper, appropriate or standard washing and size grading machines for cherries before pulping, appropriate pulping machines and proper parchment drying systems or techniques, appropriate mechanical process for sorting, hulling and size grading of parchments and mechanical devices to measure the moisture content of coffee beans. In addition, there is inadequate postharvest handling including storage, transportation, packing and packaging, and the problem of lack of modern and economic irrigation systems. Pest and disease, on the other hand, is believed to destroy around 20% of Nepal's coffee plants annually. Coffee leaf rust disease (*Hemileia vastatrix*) and white stem borer (*Xylotrechus quadripes*) are some of the recurring challenges to coffee plantations, which leave farmers with little choice – either destroy or replant.

### 3.2 Export and import of coffee

#### 3.2.1 Export of coffee from Nepal

Nepal Trade Integration Strategy 2016 (NTIS 2016) has identified Nepali coffee, popularly known as Himalayan beans, as one of the nine products having high export potential. Nepali coffee is exclusively Arabica variety and further qualifies as a specialty coffee in specific markets for its

distinct aroma, production outside the traditional tropical zone in the highlands, community-based production and organic content. Arabica variety accounts for 70% of the global coffee trade. The idea of serving Nepali highland coffee beans to domestic and foreign markets has found strong reception from established and new entrepreneurs leading to rise in Nepali coffee brands, cafes, and suppliers. Although a small player in the world coffee market, Nepal's mountain-based green bean is a high price, niche market product, which presents a new but a substantial avenue for export earnings. But as the global market experienced changes with shifts in consumer preferences in terms of quality, taste and varieties, especially towards specialty coffee, Nepal's potential to cater to a niche segment that loves specialty coffee also started becoming more evident. Organic certification started a year later, and 'Nepal Coffee' logo was registered in the Department of Industry in 2010. The collective trademark 'Nepal Coffee' is so far registered in the EU and across seven more markets (South Korea, Singapore, Japan, Norway, Switzerland, New Zealand and Hong Kong). The branding move was introduced to establish Nepal coffee brand in the global market, discourage the export of inferior coffee and gain competitive advantage and market opportunities.

Finally, coffee was recognized as one of the potential export sectors in NTIS 2016 – but missed out in its priority list – so no action plans were designed for the coffee sector. Today coffee is served everywhere across Nepal's cities and highways, while coffee drinkers gather outside home and workspace along with their favorite cups, further driving coffee culture and coffee habits. Coffee is among the few commodities in which Nepal has the consistent trade surplus status but of lately, the exports have decreased, partly indicating the increasing consumption in domestic market. In 2020, despite the pandemic, Sindhupalchowk that promotes its coffee as 'Sindhu Organic Coffee' in foreign and domestic markets, exported around one-third (7 Mt.) of their 24 Mt output to Australia, Japan and South Korea (Pande,2022). Compared to the first time coffee was exported, the country has since registered a significant increase in the overall coffee exports (Table-1 & Figure-3). However, in recent years, exports have dipped. Coffee exports reached 508.59 Mt. in the FY 2008/09, dropping considerably to 69.52 Mt. in 2021/22 (Table-1). Similarly, exports value increased to Rs 125,108,000 in FY 2008/09 also dropped to Rs 117,084,000 in 2021/22 (Table 1 & Figure 3).

**Table 1.** Status of export and import of coffee in Nepal

| Fiscal Year | Import         |                      | Export         |                      |
|-------------|----------------|----------------------|----------------|----------------------|
|             | Quantity (Mt.) | Value In NRs. (,000) | Quantity (Mt.) | Value In NRs. (,000) |
| 2007/2008   | 2.27           | 334                  | 58.20          | 22175                |
| 2008/2009   | 63.04          | 11651                | 508.59         | 125108               |
| 2009/2010   | 62.21          | 13861                | 69.04          | 24363                |
| 2010/2011   | 31.33          | 12513                | 279.76         | 93089                |
| 2011/2012   | 28.52          | 20906                | 109.44         | 43095                |
| 2012/2013   | 51.76          | 31728                | 85.86          | 45676                |
| 2013/2014   | 64.32          | 35921                | 66.19          | 52207                |
| 2014/2015   | 108.84         | 54748                | 99.59          | 99176                |
| 2015/2016   | 105.04         | 55541                | 111.17         | 107901               |
| 2016/2017   | 99.37          | 50401                | 94.60          | 84543                |
| 2017/2018   | 163.38         | 65893                | 84.22          | 93722                |
| 2018/2019   | 1262.41        | 98014.24             | 84.15          | 99703.16             |
| 2019/2020   | 266.17         | 118846.2             | 46.89          | 57726                |
| 2020/2021   | 198.67         | 106720.6             | 72.39          | 96003.34             |
| 2021/2022   | 279.82         | 127591               | 69.52          | 117084               |

Source: Trade & Export Promotion Center and Department of Customs, Nepal

Germany, Japan, Switzerland, Netherlands and Australia were the leading exports market for Nepal in FY 2020/21, contributing to around 90% of the total coffee export earnings (TEPC, 2022). Yet, overall export level is too tiny compared to a supposed combined demand for 8,000 Mt. of Nepali green beans in domestic and international markets. The low export capacity is mainly explained by limited production volume and low productivity. The high price, high demand and overwhelmingly practiced organic systems are the encouraging signs but the formidable challenge remains on fighting white stem borer and providing incentives for smallholders throughout the relatively long gestation period of coffee. In 2021, Nepal exported \$903k in coffee, making it the 109th largest exporter of coffee in the world. In the same year, coffee was the 97th most exported product in Nepal (OEC, 2021). The main destination of coffee exports from Nepal are: Germany (\$273k), Switzerland (\$260k), Japan (\$178k), Australia (\$46.9k), and Czechia (\$26k) (OEC, 2021). Likewise, in 2020, Nepal exported \$625k in coffee, making it the 110th largest exporter of coffee in the world (OEC, 2021). At the same year, coffee was the 81st most exported product in Nepal. The main destination of coffee exports from Nepal are: Germany (\$228k), Japan (\$97k), Switzerland (\$89k), Netherlands (\$71.9k), and Australia (\$37.7k). The fastest growing export markets for coffee of Nepal between 2020 and 2021 were Switzerland (\$185k), Japan (\$91.9k), and Germany (\$35.8k). Similarly, the fastest growing export markets for coffee of Nepal between 2019 and 2020 were Australia (\$20.3k), Germany (\$10.9k), and Czechia (\$7.88k). In 2018, the average tariff for Nepal in coffee was 26% (OEC, 2021).

### **3.2.2 Import of coffee in Nepal**

Growing coffee imports corroborate that coffee consumption is trending, and that it holds a promising domestic market. Almost 279.82 Mt. of coffee was imported in the FY 2021/22, a 40% growth in import compared to the previous fiscal year (Table-1 & Figure-3). There was 266.31 Mt. of coffee imported

from India, followed by 5.59 Mt. from Malaysia and 1.84 Mt. from Republic of Korea in FY 2021/22 (MoALD, 2022). In 2020, Nepal imported \$532k in coffee, becoming the 154th largest importer of coffee in the world. At the same year, coffee was the 602nd most imported product in Nepal. In 2021, Nepal imported \$884k in coffee, becoming the 152nd largest importer of coffee in the world. At the same year, coffee was the 620th most imported product in Nepal (OEC, 2021).

Nepal imports coffee primarily from: India (\$660k), Malaysia (\$134k), Thailand (\$34.1k), Italy (\$29.6k), and United Kingdom (\$14k). The fastest growing import markets in Coffee for Nepal between 2020 and 2021 were Malaysia (\$103k), Thailand (\$10.9k), and United Kingdom (\$6.23k). Similarly, the fastest growing import markets in coffee for Nepal between 2019 and 2020 were Singapore (\$3.82k) and Germany (\$1.36k) (OEC, 2021). Further, the domestic market consumes over 70% of the national production, according to Nepal Tea and Coffee Development Board. The demand is met by farming that has expanded to 44 districts, and across 31,340 small farmers. Among them, Kavrepalanchowk, Gulmi, Nuwakot, Sindhupalchowk, Kaski, Palpa, Ilam, Lalitpur, Syangja and Parbat were the leading ten producers in the FY 2020/21 contributing to almost two-third of the national production (MoALD, 2022). Whereas in a bid to expand production capacity of other districts and enhance production, Arghakhanchi, Gulmi, Syangja, Palpa and Pyuthan are enlisted in Coffee Superzone under the Prime Minister Agriculture Modernization Project. In 2018, the average tariff for Nepal in coffee was 26% (OEC, 2021). The countries with the highest import tariffs for coffee were Angola (Most Favoured Nation duty rate treatment, 26%), Burundi [Most Favoured Nation (MFN) duty rate treatment, 26%), Benin (MFN duty rate treatment, 26%), Burkina Faso (MFN duty rate treatment, 26%), and Botswana (MFN duty rate treatment, 26%). According to Observatory of Economic Complexity (OEC) in 2021, Nepal exported \$903k in coffee and imported \$884k in coffee, the net trade was \$19k (OEC, 2021).

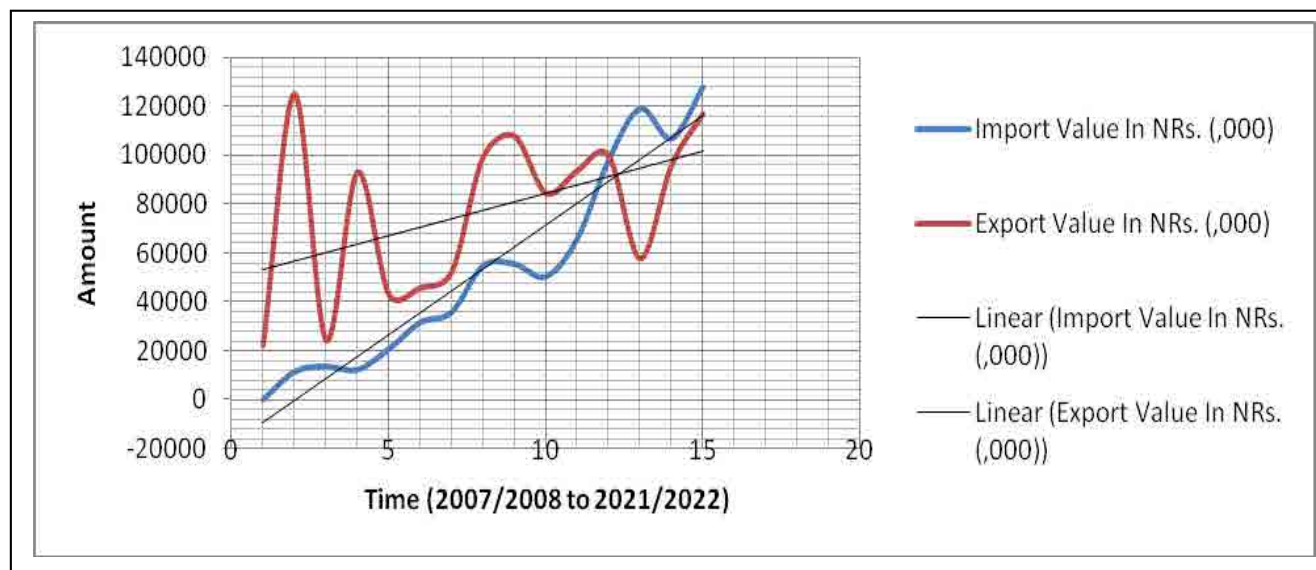


**Figure 3.** Export and import trend of coffee in Nepal

Source: Trade & Export Promotion Center and Department of Customs, Nepal

### 3.2.3 Trade promotion and branding of nepalese coffee

Nepal has registered the Nepal Coffee collective trademark in seven more markets viz, South Korea, Singapore, Japan, Norway, Switzerland, New Zealand and Hong Kong. The brand had been registered only in the European Union (EU) previously (The Kahmandu Post, 2017). Nepali coffee has been gaining popularity in the international market lately. The registration of the Nepal Coffee collective trademark has helped to boost the sales of the Nepalese coffee in the international market. According to the traders, the high quality Nepali product fetches up to \$15 per kg, about the same as world class products from top coffee producing countries like Brazil.

Himalayan Java, that sources its coffee from Ilam, runs 23 outlets across Nepal including Jomsom and Namche Bazaar and even has footprints in the US and Canada. Plantec Coffee Estate that produces Arabica coffee of Cattura variety runs a 1,100-ropani coffee estate in Nuwakot with annual production capacity of over 60 Mt. of coffee (Pande, 2022). Plantec sells its coffee under the brand name of Jalpa, among other brand names. Producers like Nepal Organic Coffee Products, Alpine, Lekali, Lumle and Kar.ma, among other prominent producers, are also expanding markets in Nepal and abroad. Entrepreneurs are also rolling up their sleeves to gain international recognition to

seize international market opportunities. In 2016, Greenland Organic Farm earned specialty certification from Specialty Coffee Association of America (Pande, 2022). Two years later, coffee produced by Lekali Coffee Estate received similar recognition becoming the first Nepali brand to be reviewed by the Californian trade publication 'Coffee Review', one of the leading coffee guides in the world. Grown at Bhirkune Village in Nuwakot, the beans scored 90 points out of 100. In 2020, its coffee rated 92 points, one of the best in South Asia (Pande, 2022). Dedicated barista institutions, along with hospitality colleges, are also growing in the cities, catering to the rising demand for skilled baristas, all thanks to growing local demand for skilled workforce and aspirations for jobs abroad where the coffee skills can prove handy.

## 4. CONCLUSION:

Coffee is a high value cash crop with environmental importance and is being popular among Nepalese since last few decades. It has been spreading in over 44 districts of the middle hills of Nepal. Coffee plantation is still a new adventure in Nepal. In late seventies, expansion of coffee took place as commercial crop to some extent when Government of Nepal imported coffee seed from India for distribution. The major shift to commercial coffee production took place in mid-eighties. However, after the year 2002, substantial increase in the export and also increase in domestic market consumption to

some extent motivated coffee producers to consider coffee as a major income generating crop. Now, coffee can be commercially produced in many parts of the country. However, there is great potentiality in mid hilly region for organic coffee production as it has got suitable climate, topography, soil, relative humidity, temperature and rainfall for Arabica Coffee. Some districts like Gulmi, Palpa, Argakhanchi, Lalitpur, Tanahu, Kavre, Sindhupalchowk, Lamjung, Kaski, Gorkha, Syangja, Parbat and Baglung are successfully growing and producing coffee beans and is increasing gradually. All Nepali Coffee is of Arabica variety, mix of bourbon and typical, grown above 800 meters up to 1,600 meters altitude with organic and eco-friendly practices by small farmers. Nepal Coffee is considered specialty coffee for its distinct flavor aroma and body as it is grown in higher altitude, away from the main coffee growing Capricorn and Cancer belt (beyond 230 latitude).

Coffee produced in Nepal is Organic and Fair-trade and is readily accepted as a Specialty Coffee in specific international markets. This will certainly help in diversifying process and will increase the income of the farmers as well as other individuals involved in coffee

processing and marketing enterprise. Nepal is exporting coffee beans mostly in Japan, America and European countries. This has been extended to other parts of the world. In spite of the government efforts, growing private sector enthusiasm, and global and domestic market surge for the Nepali coffee, and relative increase in coffee plantation land area, Nepal's coffee sector is clearly underperforming. To increase production level, more plantations are needed which would require more land parcels. For this, the government needs to unlock access to underutilized lands for commercial coffee plantations. Coffee sector is also constrained by poor research backup in Nepal. Identifying right plant varieties that best suits different geographical and agro-climatic conditions of coffee districts and managing pests and diseases would require robust coffee research centers. Policy reforms and more government support and spending targeted at small-holder farmers, cooperatives and private sector commercial plantations are therefore critical to tap the huge export potential. Government spending and policies must focus on small-holder farmers, who have limited landholding and can't invest in improving their farms and equipment, to enhance coffee yield and quality.

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# EFFECT OF ALTITUDE ON ADULT EMERGENCE, PUPAL MORTALITY AND ADULT SEX RATIO OF CHINESE CITRUS FLY, *Bactrocera minax* (ENDERLEIN) (DIPTERA: TEPHRITIDAE)

Bipin Karki<sup>1,\*</sup>, Resham Bahadur Thapa<sup>2</sup>, Debraj Adhikari<sup>2,3</sup>, Bhola Gautam<sup>2</sup> and Amrita Shedi<sup>4</sup>

<sup>1</sup> Agriculture Development Section, Shailung Rural Municipality, Dolakha, Nepal

<sup>2</sup> Agriculture and Forestry University, Chitwan, Nepal

<sup>3</sup> Plant Quarantine and Pesticide Management Centre, Hariharbhawan, Lalitpur

<sup>4</sup> Institute of Agriculture and Animal Science, Tribhuvan University, Nepal

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\*Correspondence:

bipinkarki50@gmail.com

Tel.: +977-9843255684

## ABSTRACT

Problems of Chinese Citrus Fly (CCF), *Bactrocera minax* (Enderlein) in citrus orchards have been increasing in Nepal. The reason behind the failure in CCF control is the lack of a clear and thorough understanding of the adult emergence period in different altitudes. Therefore, the pupae of CCF were collected from the soil below the infested sweet orange trees in Sunapati Rural Municipality, Ramechhap, Nepal and reared in containers of height 5 cm and diameter 6 cm (10 pupae per container) in randomized complete block design selecting different locations as treatments, viz. 1247 masl (Bethan), 1354 masl (Nagsiwa), 1443 masl (Aarukharka), 1561 masl (Sadi), 1650 masl (Dimipokhari) replicating four times. Early peak emergences of adult CCF (four weeks earlier in 4th week of April) occurred at lower altitudes as compared to the higher altitudes (in 4th week of May), where peak-emergence was recorded. Male: female ratio (range 1.05-1.37) did not differ significantly at different altitudes, while pupal mortality (25%) was found to be the highest in upper elevations. The study concludes that fly management strategy should be made according to the date of emergence at least two weeks earlier at lower elevations than in higher elevations.

## 1. INTRODUCTION

Citrus is the most popular and widely grown fruit crop in more than 140 countries of the world (FAO, 2021). In Nepal, citrus is cultivated in 50,235 ha with 32,188 ha productive area and 9.67 mt/ha productivity (MoALD, 2022). Mandarin (*Citrus reticulata*) occupies the first position in terms of area and production followed by sweet orange (*C. sinensis*) in terms of production, whereas lime in terms of area in Nepal (MoALD, 2020). Despite the well-accepted value and importance of citrus fruits in terms of income and climatic suitability, the production of citrus fruits is decreasing year after year. It is due to traditional methods of crop management, marginal production, legal and institutional constraints, inadequate irrigation, biotic and abiotic stresses and the major one is early fruit drop due to attack of the fruit flies (Gautam *et al.*, 2020). Citrus fruits are depleted by fruit flies quantitatively and qualitatively (Adhikari, Tiwari and Joshi, 2018). Plant clinic data available in Plant-wise Online Management System (POMS) in 2016 highlighted the problems of fruit flies in vegetables,

Cucurbitaceae and Solanaceae families (Adhikari, Thapa, Joshi, Pandit and Sharma, 2020). It not only causes yield loss by fruit dropping, but also limits the export trade of citrus fruits from Nepal because of the phyto-sanitary restrictions due to the fruit fly problems in the citrus orchards.

The world fauna of Tephritidae comprises nearly 4,716 species under 492 genera (Pape, Blagoderov and Mostovski, 2011). There are 27 species of fruit flies recorded in Nepal (Adhikari, Thapa, Joshi and Du, 2022). Out of them, citrus fruit loss in Nepal is commonly caused by Chinese citrus fly (CCF), *Bactrocera minax* (Enderlein), which has become a serious threat in citrus production since 2014 in central hilly districts (Sindhuli and Ramechhap) with up to 97% fruit loss (Adhikari, Tiwari and Joshi, 2018). The reasons behind the failure in fly control are a lack of clear and thorough understanding of pest biology, population dynamics, agro-ecosystem and climatic patterns (Adhikari, Thapa, Joshi, Liang and Du, 2020). The overwintering pupa of

CCF remains in the soil of depth 3-7 cm (Wu, Zhang and Liang, 2008) for 5 to 7 months (Sharma and Dahal, 2020). These species have the capacity to withstand high moisture content by lowering their respiration rate (Li *et al.*, 2019). The adult emerges from the soil in April/May which reaches to its peak in June / July and starts to decline in August (Sharma and Dahal, 2020). The pest, *B. minax* requires one year for completing its life cycle. This study presents the relationship between adult emergence, fly mortality and sex ratio in relation to altitude variations, so that management measures could be made accordingly.

## 2. MATERIALS AND METHODS

### 2.1 Site of study



**Figure 1.** Map of Nepal showing Ramechhap and research site of Sunapati Rural Municipality



**Figure 2.** Placement of pupae in the container

The experiment was done in randomized complete block design (RCBD) in Sunapati Rural Municipality,

Ramechhap, Nepal from April to June, 2021. Pupae of citrus fruit fly were collected from the soil below the infested trees of sweet orange on 10<sup>th</sup> March, 2021 from three different locations (Bethan, Aarukharka and Dimipokhari), and sheltered in small cylindrical containers of height 5 cm and diameter 6 cm containing slightly moist sandy soil below 1 cm from the top of the container at different altitudes to see its date of emergence. For this, a total of 200 pupae were collected. These pupae were reared at five different elevations (treatments) on 2<sup>nd</sup> April, 2021, viz. 1247 masl (Bethan), 1354 masl (Nagsiwa), 1443 masl (Aarukharka), 1561 masl (Sadi), 1650 masl (Dimipokhari) using 40 pupae at each elevation replicating 4 times in 4 containers (10 pupae per container). Date of emergence, male-to-female ratio and pupal mortality percentage of CCF were calculated.

### 2.2 Adult Sex Ratio (Male: female)

Male: female ratio was calculated by dividing emerged number of male adult fly by emerged number of female fly.

$$\text{Male:female ratio} = \frac{\text{Emerg ed number of male fly}}{\text{Emerg ed number of female fly}}$$

### 2.3 Mortality Percentage of Pupae

Pupal mortality percentage was calculated as follows:

$$\text{Pupal mortality percentage} = \frac{\text{Number of dead pupae inside rearing container}}{\text{total number of reared pupae}} \times 100 \%$$

### 2.4 Statistical Analysis

The data were recorded in an Excel worksheet and subjected to Genstat® 15th edition for Analysis of Variance (ANOVA). Before performing the ANOVA, data were transformed through square root transformation i.e.  $\sqrt{(x + 0.5)}$  to reduce the heterogeneity of variance. Duncan's Multiple Range Test (DMRT) at a 5% level of significance was used for mean comparison (Gomez and Gomez, 1984).

## 3. RESULTS AND DISCUSSION

The emergence of adult CCF differed significantly at different altitudes on different weeks/months of the year except for 2<sup>nd</sup> week of May and June (Table 1). The higher mean numbers of CCF emerged during the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week of April at lower altitudes (1247 masl), whereas higher adult CCF emerged only during the 4<sup>th</sup>

week of May and 1<sup>st</sup> week of June at higher altitude (1650 masl). At lower altitude, most of adults emerged during April and at higher altitude, most of them emerged during May. Similar results were found in China (Xia, Ma, Hou and Ouyang, 2018) in which adult flies of CCF emerged from March to April depending upon local temperature. A similar result was also observed by NCRP, Paripatle, Dhankuta Nepal (NCRP, 2014). The highest emergence at lower altitudes might be due to the adequate and sufficient temperature for pupal eclosion. These findings are supported by Adhikari, Thapa, Joshi, Du and GC (2021) in which they found the longer diapause intensity of 165.5 days and 164.2 days at higher altitudes as compared to shorter diapause intensity of 143.5 and 141.3 days at lower altitudes for sweet orange and lemon, respectively. Acharya and Adhikari (2019) found the delayed in emergence of Chinese citrus fly with increase in altitude due to lower temperature at higher altitudes. Xiaofei and Hui (2009) conducted a study to find out the effect of temperature on development and survival of *B. correcta* and found

that the higher temperature was the most conducive for reaching early maturity. Gautam *et al.* (2020) also found early emergence of adult Chinese citrus fly at lower elevation as compared to upper elevation. But Dong *et al.* (2013) found the termination of pupal diapause faster at long and more chilling temperature.

Adult sex ratio of CCF was found to be almost similar for all altitudes (Table 2). However, the ratio was greater than 1, which means the emergence of pupae was dominated by the male population. This result showed that male-to-female ratio was similar regardless of variation in altitudes. Regmi *et al.* (2023) also found non-significant differences among sex ratios at different altitudes. However, Chauhan *et al.* (2019) found the highest male-to-female ratio at a lower altitude of the range 1400-1474 masl. Pupal mortality was found to be the highest for upper elevations as compared to lower altitudes (Figure 3). This might be the result of high chilling temperatures during winter months.

**Table 1.** Effect of different altitudes on number of adult emergences of CCF, *B. minax* in different dates in Ramechhap district, Nepal

| Altitude range<br>(masl) | 1 <sup>st</sup><br>week<br>Apr | 2 <sup>nd</sup><br>week<br>Apr | 3 <sup>rd</sup><br>week<br>Apr | 4 <sup>th</sup><br>week<br>Apr | 1 <sup>st</sup><br>week<br>May | 2 <sup>nd</sup><br>week<br>May | 3 <sup>rd</sup><br>week<br>May | 4 <sup>th</sup><br>week<br>May | 1 <sup>st</sup><br>week<br>Jun | 2 <sup>nd</sup><br>week<br>Jun |
|--------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| 1247<br>(Bethan)         | 0.5<br>(0.71 <sup>b</sup> )    | 2.62<br>(1.62 <sup>a</sup> )   | 3.46<br>(1.86 <sup>a</sup> )   | 3.49<br>(1.87 <sup>a</sup> )   | 1.71<br>(1.31 <sup>b</sup> )   | 0.92<br>(0.96)                 | 0.5<br>(0.71 <sup>b</sup> )    | 0.5<br>(0.71 <sup>c</sup> )    | 0.5<br>(0.71 <sup>b</sup> )    | 0.5<br>(0.71)                  |
| 1354<br>(Nagsiwa)        | 0.5<br>(0.71 <sup>b</sup> )    | 0.5<br>(0.71 <sup>b</sup> )    | 1.39<br>(1.18 <sup>bc</sup> )  | 3.13<br>(1.77 <sup>a</sup> )   | 3.46<br>(1.86 <sup>a</sup> )   | 2.10<br>(1.45)                 | 0.92<br>(0.96 <sup>b</sup> )   | 0.5<br>(0.71 <sup>c</sup> )    | 0.5<br>(0.71 <sup>b</sup> )    | 0.5<br>(0.71)                  |
| 1443<br>(Aarukharka)     | 1.18<br>(1.09 <sup>a</sup> )   | 0.69<br>(0.83 <sup>b</sup> )   | 1.96<br>(1.4 <sup>b</sup> )    | 2.92<br>(1.71 <sup>a</sup> )   | 1.61<br>(1.27 <sup>b</sup> )   | 1.82<br>(1.35)                 | 1.10<br>(1.05 <sup>b</sup> )   | 0.69<br>(0.83 <sup>bc</sup> )  | 0.5<br>(0.71 <sup>b</sup> )    | 0.5<br>(0.71)                  |
| 1561<br>(Sadi)           | 0.5<br>(0.71 <sup>b</sup> )    | 0.5<br>(0.71 <sup>b</sup> )    | 0.5<br>(0.71 <sup>b</sup> )    | 1.10<br>(1.05 <sup>b</sup> )   | 0.5<br>(0.71 <sup>c</sup> )    | 1.61<br>(1.27)                 | 5.15<br>(2.27 <sup>a</sup> )   | 1.56<br>(1.25 <sup>ab</sup> )  | 1.71<br>(1.31 <sup>a</sup> )   | 0.5<br>(0.71)                  |
| 1650<br>(Dimipokhari)    | 0.5<br>(0.71 <sup>b</sup> )    | 0.5<br>(0.71 <sup>b</sup> )    | 0.92<br>(0.96 <sup>cd</sup> )  | 0.5<br>(0.71 <sup>b</sup> )    | 1.49<br>(1.22 <sup>b</sup> )   | 1.61<br>(1.27)                 | 1.39<br>(1.18 <sup>b</sup> )   | 2.43<br>(1.56 <sup>a</sup> )   | 1.39<br>(1.18 <sup>a</sup> )   | 0.84<br>(0.92)                 |
| SEM (±)                  | 0.11                           | 0.21                           | 0.25                           | 0.28                           | 0.21                           | 0.34                           | 0.31                           | 0.28                           | 0.18                           | 0.19                           |
| LSD <sub>0.05</sub>      | 0.18                           | 0.34                           | 0.39                           | 0.44                           | 0.31                           | ns                             | 0.48                           | 0.44                           | 0.27                           | ns                             |
| CV%                      | 14.7                           | 23.8                           | 20.7                           | 20.2                           | 16.2                           | 27.6                           | 25.2                           | 28.2                           | 19.3                           | 26                             |
| Grand mean               | 0.78                           | 0.92                           | 1.22                           | 1.42                           | 1.27                           | 1.26                           | 1.24                           | 1.01                           | 0.92                           | 0.75                           |
| F value                  | 0.001                          | <0.001                         | <0.001                         | <0.001                         | <0.001                         | 0.41                           | <0.001                         | 0.004                          | <0.001                         | 0.44                           |
|                          | ***                            | ***                            | ***                            | ***                            | ***                            |                                | ***                            | **                             | ***                            |                                |

CV=Coefficient of variation, Means followed by the same letter in a column are not significantly different by DMRT at 5%, \*\* significant at 1% level of significance, \*\*\* significant at 0.1% level of significance, ns non-significant. Figures in parenthesis indicate data transformed to  $\sqrt{(x + 0.5)}$ , SEM= Standard error of the mean, LSD0.05= Least significant difference at 5% level of significance.

**Table 2.** Altitude-wise adult sex ratio of CCF, *B. minax* in Sunapati Rural Municipality, Ramechhap district, Nepal

| Location    | Altitude (masl) | Male: female ratio |
|-------------|-----------------|--------------------|
| Bethan      | 1247            | 1.25               |
| Nagsiwa     | 1354            | 1.08               |
| Aarukharka  | 1443            | 1.13               |
| Sadi        | 1561            | 1.37               |
| Dimipokhari | 1650            | 1.05               |

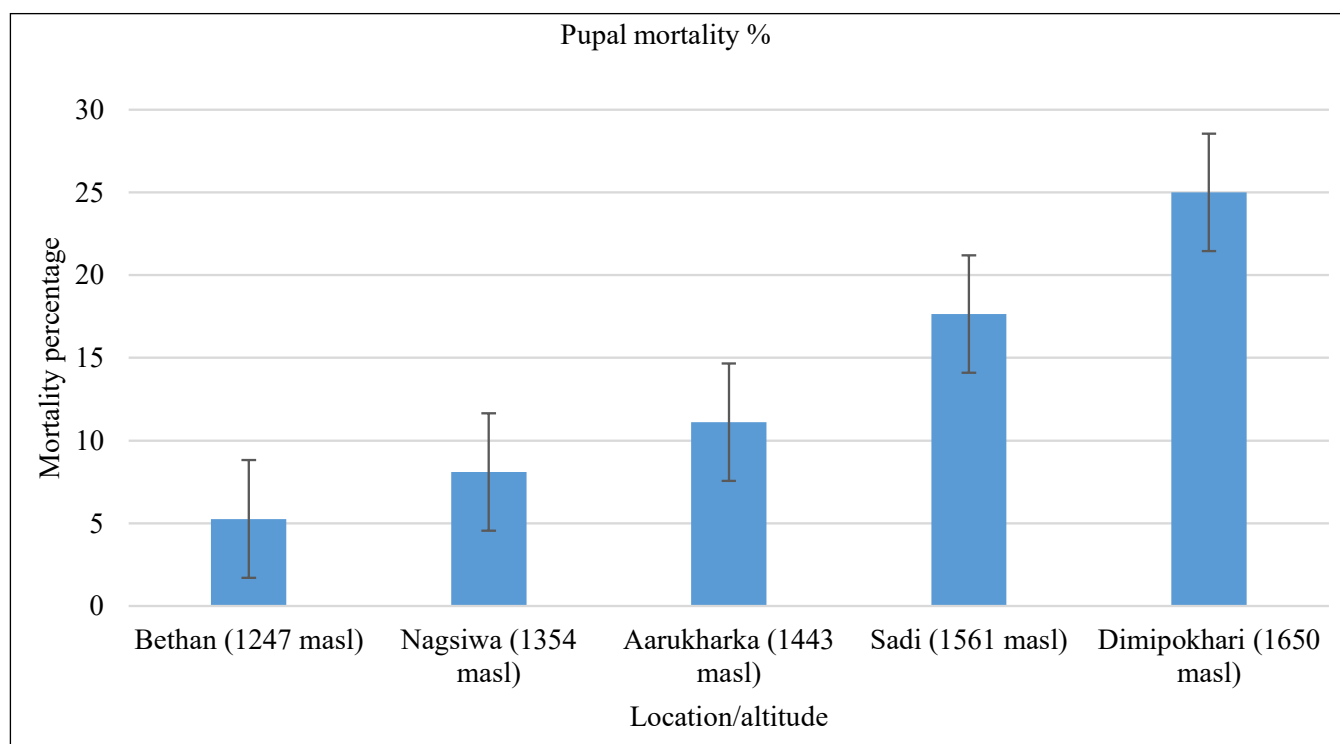


**Figure 3.** Average pupal mortality (percentage-wise)  $\pm$  SEM of CCF, *B. minax* at different altitudes in Sunapati Rural Municipality, Ramechhap district, Nepal

#### 4. CONCLUSION

The adult sex ratio of CCF, *B. minax* is independent of various altitudes, whereas pupal mortality is found to be the highest in upper elevations. Early emergence of adult fly occurred at lower altitudes as compared to the late emergence at higher altitudes. Hence, the effective management of CCF, *B. minax* is based on the date of adult emergence at different altitudes. The present finding indicates that management of CCF, *B. minax* should be practiced in the citrus orchard at least by two weeks earlier at lower altitudes than at higher elevations.

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# EFFECT OF TRANSPLANTING DATE AND SPACING ON SEED PRODUCTION OF BROCCOLI (*Brassica oleracea* var. *italica* Plenck) AT CHITWAN, NEPAL

Saraswati Shrestha<sup>1,\*</sup>, Arvind Shribastava<sup>2</sup> and Mohadatta Sharma<sup>2</sup>

<sup>1</sup> Ministry of Industry, Agriculture and Cooperative, Koshi Province, Nepal.

<sup>2</sup> Agriculture and Forestry University, Rampur, Chitwan, Nepal.

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\*Correspondence:  
saraswatish@yahoo.com  
Tel:+977-9852080278

## ABSTRACT

A study was conducted at the Agriculture and Forestry University, Rampur; Chitwan during the winter season, to find out the suitable transplanting dates and spacing for quality seed production of broccoli (*Brassica oleracea* var. *italica*) Cv. Calabrese. The experiment was laid out in split plot design with three replications, where main plot treatment consisted of transplanting dates (November 1, November 15 and November 30) and sub plot treatment consisted of spacing (60 cm × 50 cm, 60 cm × 60 cm, 60 cm × 75 cm). Since transplanting time and spacing affect vegetative traits, which are very important for seed yield. The highest seed yield (1.09 mt./ha) was obtained with November 1st planting at the spacing of 60 cm × 50 cm. So that transplanting date of November 1st with spacing 60 cm × 50 cm is better for seed production of broccoli in plain areas of Chitwan, Nepal.

## 1. INTRODUCTION

Broccoli, member of the Brassicaceae family, is a compact, rapidly developing floral vegetable that is usually harvested when the flowering heads are immature (Gray, 1982). It is one of the best and economically lucrative vegetable and more nutritious than any other vegetables of the same genus. It is a good source of carotene (575 µg), vitamin C (87 mg /100 g raw flesh), riboflavin (vitamin B2) and also rich in dietary fiber (Rashid, 1996). Broccoli also contains high concentrations of carotenoids, which are believed to have preventive qualities with regards to human cancer. It may also play a role in reducing levels of serum cholesterol. Broccoli contains a high level of sulforaphane glucosinates, which is cancer inhibiting chemicals.

Broccoli sprouts are loaded with concentrated form of sulforaphanes that enhances the cancer protective capacity of animal (Fahey *et al.*, 1997). Three-day old young broccoli sprouts contains 20-50 times more chemoprotective compounds than mature broccoli heads (Nestle, 1997). The American Cancer Society suggested the inclusion of broccoli in the human diet to reduce the risk of developing cancer (Lindsay *et al.*, 1988). The seed production is difficult in the areas having severe winter (Sinohara, 1984). So, the suitable

place for seed production is recognized where the winter is mild, spring comes earlier and rainfall is less during flowering and ripening period (Sinohara, 1984). However, one of the major problems associated with this crop in Nepal is lack of availability of high quality seeds of desired varieties (Devkota, 2000).

The total production of the vegetable seeds is 7.73 mt by government sector and 1265 mt by the private sector but the total demand of the seeds of different vegetables is 2026 mt (VDD, 2010). It is estimated that good quality seeds of improved varieties can contribute about 25-40% increase in yield (Kanwar *et al.*, 2010). Methods of seed production and planting density are two important factors which affect the quality and quantity of seed produced in cole crops. In situ method of seed production is practice in broccoli and cauliflower whereas in cabbage transplanting method is followed. These methods affect the quantity and quality of seed produced significantly (Verma & Sharma, 2000). However, in sprouting broccoli no such methods to improve the quality of seed have been studied.

The seed production of cole crops can be done in Nepal from Terai to high hilly region (VDD, 1990). In case of the broccoli Calabrese is one of the variety whose

seed production is possible in Chitwan condition (256m above sea level). Limited researches were carried out on Calabrese seed production esp. on nutrition management, techniques of seed production, time of planting and spacing. Therefore it is a very high time to carry out research on this aspect as little work has been conducted to assess the effect of date of transplanting and spacing on broccoli seed production under Rampur, Chitwan.

## 2. MATERIALS AND METHODS

### 2.1. Experimental site

The field experiment was conducted in Faculty of Agriculture, Agriculture and Forestry University,

Rampur, Chitwan, Nepal during September, 2014 to April, 2015. Chitwan valley falls under a humid subtropical type of climate. The maximum winter temperature rises up to 27°C. The hottest part of the year is April/May and June when the maximum temperature ranges between 37°C to 42°C. Rainy season starts from June and continues up to September, receiving the highest rainfall (up to 150 mm in 24 hours in some years) during June and July. The experimental site is geographically levelled at 27° 37' to 27° 46' north latitude and 83° 35" to 84° 48" east longitude with altitude of the about 256 meter above mean sea level.

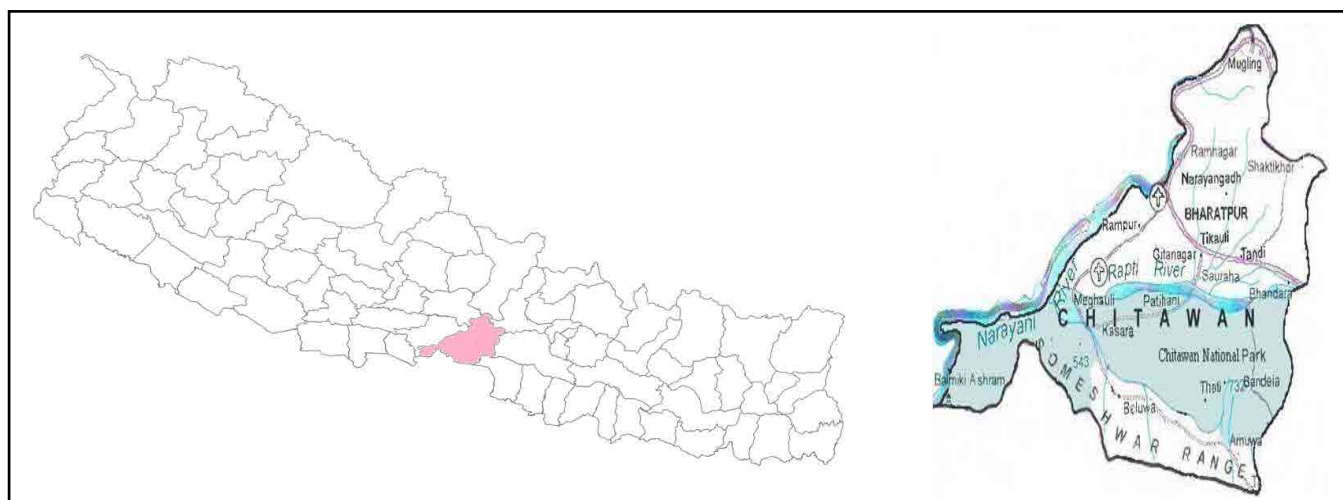


**Figure 1.** Experimental site

### 2.2. Cropping history of the experimental field

The experimental field was fallow before this planting.

### 2.3. Soil properties

Composite soil sample taken from the experimental area was air dried, ground and sieved through 2 mm sieve and subjected to soil test at soil science laboratory, Hariharbhawan, Lalitpur. The soil of experimental site sandy loam with pH value 5.4 and have low organic matter content.

**Table 1.** Physicochemical properties of experimental soil

| Physical properties of soil                 | Content (%) | Rating |
|---------------------------------------------|-------------|--------|
| Sand content                                | 68.9        |        |
| Silt content                                | 19.85       |        |
| Clay content                                | 11.25       |        |
| Soil texture                                | Sandy loam  |        |
| Chemical properties of soil                 |             |        |
| Soil pH                                     | 5.4         | Acidic |
| Total organic matter (%)                    | 2.18        | Low    |
| Total nitrogen (%)                          | 1           | Medium |
| Available phosphorus (kg ha <sup>-1</sup> ) | 63.9        | High   |
| Available potassium (kg ha <sup>-1</sup> )  | 362.4       | High   |

Source: Soil Science Laboratory, Hariharbhawan, Lalitpur

## 2.4. Agro-climatic condition of the study site

The climatic parameters like maximum and minimum temperature, average temperature, total rainfall and Relative Humidity (RH) were recorded from the agro-meteorological station of National Maize Research Center (NMRC), Rampur.

The average maximum temperature during the coldest month of the year (December to February) reached to 27°C whereas the minimum temperature during this period ranges between 6 to 10 °C. Similarly, the maximum temperature during the hottest month of the year (May to June) was measured up to 42°C. There is ample sunshine during the rainy and spring season while for winter season foggy weather prevails in most of the days. The rainy season starts from June and ends up to October. The early months (June, July, and August) of rainy season receive most of the total annual rainfall (around 80%) and winter receives occasional rainfall from the Western monsoon. The percentage of Relative humidity (RH) rises up from May (Average 50 %) and reaches maximum (100%) in some weeks of December and January (Thapa and Dangol, 1988).

## 2.5. Experimental details (Field experiment)

The field experiment was conducted between 1<sup>st</sup> October, 2014 to 30<sup>th</sup> April, 2015. The variety used was Calabrese (an open pollinated variety) which was being maintained in the Horticulture farm of the Agriculture and Forestry University, Rampur, Chitwan.

The split plot design was followed with three replication and a total of nine treatments. There were 30, 25, 20 number of plants in each experimental plot among which 12, 9, 6 and 18, 16, 14 number of plants were experimental plants and boarder plants at 50 cm × 60 cm, 60 cm × 60 cm and 60 cm × 75 cm spacing respectively. The size of individual plot was 9 m<sup>2</sup> (3 m × 3 m). The plot between the treatments was separated by 0.5 m and the replication was separated by 1m and the total area of the experiment field was 402 m<sup>2</sup>.

**Table 2.** Details of the treatments

| Factor A (Time of planting) | Factor B spacing(cm <sup>2</sup> ) |
|-----------------------------|------------------------------------|
| T1 = Nov.1                  | S1 = 60x50                         |
| T2 = Nov.15                 | S2 = 60x60                         |
| T3 = Nov.30                 | S3 = 60x75                         |

## 2.6. Data collection and analysis

Vegetative, phenological and yield attributing data measurements were taken from each plot, five inner plants were selected randomly as the sample plants and vegetative data were recorded of plant height and number of leaves. whereas phenological measurements

recorded were days to 100% flowering, 100% pod formation, number of primary and secondary branches per plant, pod length(cm), number of pod per plant, number of seed per pod and seed yield per plant (gram).

The data obtained were tabulated using MS EXCEL. Then analysis of this data was done by using computer software MSTAT-C. Mean separation was done by Duncan's Multiple Range Test (DMRT) with 5% level of significance.

## 3. RESULTS AND DISCUSSION

### 3.1. Effect on seed yield

Effect of time of planting on seed yield was highly significant (Table 3). Mean table shows that the maximum seed yield was obtained from November 1<sup>st</sup> planting (736.08 kg ha<sup>-1</sup>). But there was no significant difference between seed yield of November 15 (542.56 kg ha<sup>-1</sup>) and November 30<sup>th</sup> planting (458.80 kg ha<sup>-1</sup>) in Broccoli. The influence of spacing on seed yield was highly significant. The highest yield was obtained with spacing of 60 cm × 50 cm (815.60 kg ha<sup>-1</sup>) followed by 60 cm × 60 cm (510.84 kg ha<sup>-1</sup>) and 60 cm × 75 cm spacing (411.00 kg ha<sup>-1</sup>).

**Table 3.** Effect of time of planting and spacing on seed yield of broccoli in Chitwan, Nepal, 2014/2015

| Treatment                                     | Seed yield per plant(g) | Seed yield per ha (kg ha <sup>-1</sup> ) |
|-----------------------------------------------|-------------------------|------------------------------------------|
| Date of transplanting                         |                         |                                          |
| Nov 1                                         | 27.83 <sup>a</sup>      | 736.08 <sup>a</sup>                      |
| Nov 15                                        | 20.25 <sup>b</sup>      | 542.56 <sup>b</sup>                      |
| Nov 30                                        | 14.89 <sup>c</sup>      | 458.80 <sup>b</sup>                      |
| SEm(±)                                        | 1.13                    | 29.71                                    |
| LSD                                           | 4.44**                  | 116.70**                                 |
| Spacing                                       |                         |                                          |
| 60×50                                         | 18.54 <sup>b</sup>      | 815.60 <sup>a</sup>                      |
| 60×60                                         | 19.73 <sup>b</sup>      | 510.84 <sup>b</sup>                      |
| 60×75                                         | 24.70 <sup>a</sup>      | 411.00 <sup>c</sup>                      |
| SEm(±)                                        | 1.27                    | 22.26                                    |
| LSD (0.05)                                    | 3.91*                   | 68.59**                                  |
| Interaction (date of transplanting × Spacing) | <0.05                   | <0.01                                    |
| CV, %                                         | 18.11                   | 11.53                                    |
| Grand Mean                                    | 20.99                   | 579.15                                   |

s- not significantly different from each other based on DMRT at 5% level of significance

\* and\*\* indicate Significant at 0.05 and 0.01 level respectively.

LSD-Least Significance Difference

### 3.2. Interaction Effect Of Time Of Planting And Spacing On Seed Yield Per Plant

The result of statistical analysis showed that the interaction effect between time of planting and spacing on seed yield per plant was significant (Table 4). The highest seed yield per plant was obtained with spacing of 60 cm × 75 cm planted on 1st November which was significantly higher than the seed yield per plant recorded from other planting dates at different closer spacings. There is no significant difference in seed yield of November 30 planting with spacing 60 cm × 50 cm and 60 cm × 75 cm, while there is no significant difference on November 1<sup>st</sup> planting.

The interaction effect between time of planting and spacing on seed yield per ha was found to be highly significant (Table 5). Mean table shows that the Broccoli plant transplanted on November 1<sup>st</sup> with 60 cm × 50 cm spacing have highest seed yield per ha (1080.2 kg ha<sup>-1</sup>) which was significantly higher than the seed yield recorded from rest of the other planting dates and spacings. Mean table clearly indicates that despite of spacing, early planting (November 1<sup>st</sup>) resulted in significantly higher seed yield compared to other planting dates and spacing. The mean seed yield obtained from Nov 1<sup>st</sup> plant at different spacings was 736.08 kg ha<sup>-1</sup> where as it was 542.56 kg ha<sup>-1</sup> in November 1st planting and 458.8 kg ha<sup>-1</sup> in November 30<sup>th</sup> planting. The minimum seed yield was at spacing of 60 cm × 75 cm in November 30 planting.

**Table 4.** Interaction between time of planting and spacing on seed yield per plant of broccoli in the plain area of Chitwan, Nepal, 2014/2015.

| Spacing     | Transplanting date  |                     |                     | Mean  |
|-------------|---------------------|---------------------|---------------------|-------|
|             | Nov 1               | Nov 15              | Nov 30              |       |
| 60×50       | 26.00 <sup>b</sup>  | 16.28 <sup>c</sup>  | 13.33 <sup>d</sup>  | 18.54 |
| 60×60       | 23.81 <sup>bc</sup> | 17.37 <sup>cd</sup> | 18.00 <sup>cd</sup> | 19.73 |
| 60×75       | 33.67 <sup>a</sup>  | 27.11 <sup>ab</sup> | 13.33 <sup>d</sup>  | 24.70 |
| Mean        | 27.83               | 20.25               | 14.89               |       |
| SEm (±)     | 2.19*               |                     |                     |       |
| LSD (=0.05) | 6.76                |                     |                     |       |

s- not significantly different from each other based on DMRT

at 5% level of significance

\* and \*\* indicate Significant at 0.05 and 0.01 level respectively.

LSD-Least Significance Difference

**Table 5.** Interaction between time of planting and spacing on seed yield per ha of broccoli in the plain area of Chitwan, Nepal, 2014/2015.

| Spacing     | Transplanting dates   |                      |                      | Mean   |
|-------------|-----------------------|----------------------|----------------------|--------|
|             | Nov 1                 | Nov 15               | Nov 30               |        |
| 60×50s      | 1087.20 <sup>a</sup>  | 728.40 <sup>b</sup>  | 631.20 <sup>bc</sup> | 815.60 |
| 60×60       | 575.64 <sup>cd</sup>  | 460.08 <sup>de</sup> | 496.80 <sup>de</sup> | 510.84 |
| 60×75       | 545.40 <sup>cde</sup> | 439.20 <sup>e</sup>  | 248.40 <sup>f</sup>  | 411.00 |
| Mean        | 736.08                | 542.56               | 458.80               |        |
| SEm (±)     | 38.56                 |                      |                      |        |
| LSD (=0.05) | 38.56 **              |                      |                      |        |

s- not significantly different from each other based on DMRT at 5% level of significance

\*and\*\* indicate Significant at 0.05 and 0.01 level respectively.

LSD-Least Significance Difference

### 3.3 Effect on seed yield per plant and seed yield per ha

The November 1st planting had the highest seed weight per plant (27.827 g) and seed yield per ha (736.080 kg ha<sup>-1</sup>). November 1<sup>st</sup> planting have tall plants with vigorous growth, profuse branching, more number of flowering with longest pod length this might contribute towards more seed yield while delay planting reduced seed yield. The seedling planted on November 1<sup>st</sup> might receive the favorable weather for growing and flowering as compare to the delay plantings, which help in better seed development in pod (Kazi *et al.*, 2002). November 30 planting resulted in minimum number of flower stalks, number of pods, shortest length of pod (Firoz *et al.*, 2000).

The maximum yield of seed per plant (24.70) was observed with spacing of 60 cm × 75 cm followed by spacing of 60 cm × 60 cm (19.73 g) and 60 cm × 50 cm (18.54 g). Wider spaced plant produced higher seed yield per plant (Mehta *et al.*, 2015). It was observed that the number of leaves, number of branch per plant was higher in plants with wider spacing and the lower in closely spaced plants. This was probably due to inter plant competition in respect of light, nutrient and resources result in more seed yield per plant in wider spacing as compare to closer spacing (Mohanty & Srivastava, 2002).

As more number of leaf, stem diameter, branches and number of pod per plant were recorded at the widest spacing which contribute to increased seed yield per plot. The highest yield was recorded with spacing of 60 cm × 50 cm (815.60 kg ha<sup>-1</sup>) followed by 60 cm ×

60 cm (510.84 kg ha<sup>-1</sup>) and 60 cm × 75 cm (411.00 kg ha<sup>-1</sup>). Seed yield was maximum at closer spacing and minimum at wider spacing though seed yield per plant was maximum at wider spacing due to more number of plant per ha were recorded at closer spacing (Mihov and Antonova, 2009).

November 1<sup>st</sup> planting with spacing of 60 cm × 75 cm produced maximum (33.67 g) seed per plant where as the highest seed yield per ha was obtained with the spacing of 60 cm × 50 cm in the November 1<sup>st</sup> planting (1080.2 kg ha<sup>-1</sup>). This might be due to less number of plants per ha accommodated in wider spacing while more number of plants in closer spacing and more favorable condition accompanied by November 1<sup>st</sup> planting.

#### 4. CONCLUSION

The findings revealed that among three different time of planting November 1<sup>st</sup> planting was found as the appropriate date for seed production of broccoli in Chitwan. The time of planting and spacing played a

crucial role in growth and development of vegetative, reproductive growth and seed development of broccoli plants. Delaying on time of planting resulted in reduction in seed production. Similarly, spacing of 60 cm × 50 cm produced the maximum seed yield per ha though the seed yield per plant were found maximum with the spacing of the 60 cm × 75 cm. From this study, it can be concluded that November 1<sup>st</sup> transplanting with spacing of the 60 cm × 50 cm was found to be the best combination for producing broccoli seeds in plain areas of Chitwan.

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## EFFICACY OF CHEMICALS AND HOT WATER TREATMENTS ON QUALITY AND SHELF LIFE OF AMRAPALI VARIETY OF MANGO

Shakar Shasi Pandey<sup>1,\*</sup>, Nirajan Bhandari<sup>2</sup>, Madhav Dhital<sup>3</sup>, Arjun Kumar Shrestha<sup>3</sup> and Bishal Shrestha<sup>3</sup>

<sup>1</sup> Madan Bhandari Memorial Academy Nepal, Urlabari, Morang

<sup>2</sup> College of Natural Resource Management, Agriculture and Forestry University (AFU), Pakhribas, Dhankuta, Nepal

<sup>3</sup> Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal

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\*Correspondence:

pandeyshakar99@gmail.com

Tel.: +977-9842070219

### ABSTRACT

An experiment was conducted to evaluate the effect of hot water and chemicals on the quality and shelf life of Amrapali variety of mango at Agriculture and Forestry University, Rampur, Chitwan, Nepal. The experiment was laid out in completely randomized design with 6 treatments and 4 replications. Physiologically matured mango fruits ( $200 \pm 20$  g) were treated with distilled water for 10 minutes, hot water @  $50^\circ\text{C}$  for 10 minutes, hot water @  $55^\circ\text{C}$  for 10 minutes, carbendazim solution @ 0.1% for 10 minutes and Sodium hypochlorite solution @ 100 ppm for 2 minutes. The untreated fruits were considered as control treatment. The highest total soluble solid ( $18.15^\circ\text{Brix}$ ), the lowest physiological loss in weight (24.20%) and the lowest spoilage loss (42.05%) were observed in fruits treated with hot water at  $55^\circ\text{C}$ . Therefore, the post-harvest treatment of mango fruits with hot water at  $55^\circ\text{C}$  for 10 minutes was found to be effective for maintaining the quality and shelf life of mango.

### 1. INTRODUCTION

Mango is a king of fruits and is a popular tropical fruit of Nepal due to its attractive aroma, flavor, color, nutritional value and diverse use (Mathiazhagan *et al.*, 2022). Mango is the leading fruit of Nepal with area, production and productivity of 43,688 ha, 466,266 ton and 10.67 ton/ha respectively (MoALD, 2022). The contribution of mango in agriculture gross domestic product (AGDP) was 2.51% in the fiscal year 2021/22. The demand of mango is increasing day by day and to fulfill this demand, 13.65 million metric tons of fresh mango fruits has been imported from India and other countries in the year 2021 (FAO, 2022). This could be due to low production, high post-harvest loss and seasonal nature of production. Mango fruit is highly perishable in nature with very short shelf life ranging from 4 to 8 days at normal room temperature and 2-3 weeks in cold storage at  $13^\circ\text{C}$ , but it also varies among the cultivars (Carrillo *et al.*, 2000). After harvest, various

physiological and biochemical changes occur in the mango fruit causing significant amount of qualitative and quantitative changes during storage and transportation (Bambalele *et al.*, 2021). High moisture content, soft texture and susceptibility to various pathogens are limiting factors of longer shelf life (Makka *et al.*, 2019). The high post-harvest loss of mango is mainly associated with the harvesting technique, post-harvest handling and microbial spoilage, thus, affecting its quality and marketable period (Zheng *et al.*, 2012). In Nepal, post-harvest loss of mango varies between 20 to 30% (Bhattarai, 2018).

Chemicals like carbendazim, sodium hypochlorite, benomyl, potassium permanganate, methyl cyclopropane and non-chemical post-harvest treatments like botanical extracts, polythene film and edible coatings are mostly used to maintain fruit quality

during handling of the mango fruit (Bambalele *et al.*, 2021). However, existing post-harvest technologies are expensive and may not be accessible and adaptable to the Nepalese context (Mahajan *et al.*, 2014). Among several diseases, anthracnose (*Colletotrichum gloeosporioides*) is one of the major post-harvest diseases in all mango producing regions of the world causing significant post-harvest loss (Dodd *et al.*, 1997). Synthetic fungicides (benomyl, prochloraz, carbendazim) and chemical preservatives (1-MCP, nitric oxide, salicylic acid, GA3) are extensively used to retard respiration rate, inhibit ethylene biosynthesis, retain firmness, delay fruit ripening and control microbial spoilage (Coates *et al.*, 1993; Hong *et al.*, 2014; Banerjee *et al.*, 2015; Razzaq *et al.*, 2016). However, the growing concerns of public over the indiscriminate use of synthetic chemicals, associated health risk, potential environmental hazards and possibility of occurrence of resistant pathogen strains forced to move mango industry into safer alternatives (Yao and Tian, 2005; Dessalegn *et al.*, 2013; Bambalele *et al.*, 2021). Thus, there is an urgent need to develop low cost, accessible, environment friendly and non-chemical alternatives. Application of inorganic salts, plant extracts, edible coatings and heat treatment are other promising strategies to increase the shelf life of the mango fruit (Kumlachew *et al.*, 2014; Bambalele *et al.*, 2021). Controlled atmosphere, pulsed electric field and Gaseous ozone are some of the emerging and potential technologies in the mango fruit industry (Bambalele *et al.*, 2021). Among different non-chemical treatments, hot water treatment has gained wide international interest in quality maintenance and disease control (Kumah *et al.*, 2011; Obsa *et al.*, 2014). Mango initially free from disease at harvest develop anthracnose as fruit ripens is the major cause of post-harvest loss (Dodd *et al.*, 1997), which can be controlled by dipping in hot water (Simmonds, 2005). Reducing post-harvest losses and extending the shelf life are the key issue that needs to be addressed by using relevant technologies for reliable and long-term supply of fresh mango fruits in Nepalese market. Therefore, the present study was conducted with the objective to analyze the efficacy of chemicals and hot water treatment on quality and shelf life of mango under Chitwan condition of Nepal.

## 2. MATERIALS AND METHODS

### 2.1 Description of the experiment site

This experiment was conducted at the horticulture laboratory of Agriculture and Forestry University, Rampur, Chitwan, Nepal, during July 2018. Geographically, the

experimental site and the orchard from where mangoes are collected are located in the Terai region at 27° 40' N latitude and 84° 19' E longitudes and at an elevation of about 228 masl. The sample fruit collection area represented the area of commercial mango production in Nepal. The average temperature and relative humidity of laboratory during the research period was 29.4° C and 77.25% respectively.

### 2.2 Selection of variety and harvesting

Amrapali variety was selected from the commercial mango orchard of Chitwan, Nepal which is categorized as mid to late season variety, regular bearer, precious, best yielder and ripens during first to second week of July. Selected fruits were 200±20 gm size. Uniform sized, physiologically matured fruits were harvested with the help of pole harvester during the morning period after the dew has evaporated. They were collected in plastic crates and taken to experimental site located 100 m away from the orchard. Brushed, wounded and diseased mango fruits were sorted out.

### 2.3 Design of experiment

The experiment was carried out using Completely Randomized Design with six treatments and four replications. Each experimental unit consists of 13 fruits, of which 8 were subjected to destructive testing, while the remaining 5 were preserved for non-destructive analysis.

Treatment details

- T<sub>1</sub>: Control (no dipping)
- T<sub>2</sub>: Distilled water dipping for 10 minutes
- T<sub>3</sub>: Hot water treatment @ 50°C for 10 minutes
- T<sub>4</sub>: Hot water treatment @ 55°C for 10 minutes
- T<sub>5</sub>: Carbendazim solution @ 0.1% for 10 minutes
- T<sub>6</sub>: Sodium hypochlorite solution @ 100 ppm for 2 minutes

### 2.4 Method of treatments application

Selected fruits were kept for 10 minutes in hot water bath for hot water treatment. For chemical treatment, required amount of chemical were weighed/measured to make the required concentration of said chemicals using distilled water. 0.1% of carbendazim solution was prepared by mixing 1 gm of carbendazim in 1 litre of distilled water and 100 ppm sodium hypochlorite (NaOCl) solution was prepared by mixing 100 mg of sodium hypochlorite in 1 litre of distilled water. Fruits were kept separately

(replication wise) in a perforated plastic tray.

### 2.5 Observation taken

The observation on different qualitative and quantitative parameters was recorded at three days interval. Total soluble solids (TSS) content measured by using analogue hand held refractometer (EMRA Inc., Tokyo Japan) and expressed in terms of °Brix. Titratable acidity (TA) was determined by titration of mango juice against 0.1 N NaOH solution and expressed as percent (%) malic acid. TSS:TA ratio was determined by dividing the TSS with their corresponding TA values. Ascorbic acid contents were determined by titrating 5 ml of mango juice aliquot (obtained from 10 ml juice and 90 ml of 0.4% oxalic acid) against 2,6-dichlorophenolindophenol and expressed as mg 100 ml<sup>-1</sup> of fruit juice (Ali *et al.*, 2016). Fruit weight was measured at 3 days interval by using electronic digital weighing balance and physiological loss in weight was estimated as per the procedure of Hasan *et al.* (2020) and expressed in percentage. Spoilage loss was determined based on fruit skin defects (Holmes *et al.*, 2009) and disease incidence (Amin *et al.*, 2007). Shelf life of mango fruit was a period of time which started from harvesting and extends up to the start of rotting of fruits (Beverly, *et al.*, 1992). Shelf life of mango fruits were calculated by counting optimum marketing and eating qualities based on physiological loss in weight and spoilage loss.

### 2.6 Statistical analysis

The collected data were compiled and entered into Microsoft Excel (2016) and analysis was carried out by using software R Studio (version 3.0.3). Mean separation was carried out using Fisher's least significant difference (LSD) at 0.05 level of significance ( $P < 0.05$ ).

## 3. RESULTS AND DISCUSSION

Analysis of variance showed that significant difference was observed among different treatments with respect to TSS, TA, Ascorbic acid content, TSS/TA ratio, pulp pH, physiological loss in weight, spoilage loss and shelf life.

### 3.1 Total soluble solids

The highest TSS content (18.15°Brix) was observed in 9<sup>th</sup> days after treatment (DAT) with fruits treated with hot water @ 55°C. At 12 DAT, fruits treated with hot water @ 55°C recorded the highest TSS (17.82° Brix) which was statistically at par with hot water @ 50°C. The lowest TSS (14.0° Brix) was observed in fruits treated with NaOCl which was statistically at par with fruits dipped in distilled water. Result of TSS/TA ratio indicated that the highest TSS/TA ratio (190.04) was observed with hot water treatment @ 50°C which was statistically similar with hot water treatment @ 55°C, whereas, the fruits dipped with distilled water showed the lowest (97.67) TSS/TA ratio (Table 1).

During the storage period, TSS content and TSS/TA ratio was increased with advancement of storage period. Increasing trend of TSS was due to hydrolysis of stored polysaccharides into soluble sugar and conversion of organic acid into sugars (Wasker *et al.*, 1999). This result was supported by the findings of Rathore *et al.* (2007), who found that sharp increase in TSS content from 10 to 16.2°Brix until 6<sup>th</sup> day of storage and gradually decreased to 5.1°Brix during 15<sup>th</sup> day of storage. However, TSS content increased continuously and acidity decreased continuously. The cellulose and pectin present in mango fruits are hot water soluble and are hydrolyzed to simple sugars after hot water treatment which might be the reason of highest TSS in hot water treated fruits.

**Table 1.** Effect of chemicals and hot water treatments on TSS content and TSS/TA ratio of Amrapali variety of mango

| Treatments                        | TSS ( °Brix)         |                      |                     |                     | TSS/TA             |                    |                     |                     |
|-----------------------------------|----------------------|----------------------|---------------------|---------------------|--------------------|--------------------|---------------------|---------------------|
|                                   | 3 DAT                | 6 DAT                | 9 DAT               | 12 DAT              | 3 DAT              | 6 DAT              | 9 DAT               | 12 DAT              |
| Control                           | 14.32 <sup>abc</sup> | 16.85 <sup>abc</sup> | 16.90 <sup>ab</sup> | 15.95 <sup>ab</sup> | 16.76 <sup>b</sup> | 37.87 <sup>b</sup> | 60.32 <sup>b</sup>  | 98.26 <sup>b</sup>  |
| Distilled water for 10 minutes    | 11.72 <sup>c</sup>   | 14.80 <sup>cd</sup>  | 14.82 <sup>b</sup>  | 14.62 <sup>b</sup>  | 13.70 <sup>b</sup> | 32.00 <sup>b</sup> | 54.86 <sup>b</sup>  | 97.67 <sup>b</sup>  |
| HWT @ 50°C for 10 minutes         | 15.88 <sup>a</sup>   | 17.35 <sup>ab</sup>  | 17.52 <sup>ab</sup> | 17.45 <sup>a</sup>  | 32.62 <sup>a</sup> | 66.67 <sup>a</sup> | 123.86 <sup>a</sup> | 190.04 <sup>a</sup> |
| HWT @ 55°C for 10 minutes         | 15.55 <sup>a</sup>   | 18.07 <sup>a</sup>   | 18.15 <sup>a</sup>  | 17.82 <sup>a</sup>  | 34.71 <sup>a</sup> | 74.09 <sup>a</sup> | 129.96 <sup>a</sup> | 189.84 <sup>a</sup> |
| Carbendazim @ 0.1% for 10 minutes | 12.85 <sup>bc</sup>  | 14.47 <sup>d</sup>   | 16.07 <sup>ab</sup> | 16.05 <sup>ab</sup> | 19.86 <sup>b</sup> | 39.73 <sup>b</sup> | 73.76 <sup>b</sup>  | 107.00 <sup>b</sup> |
| NaOCl @ 100 ppm for 2 minutes     | 14.65 <sup>ab</sup>  | 15.35 <sup>bcd</sup> | 15.52 <sup>ab</sup> | 14.00 <sup>b</sup>  | 18.87 <sup>b</sup> | 44.26 <sup>b</sup> | 64.43 <sup>b</sup>  | 101.74 <sup>b</sup> |
| Mean                              | 14.16                | 16.15                | 16.49               | 15.98               | 22.75              | 49.1               | 84.53               | 130.76              |
| LSD (P=0.05)                      | 2.69 <sup>*</sup>    | 2.13 <sup>**</sup>   | 3.17 <sup>*</sup>   | 2.2 <sup>*</sup>    | 6.66 <sup>*</sup>  | 13.81 <sup>*</sup> | 32.04 <sup>*</sup>  | 46.08 <sup>*</sup>  |
| CV%                               | 8.46                 | 5.86                 | 8.59                | 6.1                 | 13.03              | 12.52              | 16.87               | 15.68               |

Means in a column followed by the same letter do not differ according to Fisher's least significant difference (LSD) at 0.05 level ( $P < 0.05$ ). \* represents significant ( $P \leq 0.05$ ), \*\* represents highly significant ( $P \leq 0.01$ ), CV is the coefficient of variation.

Due to the presence of multi OH group around polysaccharides, the solvent (hot water) creates solvating environment around polymer chain keeping polysaccharide molecules away from each other (Guo *et al.*, 2017). This result was supported by Djoua *et al.* (2009) also. Whereas, later during storage, slight decreased in TSS content was observed in all treatments as a fact that reducing sugar are converted back into polysaccharides (Anwar and Malik, 2007).

### 3.2 Titratable acidity and ascorbic acid (vitamin C) content

During the observation period, significant difference was seen among the treatments, highest TA (0.164 %) was observed in untreated fruits and the lowest TA (0.094 % & 0.096 % respectively) was observed in fruits treated with hot water @ 50°C and 55°C. Ascorbic acid content decreased gradually among all treatments along the advancement of storage period and was found highest (24.33 mg) with fruits treated with hot water @ 55°C during the course of storage period and the lowest (20.56 mg) with untreated fruits at 12 DAT (Table 2).

Continuous decrease of titratable acidity was due to the action of enzyme citric acid glyoxalase which cause the degradation of citric acid (Rathore *et al.*, 2007). The decreasing trend in ascorbic acid content was due to utilization of organic acids as a respiratory substrate during ripening, limited supply of sugar (Vala and Bhatt, 2002) and the conversion of L-ascorbic acid into dehydro-ascorbic acid upon the action of enzyme ascorbinase (Mapson, 1970). This result is similar with the findings of Wills *et al.* (2007). But preservation of ascorbic acid content in fruits treated with hot water was due to the fact that ascorbate is synthesized through Smirnoff-Wheeler (SW) pathway in fruits from D-glucose through series of intermediate products (Bulley *et al.*, 2009; Mellidou *et al.*, 2012), and D-galacturonate pathway which utilize pectin as substrate (Di Matteo *et al.*, 2010). As in both case hydrolysis of polysaccharides caused due to hot water treatment plays important role in providing substrate for ascorbic acid synthesis. This result is further supported by Srinivasan *et al.* (1973).

**Table 2.** Effect of chemicals and hot water treatments on titratable acidity (%) and ascorbic acid content (%) of Amrapali variety of mango.

| Treatments                        | TA (%)             |                     |                    |                    | Ascorbic acid (mg/100g pulp) |                     |                     |                      |
|-----------------------------------|--------------------|---------------------|--------------------|--------------------|------------------------------|---------------------|---------------------|----------------------|
|                                   | 3 DAT              | 6 DAT               | 9 DAT              | 12 DAT             | 3 DAT                        | 6 DAT               | 9 DAT               | 12 DAT               |
| Control                           | 0.854 <sup>a</sup> | 0.448 <sup>ab</sup> | 0.285 <sup>a</sup> | 0.164 <sup>a</sup> | 33.44 <sup>b</sup>           | 28.43 <sup>c</sup>  | 25.20 <sup>c</sup>  | 20.56 <sup>c</sup>   |
| Distilled water for 10 minutes    | 0.854 <sup>a</sup> | 0.465 <sup>a</sup>  | 0.268 <sup>a</sup> | 0.154 <sup>a</sup> | 33.12 <sup>b</sup>           | 28.88 <sup>c</sup>  | 25.96 <sup>bc</sup> | 21.48 <sup>bc</sup>  |
| HWT @ 50°C for 10 minutes         | 0.490 <sup>c</sup> | 0.262 <sup>de</sup> | 0.142 <sup>b</sup> | 0.094 <sup>b</sup> | 42.19 <sup>a</sup>           | 34.39 <sup>a</sup>  | 28.19 <sup>ab</sup> | 24.19 <sup>ab</sup>  |
| HWT @ 55°C for 10 minutes         | 0.452 <sup>c</sup> | 0.247 <sup>e</sup>  | 0.142 <sup>b</sup> | 0.096 <sup>b</sup> | 44.38 <sup>a</sup>           | 36.67 <sup>a</sup>  | 29.19 <sup>a</sup>  | 24.33 <sup>a</sup>   |
| Carbendazim @ 0.1% for 10 minutes | 0.654 <sup>b</sup> | 0.367 <sup>bc</sup> | 0.221 <sup>a</sup> | 0.151 <sup>a</sup> | 34.66 <sup>b</sup>           | 29.43 <sup>bc</sup> | 24.90 <sup>c</sup>  | 22.03 <sup>abc</sup> |
| NaOCl @ 100 ppm for 2 minutes     | 0.779 <sup>a</sup> | 0.352 <sup>cd</sup> | 0.241 <sup>a</sup> | 0.138 <sup>a</sup> | 40.31 <sup>a</sup>           | 33.19 <sup>ab</sup> | 26.31 <sup>bc</sup> | 23.16 <sup>abc</sup> |
| Mean                              | 0.680              | 0.357               | 0.217              | 0.133              | 38.02                        | 31.83               | 26.63               | 22.62                |
| LSD (P=0.05)                      | 0.1**              | 0.09*               | 0.07*              | 0.03*              | 5.44*                        | 3.99*               | 2.73*               | 2.84*                |
| CV%                               | 6.77               | 11.33               | 13.57              | 9.61               | 6.37                         | 5.58                | 4.56                | 5.59                 |

Means in a column followed by the same letter do not differ according to Fisher's least significant difference (LSD) at 0.05 level ( $P < 0.05$ ). \* represents significant ( $P \leq 0.05$ ), \*\* represents highly significant ( $P \leq 0.01$ ), CV is the coefficient of variation.

### 3.3 Pulp pH

The increasing trend of pH was seen along the extension of storage period up to 9<sup>th</sup> day of storage. A slight decrease in pH was noticed in distilled water, hot water treatment @ 50°C and 55°C and 0.1% carbendazim while pH of NaOCl treated fruits continuously increased during the

storage period and pH of control became static (Table 3). Higher retention of pH in fruits treated with hot water was due to the slower rate of degradation of fruit constituents like ascorbic acid resulting in increased pH (Ghosh *et al.*, 1981). Similar results were reported by Prasad and Mali (2000).

**Table 3.** Effect of chemicals and hot water as post-harvest treatments on pulp pH of Amrapali variety of mango.

| Treatments                        | Pulp pH            |                    |       |                    |
|-----------------------------------|--------------------|--------------------|-------|--------------------|
|                                   | 3 DAT              | 6 DAT              | 9 DAT | 12 DAT             |
| Control                           | 4.42 <sup>ab</sup> | 4.58 <sup>ab</sup> | 5.45  | 5.45 <sup>ab</sup> |
| Distilled water for 10 minutes    | 3.49 <sup>c</sup>  | 4.14 <sup>b</sup>  | 5.11  | 4.91 <sup>c</sup>  |
| HWT @ 50°C for 10 minutes         | 4.50 <sup>a</sup>  | 5.15 <sup>a</sup>  | 5.99  | 5.57 <sup>a</sup>  |
| HWT @ 55°C for 10 minutes         | 4.86 <sup>a</sup>  | 5.12 <sup>a</sup>  | 5.98  | 5.75 <sup>a</sup>  |
| Carbendazim @ 0.1% for 10 minutes | 4.51 <sup>a</sup>  | 4.26 <sup>b</sup>  | 5.91  | 5.65 <sup>a</sup>  |
| NaOCl @ 100 ppm for 2 minutes     | 3.81 <sup>bc</sup> | 4.35 <sup>b</sup>  | 5.00  | 5.05 <sup>bc</sup> |
| Mean                              | 4.27               | 4.6                | 5.57  | 5.4                |
| LSD (P=0.05)                      | 0.63*              | 0.71*              | NS    | 0.52*              |
| CV%                               | 6.6                | 6.86               | 8.11  | 4.27               |

Means in a column followed by the same letter do not differ according to Fisher's least significant difference (LSD) at 0.05 level ( $P < 0.05$ ). NS represents non-significant, \* represents significant ( $P \leq 0.05$ ), CV is the coefficient of variation.

### 3.4 Physiological loss in weight

The result revealed that PLW was highest (32.62%) in distilled water dipped fruits which was statistically at par with control and NaOCl whereas, PLW was the lowest (24.20%) on hot water treatment @ 55°C. Spoiled fruits percent increased significantly after 6<sup>th</sup> days of storage in all treatments. During 12<sup>th</sup> DAT, the highest spoilage (69.20%) was observed in distilled water dipped fruits which was similar with control and NaOCl treated fruits. The lowest spoilage loss (42.05%) was recorded in hot water treated fruits @ 55°C (Table 4).

A significant increase in physiological loss in weight and increasing trend of spoilage loss was observed

irrespective of the treatments as the storage period prolonged. The loss in weight was due to more loss of moisture as evapo-transpiration and respiration through uninterrupted atmospheric column (Reddy, 2015). The lower weight loss in hot water treated fruits attributed to slow breakdown of respiratory substrate due to inactivation of enzyme (Djioua *et al.*, 2009). This result is supported by Vala and Bhatt (2002). Hot water treatment can effectively suppress the growth of pathogens, may disinfect the fruit surface and inhibit the latent infection, thereby improved the shelf life (Mansour *et al.*, 2006; Shiesh *et al.*, 2017). Additionally, hot water treatment act as an elicitor for the activation of the defensive response in harvested fruits (Huan *et al.*, 2017).

**Table 4.** Effect of chemicals and hot water treatments on PLW and spoilage loss of Amrapali variety of mango

| Treatments                        | WPLW (%)           |                     |                     |                     | Spoilage loss (%) |                     |                     |                    |
|-----------------------------------|--------------------|---------------------|---------------------|---------------------|-------------------|---------------------|---------------------|--------------------|
|                                   | 3 DAT              | 6 DAT               | 9 DAT               | 12 DAT              | 3 DAT             | 6 DAT               | 9 DAT               | 12 DAT             |
| Control                           | 8.23 <sup>a</sup>  | 17.52 <sup>a</sup>  | 23.61 <sup>a</sup>  | 32.33 <sup>a</sup>  | 0.00              | 12.18 <sup>ab</sup> | 32.87 <sup>a</sup>  | 67.92 <sup>a</sup> |
| Distilled water for 10 minutes    | 8.41 <sup>a</sup>  | 17.30 <sup>a</sup>  | 21.08 <sup>ab</sup> | 32.63 <sup>a</sup>  | 0.00              | 12.95 <sup>a</sup>  | 31.90 <sup>a</sup>  | 69.20 <sup>a</sup> |
| HWT @ 50°C for 10 minutes         | 6.63 <sup>b</sup>  | 13.41 <sup>bc</sup> | 16.57 <sup>c</sup>  | 26.36 <sup>b</sup>  | 0.00              | 8.78 <sup>c</sup>   | 18.15 <sup>b</sup>  | 46.72 <sup>b</sup> |
| HWT @ 55°C for 10 minutes         | 6.42 <sup>b</sup>  | 12.75 <sup>c</sup>  | 15.34 <sup>c</sup>  | 24.20 <sup>b</sup>  | 0.00              | 9.25 <sup>c</sup>   | 17.95 <sup>b</sup>  | 42.05 <sup>b</sup> |
| carbendazim @ 0.1% for 10 minutes | 6.25 <sup>b</sup>  | 15.95 <sup>ab</sup> | 17.47 <sup>bc</sup> | 29.00 <sup>ab</sup> | 0.00              | 10.21 <sup>bc</sup> | 23.96 <sup>ab</sup> | 47.73 <sup>b</sup> |
| NaOCl @ 100 ppm for 2 minutes     | 7.46 <sup>ab</sup> | 17.02 <sup>a</sup>  | 18.69 <sup>bc</sup> | 32.45 <sup>a</sup>  | 0.00              | 10.06 <sup>bc</sup> | 26.42 <sup>ab</sup> | 65.09 <sup>a</sup> |
| Mean                              | 7.23               | 15.66               | 18.79               | 29.5                | 0.00              | 10.57               | 25.21               | 56.45              |
| LSD (P=0.05)                      | 1.23*              | 2.98*               | 3.7*                | 5.46*               | 0.00              | 2.46*               | 9.44*               | 13.79*             |
| CV%                               | 7.61               | 8.47                | 8.76                | 8.24                | 0.00              | 10.36               | 16.67               | 10.87              |

Means in a column followed by the same letter do not differ according to Fisher's least significant difference (LSD) at 0.05 level ( $P < 0.05$ ). \* represents significant ( $P \leq 0.05$ ), CV is the coefficient of variation.

### 3.5 Shelf life

Shelf life of mango fruits was significantly affected by different post-harvest treatments. Results revealed that the longest shelf life (14.25 days) of mango fruits was observed in the fruits treated with hot water @ 55°C. The shortest shelf life of 9.50 days was observed in the fruits dipped in distilled water for 10 minutes (Table 5). The longest shelf life of fruits treated with hot water was possibly due to the reduced rate of physico-chemical changes, reduced weight loss and minimal disease severity and the suppression of microbial growth (Hoque *et al.*, 2017).

**Table 5.** Effect of chemicals and hot water treatments on Shelf life of Amrapali variety of mango

| Treatments                        | Shelf life         |
|-----------------------------------|--------------------|
| Control                           | 9.75 <sup>b</sup>  |
| Distilled water for 10 minutes    | 9.50 <sup>b</sup>  |
| HWT @ 50°C for 10 minutes         | 13.50 <sup>a</sup> |
| HWT @ 55°C for 10 minutes         | 14.25 <sup>a</sup> |
| carbendazim @ 0.1% for 10 minutes | 13.50 <sup>a</sup> |
| NaOCl @ 100 ppm for 2 minutes     | 10.00 <sup>b</sup> |
| Mean                              | 11.75              |
| LSD (P=0.05)                      | 1.33 <sup>**</sup> |
| CV%                               | 7.64               |

Means in a column followed by the same letter do not differ according to Fisher's least significant difference (LSD) at 0.05 level ( $P < 0.05$ ). \*\* represents highly significant ( $P \leq 0.01$ ), CV is the coefficient of variation.

### 4. CONCLUSION

The present study concluded that hot water treatment of mango fruits at 55°C was found to be effective for marinating quality and shelf life of mango due to its superior performance for most of the important post-harvest parameters (TSS, PLW, vitamin C, pH and spoilage loss). Considering the quality, consumer's preferences, environmental aspects and cost of application, the present result clearly indicated that the hot water treatment at 55°C can be alternative of synthetic chemicals in post-harvest management of mango.

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# EFFICACY OF SOME PLANT EXTRACTS AGAINST ROOT ROT DISEASE OF GREEN OAK LETTUCE (*Lactuca sativa* var. *Crispa*) CAUSED BY *Pythium* sp. GROWN IN A HYDROPONIC SYSTEM

Lakshya Bahadur Chaudhary<sup>1,2,\*</sup>, Pimjai Meetum<sup>1</sup>, Mana Kanjanamaneesathian<sup>1</sup>, Roshan Adhikari<sup>1,2</sup> and Rachsawan Mongkol<sup>1</sup>

<sup>1</sup> Faculty of Animal Science and Agricultural Technology, Silpakorn University Phetchaburi IT campus, Cha-Am, Phetchaburi, Thailand

<sup>2</sup> Ministry of Agriculture and Livestock Development, Government of Nepal

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\*Correspondence:  
lakshyacsu@gmail.com  
Tel: +977-9841500385

## ABSTRACT

An antifungal activity of the twelve plant extracts were tested against *Pythium* sp., the causal agents of root rot diseases grown in hydroponic systems. The crude extracts of *Acorus calamus* and *Syzygium aromaticum* completely inhibited the mycelial growth of *Pythium* sp. in the in vitro condition at 5,000 ppm, whilst *Curcuma zedoaria* and other plant extracts had show lesser inhibitory activity. Out of these twelve plant extracts selecting *A. calamus*, *S. aromaticum* and *C. zedoaria* for further in vivo test in nursery raising seedling in plastic tray of hydroponic system by inoculating *Pythium* sp. mycelial developed on agar medium, *A. calamus* rhizome extract exhibited highest inhibitory effect against % root tip colonized by *Pythium* sp. of 5.85 to 48 followed by *S. aromaticum* floral bud 9.97 to 60 and rhizome of *C. zedoaria* extract 28.32 to 90 at 2000 ppm on seven and twenty one days after inoculation respectively. The concentrations and application methods of these extracts should be further investigated to control the root rot disease of lettuce in the hydroponic system.

## 1. INTRODUCTION

Vegetable cultivation in hydroponic system is considered as growing plants with or without use of substrate (e.g. gravel, sand, sawdust, vermiculite, rockwool, sponge or peatmoss) to provide mechanical support to the plants where nutrients are supplied through solution (Jensen, 1997; Jensen & Collins, 1985). Mostly, hydroponic systems are enclosed inside greenhouses or shade nets to control temperature, reduce water loss from evaporation water loss and to protect from diseases, pest and against the elements of weather such as wind and rain. Besides the advantages of hydroponics growing system through avoidance of soil borne pathogen, there might be introduction of pathogen through people moving in and out of the greenhouse, dust on the uncovered system, infected seeds, propagation materials, pests etc. (Paulitz, 1997).

For higher production and productivity, application of chemical fungicides is not an option for disease control in vegetables grown hydroponically as it would result in the rejection of the produce by the consumers who perceive vegetables grown hydroponically as a pesticide-free produce and are willing to pay higher prices for these commodities (Kanjnamaneesathian, 2015).

The negative environmental impact of pesticides used for pest and disease control is intensively increasing every day, especially in vegetable production system and the consumer are now getting curious about fresh and organic products for their better health and sustainable development. For this reason, several alternative methods of reducing pesticide are being developed among which one of the effective methods

is to use plant extracts which incorporating natural antifungal substances. Antifungal effects of plant and plant products are emerging every day. As antifungal substances, obtained from plants have no side effect against environment, they are showing significant advantages to the environment and organic management system for pest and disease. So, conducting a research of alternative control methods based on plant extract comes into prominence for minimizing use of commercial pesticide and development of sustainable agriculture production in hydroponics.

Plant extracts, essential oils, gums, resins etc. have been shown to exert biological activity against plant fungal pathogens *in vitro* and *in vivo* and can be used as bio-fungicidal products (Fawzi *et al.*, 2009; Jalili *et al.*, 2010; Romanazzi *et al.*, 2012). These products are generally assumed to be more acceptable and less hazardous for the ecosystems and could be used as alternative remedies for treatment of plant diseases (Chuang *et al.*, 2007). Natural plant products have a narrow target range with specific mode of action, therefore are suitable for a specific target, mostly nontoxic for antagonistic microorganisms, show limited field persistence and have a shorter shelf life and no residual threats.

Antifungal activity of plant extracts has been reported (Cowan, 1999; Grainge & Ahmed, 1988; Kurita *et al.*, 1981; Wilson *et al.*, 1997). These researches have indicated that antimicrobial components of the plant extracts act by crossing the cell membrane and interacting with the enzymes and proteins of the membrane, leading to produce a flux of protons towards the cell exterior which induces change in the cell and ultimately their death (Omidbeygi *et al.*, 2007; Pane *et al.*, 2011). Other researcher attributed the inhibitory effect of these plant extracts to hydrophobicity characters of these plant extracts and their components. This enables them to make partition in the lipids of the cell wall and cell membrane, causing leakage of ions and other cell contents which may lead to cell death (Burt, 2004).

Plant extracts are generally prepared by using alcohol or

other aqueous solutions, either by hot or cold extraction methods. Crude and alcohol extract of several plants have been screened and tested for their potential antimicrobial activities against plant pathogenic microbes such as virus, bacteria, fungi and protozoa (Abere *et al.*, 2007; Afolayan, 2003).

This research study was aimed at evaluating the potentials plant extracts of *S. aromaticum*, *C. zedoaria*, *A. calamus* in the biological control of *Pythium* sp. causing root rot in hydroponic system. This study pursues highlights on useful and sustainable alternative of costly and non-eco-friendly synthetic fungicides to control of fungal diseases of lettuce grown hydroponically.

## 2. MATERIALS AND METHODS

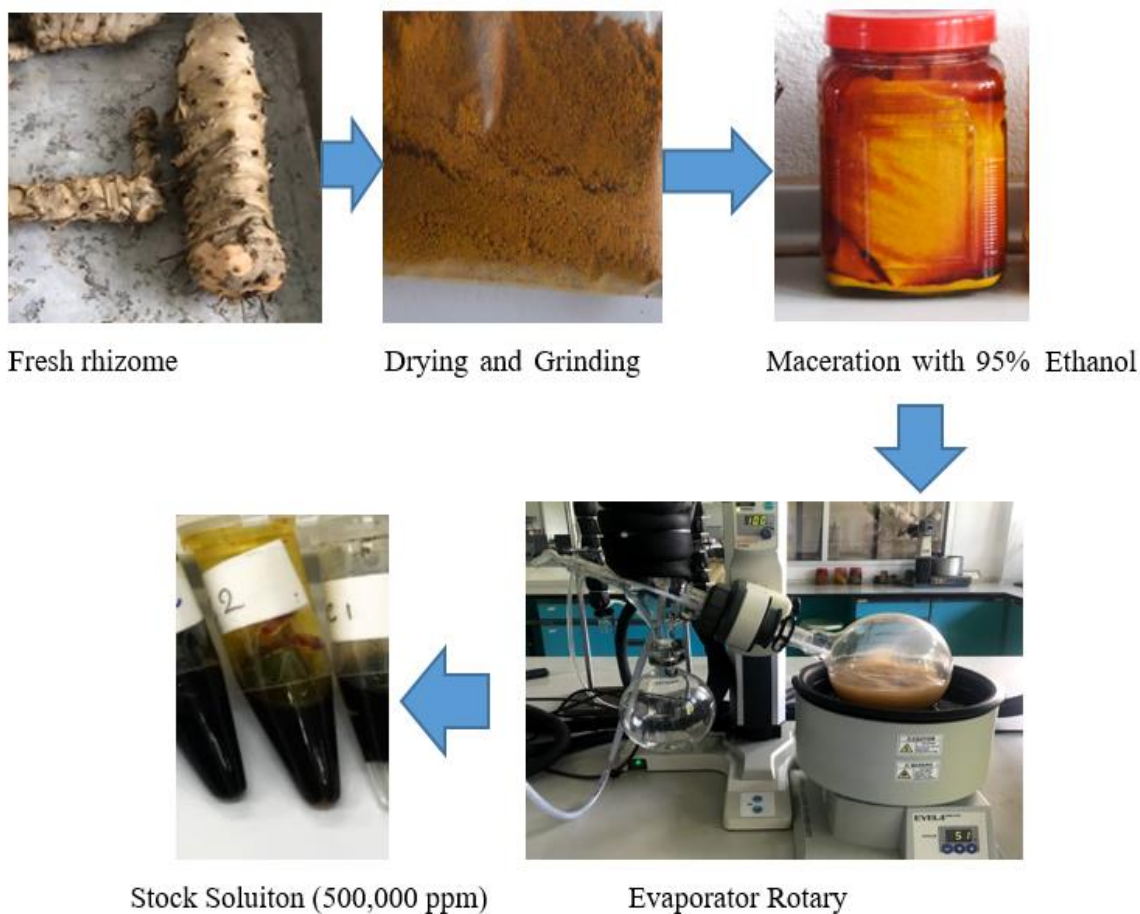
### 2.1. Collection and Preparation of Plant Extracts

Plant materials used for tests were either collected from Hua-Hin district, Prachub Khiri khan province and Cha-Am district, Phetchaburi province or purchased from "Tai-Hua-Chan" medical plant shops, Bangkok, Thailand in 2018 (Table 1). Most of the collected plants were locally and widely available in Nepal and Thailand. The fresh plant parts were washed with tap water, air dried and then weighted. These plant parts were cut into small pieces, packed to aluminum trays and placed in the oven at 60°C for 24 hours. Each dried plant part was weighted and then grinded into powder which was used to prepare the crude extract.

The powdered plant parts (such as flowers, leaves, rhizomes and buds) were separately soaked in 95% ethanol in the glass bottles for maceration. These mixtures were incubated at room temperature (26-32°C) for three days. After incubation, the powdered plant parts were filtered with the muslin cloth and the liquid part was collected in the round bottom flasks. The liquid was then evaporated by a rotary evaporator at 100 rpm for 20 min (at 50-56°C) to obtain the crude extracts. These crude extracts were weighted. The stock solutions of each crude extract were prepared by dissolving 0.5 g of each crude extract in one mL of acetone to obtain 500,000 ppm stock solution for further use (Figure 1)

**Table 1.** List of plant species for extraction and efficacy testing against fungi.

| Family         | Scientific name            | Common name   | Nepali name  | Part of plant                   | Collected place               |
|----------------|----------------------------|---------------|--------------|---------------------------------|-------------------------------|
| Acoraceae      | <i>Acoros calamus</i>      | Sweet flag    | Bojo         | Rhizome                         | Medicinal plant shop, Bangkok |
| Asteraceae     | <i>Azeratum conizoides</i> | Goatweed      | Gande        | Aerial part                     | Cha-Am, Phetchaburi           |
| Asteraceae     | <i>Eupatorium odoratum</i> | Siam weed     | Aule Banmara | Aerial part                     | Hua-Hin, Prachuabkhirikhan    |
| Asteraceae     | <i>Tagetes erecta</i>      | Marigold      | Saypatri     | Aerial part                     | Local market, Phetchaburi     |
| Clusiaceae     | <i>Garcinia mangostana</i> | Mangosteem    | Mangosteem   | Inflorescence<br>Fruit pericarp | Local market, Phetchaburi     |
| Fabaceae       | <i>Cassia spectabilis</i>  | Yellow cassia | Rajbrikshya  | Flower                          | Hua-Hin, Prachuabkhirikhan    |
| Meliaceae      | <i>Azadiracta indica</i>   | Neem          | Neem         | Leaf                            | Hua-Hin, Prachuabkhirikhan    |
| Myrtaceae      | <i>Syzygium aromaticum</i> | Clove         | Lwang        | Floral bud                      | Medicinal plant shop, Bangkok |
| Plantaginaceae | <i>Scoparia dulcis</i>     | Sweet-broom   | Patal Mishri | Whole plant                     | Hua-Hin, Prachuabkhirikhan    |
| Verbenaceae    | <i>Lantana camara</i>      | Lantana weed  | Banmara      | Aerial part                     | Cha-Am, Phetchaburi           |
| Zingiberaceae  | <i>Alpinia galangal</i>    | Galangal      | Haledo       | Rhizome                         | Cha-Am, Phetchaburi           |
| Zingiberaceae  | <i>Curcuma zedoaria</i>    | Zedoary       | Seto Besar   | Rhizome                         | Cha-Am, Phetchaburi           |


**Figure 1.** Preparation of crude extract by maceration technique

## 2.2. Laboratory screening of the antifungal activity of plant extract

An antifungal activity of the selected plant extracts was tested against both *Pythium* sp., using food poisoning technique (Kumar *et al.*, 2008). Each plant extract stock solution (0.1 mL) was added to 9.9 mL of sterile PDA at 55°C. The mixture prepared was poured into the petri dishes to attain final concentration of 5,000 ppm. Nil control medium was prepared by mixing acetone (0.1 mL) in the sterile PDA (9.9 mL). The growing mycelia from the seven-day-old culture of both pathogens on PDA were cut and placed onto the centre of petri-plates filled with each plant extract individually. Sterile cork borer (6mm in diameter) was used to cut the growing mycelia and then transferred aseptically onto the centre of PDA which had been mixed the crude extracts. The plates were then incubated at room temperature (at 26-32°C) for seven days. The diameter of the fungal colony of the treatments and nil control were measured and the percentage of mycelial growth inhibition (M) was calculated (Wang *et al.*, 2005).

$$M = [(A-B) / A] \times 100$$

Where A is the colony diameter of the control treatment and B is the colony diameter of the treated crude extracts.

## 2.3. Efficacy testing of the plant extracts to suppress root colonization by *Pythium* sp. in the plastic tray

*A. calamus*, *S. aromaticum* and *C. zedoaria* were selected to test their efficacy against *Pythium* sp. The lettuce seedlings were raised in the seed beds which had been drenched with the mixture of extract solution and nutrients (at 2,000 ppm) for seven days. After seven days, the seedlings were transplanted to the hydroponic unit.

One hundred lettuce seeds were germinated in polyurethane sponge (23x25x2.5 cm in dimension) which had been moisten with distilled water. After 12 days, these seedlings were transferred to plastic tray which contained the mixture of distilled water (4 L) and nutrient solution A (3 mL L<sup>-1</sup> of water) and B 3 mL L<sup>-1</sup> of water). Twenty seedlings were planted in each plastic tray in the experiment which consisted of eight treatments with four replications and each trays contains 20 lettuce seedlings (Table 2)(Kobayashi & Yamaji, 2022; Lee *et al.*, 2006).

**Table 2.** Lettuce seedlings treated with different plant extracts solution

| Name of treatment                         | Replication | Number of plants / tray | Total plants/ treatment |
|-------------------------------------------|-------------|-------------------------|-------------------------|
| <i>A. calamus</i> + <i>Pythium</i> sp.    | 4           | 20                      | 80                      |
| <i>A. calamus</i>                         | 4           | 20                      | 80                      |
| <i>S. aromaticum</i> + <i>Pythium</i> sp. | 4           | 20                      | 80                      |
| <i>S. aromaticum</i>                      | 4           | 20                      | 80                      |
| <i>C. zedoaria</i> + <i>Pythium</i> sp.   | 4           | 20                      | 80                      |
| <i>C. zedoaria</i>                        | 4           | 20                      | 80                      |
| <i>Pythium</i> sp. (Control)              | 4           | 20                      | 80                      |
| Nutrient (Nil Control)                    | 4           | 20                      | 80                      |

Two days after the seedlings were transferred to the hydroponic unit in plastic tray, eight grams of plant extracts were added to the nutrient solution 2,000 ml to maintain 2,000 ppm. Twelve agar plugs of the 5-day-old of the mycelia of *Pythium* sp. growing on PDA were inoculated into the plastic tray. Root tip of the lettuce which had been colonized with *Pythium* sp. was

assessed. Three lettuce seedlings from each plastic tray were sampled and the root was cleaned with tap water and distilled water twice. The root was subsequently dipped into 1% sodium hypochlorite solution for 40 seconds and then rinsed twice with distilled water. Ten root tips (2-4 mm in length) were selected randomly from seedlings by cutting the secondary root tips and

they were placed onto the Petri plates containing 10 mL PDA. The growing mycelia of *Pythium* sp. from the root tissues on PDA were observed in 7 days and 21 days of inoculation. Percentage root colonization per plant were calculated (Bhale, 2018):

$$\% \text{ Root colonization} = \frac{\text{Total } Pythium \text{ sp. mycelial colony developed on petri plate}}{\text{Total root tips placed on the petri plates}} \times 100$$

#### 2.4. Statistical Analysis

Data collected from this research were analyzed using Microsoft excel 2013 and R-Program software. Duncan Multiple Range Test (DMRT) analysis was used to compare the means using R-Program version 3.5.1.

### 3. RESULTS AND DISCUSSION

#### 3.1. In vitro inhibition of *Pythium* sp. by plant extracts on PDA

*In vitro* efficacy of plant extracts were examined against *Pythium* sp. using poison food technique. Among 13 ethanol crude extracts from 12 plant species, rhizome extract of *A. calamus* and floral bud of *S. aromaticum* completely inhibited *Pythium* sp., followed by rhizome of *C. zedoaria* (54.15%), aerial part of *E. odoratum* (40.62%), rhizome of *A. galanga* (40%) and fruit pericarp of *G. mangostana* (32.92%), compared with acetone only as control treatment. The other ethanol crude extracts with lesser than 10% inhibition were flower of *C. spectabilis* (8%), whole plant of *S. dulcis* (5.54%), aerial part of *A. conizoides* (2.77%) and aerial part of *L. camara* (1.23%). Some crude extracts such as aerial part and inflorescence of *T. erecta* and leaf of *A. indica*, did not inhibit the mycelial growth of *Pythium* sp. (Table 3 and Figure 2).

**Table 3.** Efficacy of crude extracts against mycelial growth of *Pythium* sp. on PDA

| Family              | Scientific name       | Plant parts    | Inhibition (%)± SD |
|---------------------|-----------------------|----------------|--------------------|
| Acoraceae           | <i>A. calamus</i>     | Rhizome        | 100±0.00a          |
| Asteraceae          | <i>A. conizoides</i>  | Aerial part    | 2.77±1.05d         |
| Asteraceae          | <i>E. odoratum</i>    | Aerial part    | 40.62±0.64bc       |
| Asteraceae          | <i>T. erecta</i>      | Aerial part    | -47.69±0.90e       |
|                     |                       | Inflorescence  | -54.15±0.61e       |
| Clusiaceae          | <i>G. mangostana</i>  | Fruit pericarp | 32.92±0.39bcd      |
| Fabaceae            | <i>C. spectabilis</i> | Flower         | 8.00±0.91cd        |
| Meliaceae           | <i>A. indica</i>      | Leaf           | -46.46±0.58e       |
| Myrtaceae           | <i>S. aromaticum</i>  | Floral bud     | 100±0.00a          |
| Plantaginaceae      | <i>S. dulcis</i>      | Whole plant    | 5.54±1.18d         |
| Verbenaceae         | <i>L. camara</i>      | Aerial part    | 1.23±0.28d         |
| Zingiberaceae       | <i>A. galangal</i>    | Rhizome        | 40±0.83bc          |
| Zingiberaceae       | <i>C. zedoaria</i>    | Rhizome        | 54.15±0.03b        |
| Control             |                       |                | 0.00±0.00          |
| CV                  |                       |                | 16.09              |
| LSD <sub>0.05</sub> |                       |                | 1.211              |

\*Indicate significant effects at p<0.01 (DMRT) within a column.

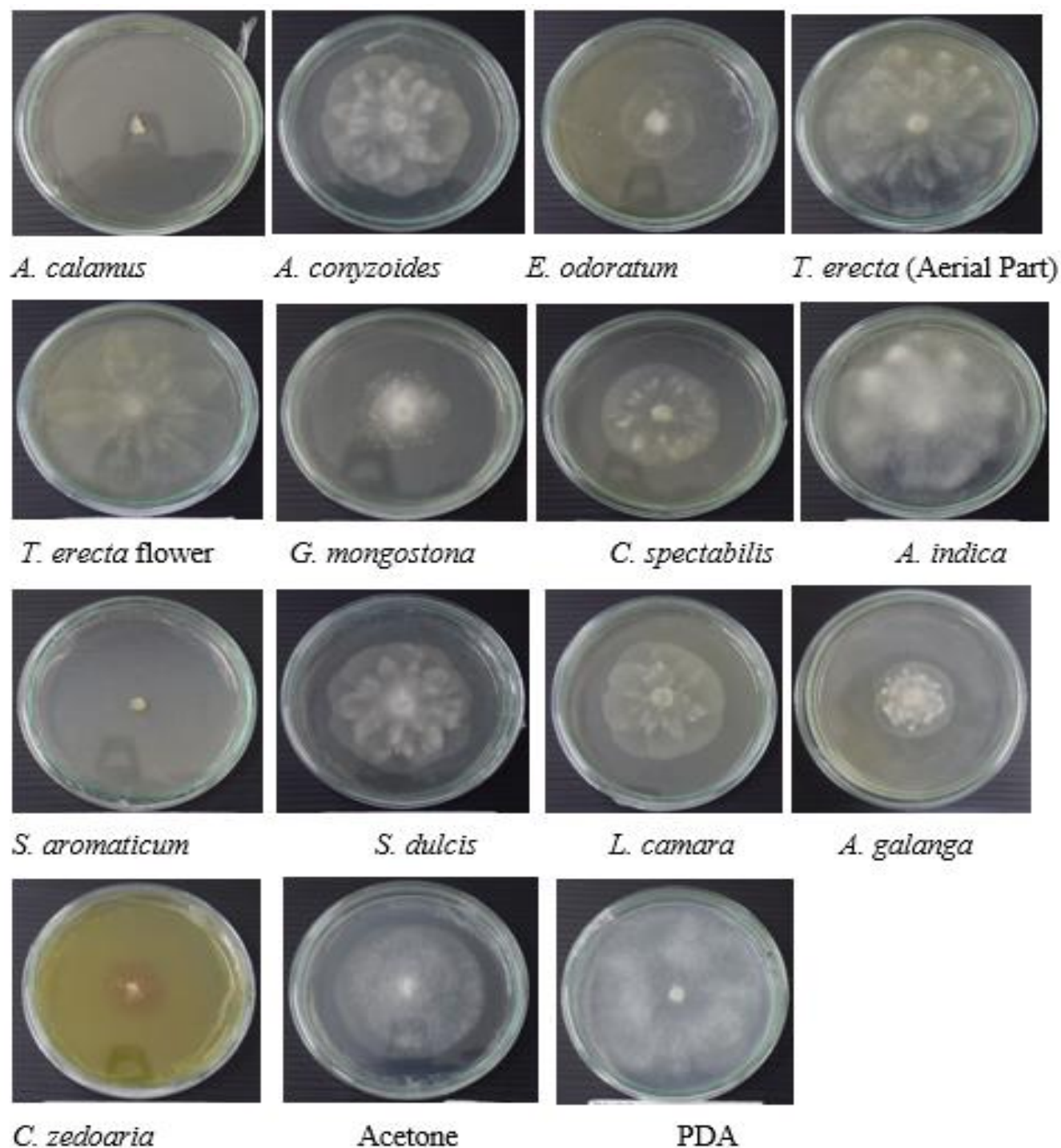


**Figure 2.** *In vitro* efficacy of different plant crude extracts against *Pythium* sp. at 5,000 ppm

### 3.2. Efficacy of plant extracts in reducing the capacity of *Pythium* sp. to colonize lettuce root

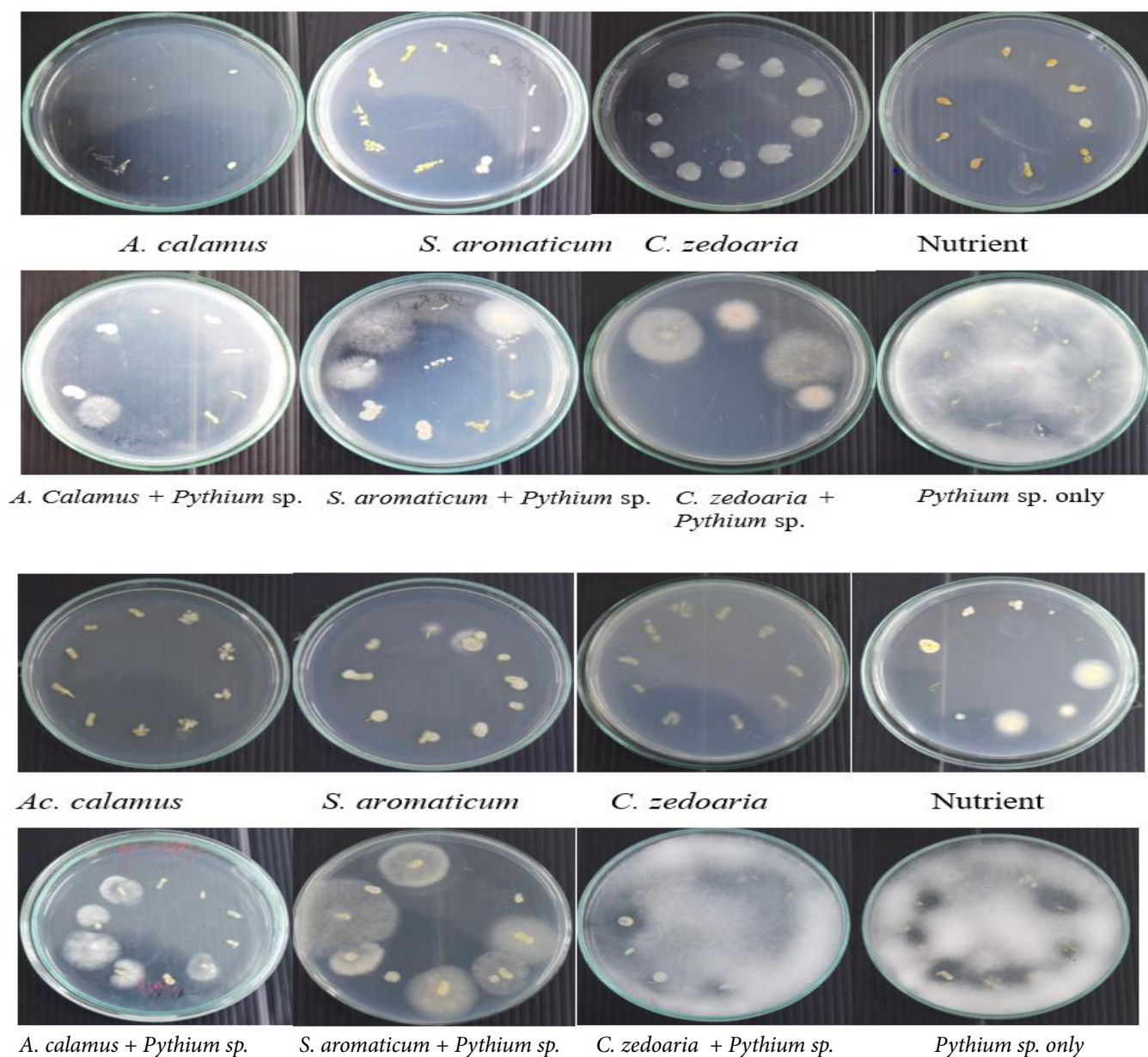
Based on the percentage of root tips which were

colonized by *Pythium* sp., *A. calamus* was effective in reducing the percentage of root colonization followed by *S. aromaticum* and *C. zedoaria* as shown in table 4 and Figure 3.

**Table 4.** Percent root tip of lettuce colonized by *Pythium* sp.

| Name of Treatments                        | % Colonization on 7 Day | % Colonization on 21 Day |
|-------------------------------------------|-------------------------|--------------------------|
| <i>A. calamus</i> + <i>Pythium</i> sp.    | 5.825±0.56c             | 48±29.49b                |
| <i>S. aromaticum</i> + <i>Pythium</i> sp. | 9.975±1.053c            | 60±21.21b                |
| <i>C. zedoaria</i> + <i>Pythium</i> sp.   | 28.32±0.794b            | 90±14.14a                |
| <i>Pythium</i> sp.                        | 90±0.901a               | 90±10.00a                |
| Nutrient                                  | 6.67±0.722c             | 10±14.14                 |
| <i>A. calamus</i>                         | 0±00c                   | 0±00c                    |
| <i>S. aromaticum</i>                      | 0±00c                   | 0±00c                    |
| <i>Cu. zedoaria</i>                       | 0±00c                   | 0±00c                    |

\*\* indicate significant effects at  $p < 0.01$  (by Duncan multiple range test) within a column.

**Figure 3.** Percentage colonized by *Pythium* sp. on root tips of lettuce at 7 and 21 Day

### 3.3 Discussion

Antifungal activity of plant extracts has been reported (Cowan, 1999; Grainge & Ahmed, 1988; Kurita *et al.*, 1981; Wilson *et al.*, 1997). These researches have indicated that antimicrobial components of the plant extracts act by crossing the cell membrane and interacting with the enzymes and proteins of the membrane, leading to produce a flux of protons towards the cell exterior which induces change in the cell and ultimately their death (Omidbeygi *et al.*, 2007; Pane *et al.*, 2011). Other researcher attributed the inhibitory effect of these plant extracts to hydrophobicity characters of these plant extracts and their components. This enables them to make partition in the lipids of the cell wall and cell membrane, causing leakage of ions and other cell contents which may lead to cell death (Burt, 2004).

*A. calamus*, *C. spectabilis*, *C. zedoaria*, *S. aromaticum* were reported to possess the antimicrobial, antibacterial, antifungal, anti-protozoal, anti-inflammatory and antioxidant activities (Ayoola *et al.*, 2008; Latha *et al.*, 2009; Sangetha *et al.*, 2008; Umamaheshwari & Rekha, 2018; Yusuf *et al.*, 2001). Plant extracts could also be used against plant pathogenic fungi (Yulia, 2005).

In this study, twelve plants were selected for screening to determine their efficacy against *Pythium* sp. an important causal agents of root rot diseases of lettuce grown hydroponically. The results showed that the crude ethanol extracts of rhizome of *A. calamus*, *C. zedoaria* and *A. galanga*; floral bud of *S. aromaticum*, and fruit pericarp of *G. mangostana* have potential plant for farmers to use in hydroponic system to control root rot. Rhizome extract of *A. calamus* completely inhibited *Pythium* sp. in vitro at 5,000 ppm. Koohakan (2009) also reported that ethanol extract of rhizome of *A. calamus* showed the efficacy to completely suppress both *P. aphanidermatum* and *P. myriotylum* at 5,000 ppm on agar tests. Thus, the extract of *A. calamus* at 5,000 ppm was confirmed to be the appropriate choice for use in further research. The antifungal compound present in the crude extract may cause fluid leakage from cells leading to fungal cell death and the overall suppression to the fungal colony (Phongpaichit *et al.*, 2005). However, various factors such as the choice of solvents, concentrations of the solvent and extraction methods may have contributed to the quality, quantity and efficacy of the yield. Khruasanit (2004) reported

that dichloromethane was found to be an effective solvent for extracting the active compounds from *A. calamus*, whilst methanol was unable to extract the active compounds and the product was not inhibitory to *Pythium* sp.

The inhibitory activity of plant extracts observed in the study could be attributed to the presence of biologically active anti-fungal compounds. The active compounds from ethanolic extracts of *S. aromaticum* and *A. galanga* were characterized as eugenol and 1'-acetoxychavicol acetate (ACA) respectively against the mycelial growth and conidial germination of *Curvularia lunata* and *Fusarium sacchari* (Madi *et al.*, 2020). 1'-Acetoxychavicol acetate is identified as active compound from *A. galangal* that inhibited the growth of *Phytophthora nicotianae* and *Alternaria porri*, with minimum inhibition concentration values of 15.6 and 31.5 µg mL<sup>-1</sup>, respectively (Mongkol *et al.*, 2015).

Nevertheless, in this study the ethanol crude extract of *A. calamus*, *S. aromaticum* and *C. zedoaria* were found to be effective to suppress *Pythium* sp. both *in vitro* and *in vivo* conditions.

### 4. CONCLUSION

The results are showed that the crude ethanol extracts of rhizome of *A. calamus*, *C. zedoaria* and floral bud of *S. aromaticum*, have potential plant for farmers to use in hydroponic system to control root rot. Rhizome extract of *A. calamus* completely inhibited *Pythium* sp. in vitro at 5,000 ppm. Ethanol crude extract of *A. calamus*, *S. aromaticum* and *C. zedoaria* was found to be effective to suppress both *Pythium* sp. in the laboratory tests as well as in root tips of nursery seeding before transplanting it into main hydroponic systems. Based on the both *in vitro* and *in vivo* results of selected plant extracts these plant extract could be potential bio-fungicide for hydroponic system while phytotoxicity and compatibility to the plant of these potential bio-fungicides should be considered for further research before applying it in the system.

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# EVALUATION OF DIFFERENT GROWING MEDIA FOR TOMATO AND SWEET PEPPER SEEDLINGS RAISING IN POKHARA, NEPAL

Santosh Lohani<sup>1,\*</sup>, Saroj Adhikari<sup>1</sup>, Lok Nath Aryal<sup>1</sup>, Yubraj Bhusal<sup>2</sup>, Manahar Kadariya<sup>3</sup> and Sunil Aryal<sup>1</sup>

<sup>1</sup> Horticulture Research Station, Malepatan, Nepal Agricultural Research Council

<sup>2</sup> National Agricultural Technology Information Center, Nepal Agricultural Research Council

<sup>3</sup> Agriculture and Livestock Development Division, Pokhara Metropolitan City, Kaski

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\*Correspondence

lohanisantos@gmail.com

Tel: +977-9845113879

## ABSTRACT

Nine different compositions of growing media were evaluated for growing tomato and capsicum in two consecutive years (2020 and 2021). Experiments were conducted in Randomized Complete Block Design (RCBD) under rain shelter (poly house) with three replications. The growing media were Cocopeat, FYM+Cocopit+Perlite, FYM+Cocopit+Vermiculite, FYM+Perlite, FYM+Soil, FYM+Vermiculite, Vermicompost+Perlite, Vermicompost +Soil and Vermicompost+Vermiculite. There were significant ( $P<0.05$ ) differences in the observed parameters like number of seedlings emergence, number of seedling establishment, number of leaves, seedling height and diameter of seedling stem among the tested growing media. The highest seedling emergence of tomato and sweet pepper were recorded in cocopeat as a growing media. After 30 days after seed sowing (DAS) in tomato, the highest number of seedling (103.5) were found in Cocopeat and the tallest seedling height (19.92 cm) was found in Vermicompost+perlite. Similarly, in Capsicum (40DAS), the highest number of seedling (83.5) were found for the growing media Cocopeat only, followed by Vermicompost + Perlite (78) and the tallest seedling height (13.89 cm) was found in Vermicompost+Vermiculite grown seedlings. The good root plug formation was found for the use of cocopeat. Therefore, use of vermicompost or FYM in the mixed form with cocopeat or the soil as a growing media would be sustainable technology for commercial seedling production of vegetables.

## 1. INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is a member of Solanaceae family, one of the most popular and widely grown vegetable crops in the world (Sharma & Singh, 2015). Tomato fruits can be consumed fresh or processed (Eivazi *et al.*, 2013) and rich source of minerals and vitamins (Wilcox *et al.*, 2003; Eivazi *et al.*, 2013). Similarly, Sweet pepper (*Capsicum annuum* L.) belongs to the family Solanaceae and it is rich in vitamin C (Arancon *et al.*, 2010; El-Sayed *et al.*, 2015). Capsicum is consumed for their colour, taste and nutritional value (Perez-Lopez *et al.*, 2007). The cultivation of Solanaceous crops like Tomato and sweet pepper starts with the transplanting of seedlings in the main growing field. Healthy and robust seedlings production is utmost necessary because of great effect of the seedlings on the final crop establishment and yield. The expensive seeds (particularly of hybrids) of high value crops like tomato, brinjal, capsicum and cucumber etc. are being transplanted after growing in nursery under close observation and care to achieve

maximum germination and healthy seedling production. Climatic conditions and seed sowing media have significant impact on seedling development (Hazarika *et al.*, 2022). Seedling production in nursery bed can produce stress in plants during transplanting (Demir *et al.*, 2010). Use of different growth media mainly helps to proper growth in nursery as well as easy adaptation in the field (Sterrett, 2001).

Growing media are substrates, other than soils for plants grown. These are organic materials like peat, compost, tree bark, coconut (*Cocos nucifera* L.) coir, poultry feathers and inorganic materials such as clay, perlite, vermiculite, and mineral wool (Grunert *et al.*, 2008) or mixes such as peat and perlite; coir and clay, peat and compost (Nair *et al.*, 2011). Growing media has direct impacts on seed germination, seedling emergence, seedling growth and quality of seedlings in a nursery stage (Unal, 2013). Growing media is source for seeds sowing and source of

plant nutrients (Indriyani *et al.*, 2011). Besides this it also supports the root system to stand the plant (Abad *et al.*, 2005).

Seedlings raised soil beds are prone to root damage during uprooting. Most of the commercial farmers are using seedling growing plug trays on vegetable seed germination and transplant establishment in various growing media (Nair *et al.*, 2011). So adoption of plug-tray nursery raising technology is increasing. This technique helps vigorous root development as well as prevents the damage during transplanting. The use of this technology can grow large number of seedlings and it has high significance when the seedlings should be raised in semi/controlled conditions. For the commercial seedling production by use of plug tray there is use of cocopeat or vermicompost for the growing media. The use of only cocopeat for growing media require additional nutrient supplement during seedling growth and use of vermicompost will be higher cost for the farmers. For the production of seedlings under protected structure by using different growing media on plug tray should be evaluated. Some nurseries are using the mineral rocks for the seedling/sapling production of ornamental plants and some progressive farmers of Nepal also using cocopeat and vermicompost or cocopeat and FYM in 1:1 ratio by volume. Selection of cheaper and easily available organic materials for production of seedling is the way of sustainable seedling production as use of media. So the validation and verification of alternative growing media for plug tray in mixture of different concentration is necessary. For the healthy and economically viable seedling production, evaluation of different growing media with their mixture is necessary to verify the technology for commercial purpose. Very few researches or studies were carried out in Nepal to evaluate the quality parameters of the seedling by using growing media for seedling production of sweet pepper and tomato. Therefore, the aim of this study was to evaluate different growing media for the germination, growth and development of seedlings in plug tray under rain shelter plastic house.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site

The trial was conducted at Horticulture Research Station (HRS), Malepatan, Pokhara, Kaski district during the two consecutive seasons, of March, 2020 and April, 2021 under rain shelter. The station is located at about 28°13'6.8" N latitude and 83°58'27.72" E longitude with an elevation of 848 masl. The station has sub-tropical humid type of climate.

**Table 1.** Meteorological data of HRS, Malepatan for open field in 2020 and 2021

| Year /<br>Month | Temperature (°C) |         |         | Rainfall<br>(mm) | Relative<br>Humidity<br>(%) |
|-----------------|------------------|---------|---------|------------------|-----------------------------|
|                 | Maximum          | Minimum | Average |                  |                             |
| 2020            |                  |         |         |                  |                             |
| March           | 25.1             | 11.1    | 18.1    | 108.1            | 65.5                        |
| April           | 27.5             | 14.2    | 20.85   | 238.6            | 64.5                        |
| May             | 28.5             | 17.2    | 22.85   | 488.2            | 73.3                        |
| June            | 29.7             | 21.3    | 25.5    | 936.8            | 83.3                        |
| 2021            |                  |         |         |                  |                             |
| March           | 27.19            | 15.62   | 21.4    | 58.1             | 77.77                       |
| April           | 32.01            | 17.54   | 24.77   | 71.9             | 56.15                       |
| May             | 29.96            | 21.02   | 25.49   | 283.4            | 73                          |
| June            | 29.15            | 22.56   | 25.85   | 965.21           | 87.76                       |

Source: Department of hydrology and meteorology, meteorological forecasting division, Pokhara, Kaski, Nepal

### 2.2 Growing Media

#### 2.2.1 Soil

Soil is the universal media for the seedling growth and widely used for the most of the vegetable crops to raise their seedlings. We used top soil of this farm for the preparation of growing media. Soil is mixed with either FYM or vermicompost which increase its nutrient content and improve the soil condition for germination and seedling establishment.

#### 2.2.2 Vermiculite

Vermiculite used in this study is micaceous mineral insoluble in water which absorbs large quantities of water and helps in aeration. It is normally mixed with other growth media because it contains magnesium and potassium minerals.

#### 2.2.3 Perlite

It is gray colored, light sterile volcanic material containing no nutrients. Despite holding water, it is used for the improving of drainage and percent of aeration.

#### 2.2.4 Cocopeat

It is by product of coconut processing for the fiber production. It has very low of nutrient content. It has excellent aeration due to its fine structure and it holds more moisture than other media.

### 2.2.5 Farmyard manure (FYM)

It is the organic manure comes from the excreta of livestock raised in farm.

### 2.2.6 Vermicompost

Vermicompost is the organic manure comes from the digestion of organic matter by earthworms and aerobic decomposition.

### 2.3 Growing pot

Plastic seedling trays (also known as plug trays or pro-trays) having 105 cavities (plugs) were used for the experiment and the type of cavities were inverted pyramid shaped and having the size of cup was 3x3x4 cm<sup>3</sup>.

### 2.4 Experimental design

Nine different compositions of growing media were used for the Tomato and Capsicum seedling production. To compare the efficiency of different growing media for the seedling production; we made nine different growing media on volume basis and filled in pro-trays. Individual tray having one type of growing media is called experimental unit and they were put under RCBD design having three replications of each treatment. Trays were placed down under rain shelter for the seedling growth and application of water was performed by the help of watering can.

**Table 2.** Growing media treatment details used for the experiment

| S.N. | Symbol         | Composition on volume basis | Treatment                  |
|------|----------------|-----------------------------|----------------------------|
| T1   | Cocopeat       | 100%                        | Cocopeat                   |
| T2   | F+C+P          | 1:1:1                       | FYM+Cocopeat + Perlite     |
| T3   | F+C+V          | 1:1:1                       | FYM+Cocopeat + Vermiculite |
| T4   | F+P            | 1:1                         | FYM+Perlite                |
| T5   | F+S            | 1:1                         | FYM+Soil(Control)          |
| T6   | F+V            | 1:1                         | FYM+Vermiculite            |
| T7   | Vermicompost+P | 1:1                         | Vermicompost+Perlite       |
| T8   | Vermicompost+S | 1:1                         | Vermicompost+Soil          |
| T9   | Vermicompost+V | 1:1                         | Vermicompost+Vermiculite   |

### 2.5 Plant materials

The used plant materials were Srijana hybrid variety of Tomato and California Wonder variety (OP) of Capsicum. The Srijana seed was own production of this farm and its germination test report was 100% while Sweet pepper seed was purchased from the seed market of Pokhara so the germination test of sweet pepper in lab was not performed.

### 2.6 Data collection

In the first year, the date of seed sowing was on March 2, 2020 and in the second year, the date of seed sowing was on April 29, 2021. The number of seed sowing per tray was 105. From each plastic tray which represents experiment units of growing media, we used five sample plants from the each tray for the evaluation of plant parameters. In tomato; the emergence data were recorded in the tenth day of sowing while in case of sweet pepper the data recording on emergence were done at 15 days after sowing (DAS). The data on growth parameters were observed at 30 DAS for tomato and 40 DAS for Capsicum. During this experiment no extra fertilizer were applied after seed germination for the growth and development so the growth of seedlings became little bit slower for transplanting in terms of height.

The data on plant height, stem diameter, number of leaves and length of root were measured from each treatment. The plant height was measured from the plant stem crown to the growing point of the plant. A measuring scale was used to measure the height. The stem diameter was measured by Vernier caliper at the base of seedling just 2 cm above the soil surface. The number of leaves were counted from the sample plant and the finally the length of root was measured by scale.

### 2.7 Data Analysis

The observed parameters were recorded and maintained on spread sheet. Analysis of variance was performed using R (version 3.6.3) and R Studio1.3.1093. Means were compared by Duncan's Multiple Range Test (DMRT) at 5% level of significance.

## 3. RESULTS AND DISCUSSION

### 3.1. Phenotypic condition of seedling during uprooting

Seedlings vigor and root plug formation were recorded by visual observation from sample seedlings. The growth vigor and root plug formation were presented in Table 3.

**Table 3.** The phenotypic growth of seedlings and the root plug condition of Tomato (30 DAS) and Capsicum (40 DAS) at HRS, Malepatan, Kaski in 2020 and 2021.

| Treatment                 | Seedling vigor                            | Root plug formation                                      |
|---------------------------|-------------------------------------------|----------------------------------------------------------|
| Cocopeat                  | Stunted growth and yellowing of seedlings | Good plug                                                |
| FYM+Cocopeat +Perlite     | Slightly yellow was started in seedling   | Good plug                                                |
| FYM+Cocopeat +Vermiculite | Healthy growth                            | Poor plug                                                |
| FYM+Perlite               | Healthy growth                            | Good plug                                                |
| FYM+ Soil                 | Healthy growth                            | Good plug                                                |
| FYM+Vermiculite           | Healthy growth                            | Plug formation was very poor                             |
| Vermicompost+Perlite      | Healthy growth                            | Plugging poor but quite better than use of vermiculite   |
| Vermicompost+Soil         | Healthy growth                            | Plugging was not good but better than use of vermiculite |
| Vermicompost+ Vermiculite | Healthy growth                            | Poor plug                                                |

### 3.2. Effect of growing media on the tomato seedling

There was significant ( $P<0.001$ ) difference in number of seedling emergence at 10 DAS among the tested growing media (Table 4). The highest number of seedling emergence (104.33) was found for the growing media Cocopeat only followed by Vermicompost+Soil (101.66), Vermicompost+Perlite (99), Vermicompost+Vermiculite (89.66), FYM+Cocopeat + Perlite (89.33). The lowest number of seedling established were occurred in FYM+Perlite (76.66) which was at par with FYM+Soil (78.83) and FYM+Vermiculite (79.16). Similarly there was significant ( $P<0.001$ ) difference in number of seedling established at 30 DAS among the tested growing media (Table 3). The highest number of seedling (103.5) was found for the growing media Cocopeat followed by Vermicompost+ Soil (99.16), Vermicompost+ Perlite (95.83), FYM+Cocopeat+Perlite (86.66), Vermicompost+Vermiculite (85.66). The lowest number of seedling established were occurred in FYM+ Perlite (72.5) which was at par with FYM+Vermiculite (74.16) and FYM+Soil (75.33).

**Table 4.** Effect of growing media on the no. of seedlings established and no. of leaves per seedling of tomato at HRS, Malepatan in 2020 and 2021.

| Treatment           | No. of seedling emergence (10 DAS) |                      |                     | No. of seedlings (30 DAS) |                  |                     | No. of leaves per seedling (30 DAS) |                     |                     |
|---------------------|------------------------------------|----------------------|---------------------|---------------------------|------------------|---------------------|-------------------------------------|---------------------|---------------------|
|                     | 2020                               | 2021                 | Mean                | 2020                      | 2021             | Mean                | 2020                                | 2021                | Mean                |
| Cocopeat            | 105.00 <sup>a</sup>                | 103.66 <sup>a</sup>  | 104.33 <sup>a</sup> | 105.00 <sup>a</sup>       | 102 <sup>a</sup> | 103.50 <sup>a</sup> | 4.00 <sup>c</sup>                   | 5.46 <sup>abc</sup> | 4.73 <sup>bcd</sup> |
| F+C+P               | 102.66 <sup>b</sup>                | 76.00 <sup>c</sup>   | 89.33 <sup>d</sup>  | 101.33 <sup>b</sup>       | 72 <sup>c</sup>  | 86.66 <sup>d</sup>  | 4.00 <sup>c</sup>                   | 5.06 <sup>abc</sup> | 4.53 <sup>cd</sup>  |
| F+C+V               | 104.33 <sup>ab</sup>               | 61.66 <sup>d</sup>   | 83.00 <sup>c</sup>  | 103.66 <sup>a</sup>       | 55 <sup>d</sup>  | 79.33 <sup>c</sup>  | 4.46 <sup>bc</sup>                  | 3.33 <sup>c</sup>   | 3.9 <sup>d</sup>    |
| F+P                 | 90.00 <sup>c</sup>                 | 63.33 <sup>d</sup>   | 76.66 <sup>g</sup>  | 86.00 <sup>c</sup>        | 59 <sup>d</sup>  | 72.50 <sup>g</sup>  | 5.06 <sup>ab</sup>                  | 6.86 <sup>a</sup>   | 5.96 <sup>a</sup>   |
| F+S                 | 96.00 <sup>d</sup>                 | 61.66 <sup>d</sup>   | 78.83 <sup>fg</sup> | 92.66 <sup>d</sup>        | 58 <sup>d</sup>  | 75.33 <sup>f</sup>  | 5.00 <sup>ab</sup>                  | 5.26 <sup>abc</sup> | 5.13 <sup>abc</sup> |
| F+V                 | 78.33 <sup>g</sup>                 | 80.00 <sup>c</sup>   | 79.16 <sup>f</sup>  | 73.33 <sup>g</sup>        | 75 <sup>c</sup>  | 74.16 <sup>fg</sup> | 4.46 <sup>bc</sup>                  | 4.00 <sup>bc</sup>  | 4.23 <sup>cd</sup>  |
| Vermicompost+P      | 100.00 <sup>c</sup>                | 98.00 <sup>b</sup>   | 99.00 <sup>c</sup>  | 96.66 <sup>c</sup>        | 95 <sup>b</sup>  | 95.83 <sup>c</sup>  | 5.40 <sup>a</sup>                   | 6.06 <sup>ab</sup>  | 5.73 <sup>ab</sup>  |
| Vermicompost+S      | 103.33 <sup>ab</sup>               | 100.00 <sup>ab</sup> | 101.66 <sup>b</sup> | 101.33 <sup>b</sup>       | 97 <sup>b</sup>  | 99.16 <sup>b</sup>  | 4.93 <sup>ab</sup>                  | 4.13 <sup>bc</sup>  | 4.53 <sup>cd</sup>  |
| Vermicompost+V      | 80.66 <sup>f</sup>                 | 98.66 <sup>b</sup>   | 89.66 <sup>d</sup>  | 76.33 <sup>f</sup>        | 95 <sup>b</sup>  | 85.66 <sup>d</sup>  | 4.80 <sup>ab</sup>                  | 4.00 <sup>bc</sup>  | 4.4 <sup>cd</sup>   |
| Grand Mean          | 95.59                              | 82.55                | 89.07               | 92.92                     | 78.66            | 85.79               | 4.68                                | 4.91                | 4.79                |
| SEM $\pm$           | 0.93                               | 6.16                 | 1.64                | 1.07                      | 7.11             | 1.34                | 0.10                                | 1.43                | 0.37                |
| LSD <sub>0.05</sub> | 1.67***                            | 4.29***              | 2.22***             | 1.79***                   | 4.61***          | 2.01***             | 0.56***                             | 2.07*               | 1.06*               |
| CV%                 | 1.01                               | 3.00                 | 1.44                | 1.11                      | 3.38             | 1.35                | 7.00                                | 24.38               | 12.77               |

\* $P<0.05$ , \*\* $P<0.01$  and \*\*\* $P<0.001$ , CV; Coefficient of variation, LSD; Least significant difference,  $\pm$ ; standard error of mean (SEM), means within the column followed by the same letter are not significantly different at a 5% level of significance by DMRT.

There were significant ( $P<0.01$ ) difference for the number of leaves per seedling among the tested growing media (Table 4). The highest number of leaves per seedling (5.96) was recorded for the FYM+Perlite which was at par with Vermicompost+Perlite (5.73) and FYM+Soil (5.13) followed by Cocopeat (4.73) and FYM+Cocopeat+Perlite (4.53). The lowest number of leaves (3.9) recorded for FYM+Cocopeat+Vermiculite.

There was significant ( $P<0.001$ ) difference in seedling height at 30 DAS among the tested growing media (Table 5). The tallest seedling height (19.92 cm) was found in the media Vermicompost+Perlite followed by FYM+Perlite (15.78 cm) and Vermicompost+Soil

(12.86 cm). The shortest seedling height occurred in Cocopeat (8.93 cm) which was at par with the growing media FYM+Cocopeat+ Vermiculite (8.97 cm) and FYM+Vermiculite (10.45 cm) and Vermicompost+ Vermiculite (11.15 cm).

The stem diameter of the seedling at 30 DAS were significant ( $P<0.01$ ) among the tested growing media (Table 5). The highest stem diameter was recorded for Vermicompost+Perlite (2.93 mm) followed by FYM+Perlite (2.33 mm), Vermicompost+Soil (2.24), FYM+ Cocopeat +Vermiculite (2.13 mm), FYM+ Cocopeat + Perlite (2.06) and the smallest stem diameter was occurred in Cocopeat (1.91 mm).

**Table 5.** Effect of growing media on the seedling's height, stem diameter and length of roots at 30 DAS of tomato at HRS, Malepattan in 2020 and 2021.

| Treatment           | Plant height(cm)      |                     |                      | Stem diameter(mm)  |                     |                   | Root length(cm) |                     |                      |
|---------------------|-----------------------|---------------------|----------------------|--------------------|---------------------|-------------------|-----------------|---------------------|----------------------|
|                     | 2020                  | 2021                | Mean                 | 2020               | 2021                | Mean              | 2020            | 2021                | Mean                 |
| Cocopeat            | 6.83 <sup>c</sup>     | 11.02 <sup>de</sup> | 8.93 <sup>c</sup>    | 1.96 <sup>c</sup>  | 1.86 <sup>bcd</sup> | 1.91 <sup>b</sup> | 7.1             | 10.98 <sup>ab</sup> | 9.04 <sup>abc</sup>  |
| F+C+P               | 7.83 <sup>de</sup>    | 16.54 <sup>c</sup>  | 12.18 <sup>cd</sup>  | 1.93 <sup>c</sup>  | 2.19 <sup>b</sup>   | 2.06 <sup>b</sup> | 9.5             | 7.83 <sup>bcd</sup> | 8.66 <sup>abcd</sup> |
| F+C+V               | 8.83 <sup>bcd</sup>   | 9.11 <sup>e</sup>   | 8.97 <sup>c</sup>    | 2.93 <sup>ab</sup> | 1.33 <sup>d</sup>   | 2.13 <sup>b</sup> | 9.76            | 3.66 <sup>d</sup>   | 6.71 <sup>cd</sup>   |
| F+P                 | 11.00 <sup>ab</sup>   | 20.56 <sup>b</sup>  | 15.78 <sup>b</sup>   | 2.53 <sup>cd</sup> | 2.14 <sup>bc</sup>  | 2.33 <sup>b</sup> | 10.39           | 9.86 <sup>abc</sup> | 10.12 <sup>ab</sup>  |
| F+S                 | 10.00 <sup>abcd</sup> | 15.07 <sup>c</sup>  | 12.53 <sup>cd</sup>  | 2.15 <sup>c</sup>  | 1.89 <sup>bcd</sup> | 2.02 <sup>b</sup> | 10.04           | 11.13 <sup>ab</sup> | 10.58 <sup>ab</sup>  |
| F+V                 | 6.86 <sup>c</sup>     | 14.04 <sup>cd</sup> | 10.45 <sup>de</sup>  | 2.03 <sup>c</sup>  | 2.07 <sup>bcd</sup> | 2.05 <sup>b</sup> | 7.71            | 9.10 <sup>bc</sup>  | 8.4b <sup>cd</sup>   |
| Vermicompost+P      | 11.83 <sup>a</sup>    | 28.02 <sup>a</sup>  | 19.92 <sup>a</sup>   | 2.63 <sup>bc</sup> | 3.22 <sup>a</sup>   | 2.93 <sup>a</sup> | 7.41            | 14.73 <sup>a</sup>  | 11.07 <sup>a</sup>   |
| Vermicompost+S      | 10.33 <sup>abc</sup>  | 15.39 <sup>c</sup>  | 12.86 <sup>c</sup>   | 3.13 <sup>a</sup>  | 1.36 <sup>cd</sup>  | 2.24 <sup>b</sup> | 10.04           | 10.97 <sup>ab</sup> | 10.50 <sup>ab</sup>  |
| Vermicompost+V      | 8.16 <sup>cde</sup>   | 14.14 <sup>cd</sup> | 11.15 <sup>cde</sup> | 2.23 <sup>de</sup> | 1.76 <sup>bcd</sup> | 1.99 <sup>b</sup> | 8.08            | 4.81 <sup>cd</sup>  | 6.45 <sup>d</sup>    |
| Grand Mean          | 9.07                  | 15.99               | 12.53                | 2.39               | 1.98                | 2.18              | 8.89            | 9.23                | 9.06                 |
| SEM±                | 1.48                  | 3.94                | 1.58                 | 0.03               | 0.17                | 0.05              | 4.64            | 7.71                | 1.81                 |
| LSD <sub>0.05</sub> | 2.10***               | 3.44***             | 2.17***              | 0.31***            | 0.71**              | 0.39**            | ns              | 4.80**              | 2.33**               |
| CV%                 | 13.40                 | 12.42               | 10.04                | 7.50               | 20.80               | 10.54             | 24.22           | 30.08               | 14.86                |

ns; non-significant, \* $P<0.05$ , \*\* $P<0.01$  and \*\*\* $P<0.001$ , CV; Coefficient of variation, LSD; Least significant difference, ±; standard error of mean (SEM), means within the column followed by the same letter are not significantly different at a 5% level of significance by DMRT.

There were found significant ( $P<0.01$ ) difference among the tested growing media for the root length (Table 5). The highest length of root was recorded for the growing media Vermicompost+Perlite (11.07 cm) which was at par with FYM+Soil (10.58 cm), Vermicompost+Soil (10.5 cm) and the shortest length of root was found for the growing media Vermicompost+Vermiculite (6.45cm) which was similar with FYM+ Cocopeat +Vermiculite (6.71 cm) and FYM+ Cocopeat +Perlite (8.66cm).

### 3.3. Effect of growing media on the sweet pepper seedling

There was significant ( $P<0.001$ ) difference in number of seedling emergence at 15 DAS among the tested growing media (Table 6). The highest number of seedling

emergence (86.16) was found for the growing media Cocopeat only followed by Vermicompost + Perlite (82.66), Vermicompost+Vermiculite (81.5), FYM+Perlite (77.83), FYM+ Cocopeat + Perlite (76.33). The lowest numbers of seedling established were occurred in Vermicompost+Soil (65.5). Likewise, there was significant ( $P<0.001$ ) difference in number of seedling established at 40 DAS among the tested growing media (Table 5). The highest number of seedling (83.5) was found for the growing media Cocopeat only followed by Vermicompost + Perlite (78), Vermicompost+Vermiculite (77), FYM+Cocopeat+ Perlite (73), FYM+Perlite (72.5). The lowest number of seedling established were occurred in Vermicompost+Soil (62) which was at par with FYM+ Vermiculite (63) and FYM+Soil (65.5).

**Table 6.** Effect of growing media on the no. of seedlings established and no. of leaves per seedling of sweet pepper at HRS, Malepattan in 2020 and 2021.

| Treatment           | No. of seedling emergence (15 DAS) |                     |                     | No. of seedlings (40 DAS) |                  |                    | No. of leaves per seedling (40 DAS) |                    |                    |
|---------------------|------------------------------------|---------------------|---------------------|---------------------------|------------------|--------------------|-------------------------------------|--------------------|--------------------|
|                     | 2020                               | 2021                | Mean                | 2020                      | 2021             | Mean               | 2020                                | 2021               | Mean               |
| Cocopeat            | 89.66 <sup>a</sup>                 | 82.66 <sup>b</sup>  | 86.16 <sup>a</sup>  | 87 <sup>a</sup>           | 80 <sup>ab</sup> | 83.5 <sup>a</sup>  | 4.93                                | 3.53 <sup>c</sup>  | 4.23 <sup>d</sup>  |
| F+C+P               | 86.00 <sup>a</sup>                 | 66.66 <sup>d</sup>  | 76.33 <sup>cd</sup> | 83 <sup>b</sup>           | 63 <sup>d</sup>  | 73.0 <sup>c</sup>  | 5.93                                | 3.80 <sup>de</sup> | 4.86 <sup>cd</sup> |
| F+C+V               | 87.66 <sup>a</sup>                 | 61.33 <sup>e</sup>  | 74.50 <sup>d</sup>  | 84 <sup>ab</sup>          | 57 <sup>e</sup>  | 70.5 <sup>c</sup>  | 5.86                                | 5.20 <sup>bc</sup> | 5.53 <sup>bc</sup> |
| F+P                 | 71.66 <sup>d</sup>                 | 84.00 <sup>ab</sup> | 77.83 <sup>c</sup>  | 66 <sup>d</sup>           | 79 <sup>b</sup>  | 72.5 <sup>c</sup>  | 5.93                                | 6.40 <sup>ab</sup> | 6.16 <sup>ab</sup> |
| F+S                 | 64.33 <sup>e</sup>                 | 75.00 <sup>c</sup>  | 69.66 <sup>e</sup>  | 60 <sup>e</sup>           | 71 <sup>c</sup>  | 65.5 <sup>d</sup>  | 5.13                                | 5.53 <sup>bc</sup> | 5.33 <sup>bc</sup> |
| F+V                 | 79.66 <sup>b</sup>                 | 58.33 <sup>e</sup>  | 69.00 <sup>e</sup>  | 74 <sup>c</sup>           | 52 <sup>f</sup>  | 63.0 <sup>de</sup> | 6.13                                | 7.33 <sup>a</sup>  | 6.73 <sup>a</sup>  |
| Vermicompost+P      | 87.00 <sup>a</sup>                 | 78.33 <sup>c</sup>  | 82.66 <sup>b</sup>  | 83 <sup>b</sup>           | 73 <sup>c</sup>  | 78.0 <sup>b</sup>  | 5.53                                | 5.53 <sup>bc</sup> | 5.53 <sup>bc</sup> |
| Vermicompost+S      | 61.00 <sup>e</sup>                 | 70.00 <sup>d</sup>  | 65.50 <sup>f</sup>  | 58 <sup>e</sup>           | 66 <sup>d</sup>  | 62.0 <sup>e</sup>  | 6                                   | 5.60 <sup>bc</sup> | 5.80 <sup>bc</sup> |
| Vermicompost+V      | 75.33 <sup>c</sup>                 | 87.66 <sup>a</sup>  | 81.50 <sup>b</sup>  | 71 <sup>c</sup>           | 83 <sup>a</sup>  | 77.0 <sup>b</sup>  | 6                                   | 4.93 <sup>cd</sup> | 5.46 <sup>bc</sup> |
| Grand Mean          | 78.03                              | 73.77               | 75.90               | 74                        | 69.33            | 71.66              | 5.71                                | 5.31               | 5.51               |
| SEM±                | 4.09                               | 4.83                | 3.13                | 4.44                      | 4.19             | 3.42               | 0.30                                | 0.51               | 0.24               |
| LSD <sub>0.05</sub> | 3.50***                            | 3.80***             | 3.06***             | 3.64***                   | 3.54***          | 3.20***            | ns                                  | 1.23***            | 0.86**             |
| CV%                 | 2.59                               | 2.97                | 2.32                | 2.84                      | 2.95             | 2.58               | 9.62                                | 13.46              | 9.02               |

ns; non-significant, \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001, CV; Coefficient of variation, LSD; Least significant difference, ±; standard error of mean, means within the column followed by the same letter are not significantly different at a 5% level of significance by DMRT.

There were significant (P<0.01) difference for the number of leaves per seedling among the tested growing media (Table 6). The highest number of leaves per seedling (6.73) was recorded for the FYM+Vermiculite which was at par with FYM+ Perlite (6.16) followed by Vermicompost+Soil (5.8), Vermicompost+Perlite (5.53) and Vermicompost + Vermiculite (5.46). The lowest number of leaves (4.23) recorded for Cocopeat which was at par with FYM+Cocopeat+Perlite (4.86).

There was significant (P<0.001) difference in seedling height at 40 DAS among the tested growing media (Table 7). The tallest seedling height (13.89 cm) was found in the media Vermicompost+Vermiculite which was at par with FYM+ Vermiculite (12.75 cm) followed by Vermicompost+Soil (11.74 cm) and FYM+Perlite (10.74 cm). The shortest seedling height occurred in Cocopeat (5.43 cm).

**Table 7.** Effect of growing media on the seedling's height, stem diameter and length of roots at 40 DAS of sweet pepper at HRS, Malepattan in 2020 and 2021.

| Treatment           | Plant height(cm)    |                     |                     | Stem diameter(mm)  |                    |                    | Root length(cm)    |                    |                     |
|---------------------|---------------------|---------------------|---------------------|--------------------|--------------------|--------------------|--------------------|--------------------|---------------------|
|                     | 2020                | 2021                | Mean                | 2020               | 2021               | Mean               | 2020               | 2021               | Mean                |
| Cocopeat            | 6.50 <sup>c</sup>   | 4.36 <sup>c</sup>   | 5.43 <sup>f</sup>   | 1.56 <sup>d</sup>  | 0.51 <sup>d</sup>  | 1.04 <sup>c</sup>  | 6.13 <sup>c</sup>  | 5.88 <sup>b</sup>  | 6.01 <sup>cd</sup>  |
| F+C+P               | 8.90 <sup>abc</sup> | 5.56 <sup>de</sup>  | 7.23 <sup>c</sup>   | 1.83 <sup>cd</sup> | 0.57 <sup>d</sup>  | 1.20 <sup>c</sup>  | 6.80 <sup>bc</sup> | 3.93 <sup>b</sup>  | 5.36 <sup>d</sup>   |
| F+C+V               | 8.86 <sup>abc</sup> | 7.32 <sup>d</sup>   | 8.09 <sup>c</sup>   | 2.53 <sup>ab</sup> | 0.89 <sup>c</sup>  | 1.71 <sup>b</sup>  | 7.46 <sup>bc</sup> | 6.18 <sup>ab</sup> | 6.82 <sup>bcd</sup> |
| F+P                 | 10.83 <sup>a</sup>  | 10.65 <sup>c</sup>  | 10.74 <sup>cd</sup> | 2.06 <sup>bc</sup> | 1.23 <sup>b</sup>  | 1.65 <sup>b</sup>  | 7.13 <sup>bc</sup> | 6.06 <sup>ab</sup> | 6.60 <sup>bcd</sup> |
| F+S                 | 7.10 <sup>bc</sup>  | 10.81 <sup>c</sup>  | 8.95 <sup>de</sup>  | 2.20 <sup>bc</sup> | 1.25 <sup>b</sup>  | 1.72 <sup>b</sup>  | 7.96 <sup>b</sup>  | 4.70 <sup>b</sup>  | 6.33 <sup>bcd</sup> |
| F+V                 | 11.66 <sup>a</sup>  | 13.84 <sup>b</sup>  | 12.75 <sup>ab</sup> | 2.73 <sup>a</sup>  | 1.38 <sup>ab</sup> | 2.05 <sup>a</sup>  | 9.43 <sup>a</sup>  | 5.83 <sup>b</sup>  | 7.63 <sup>bc</sup>  |
| Vermicompost+P      | 7.66 <sup>bc</sup>  | 12.19 <sup>bc</sup> | 9.93 <sup>d</sup>   | 2.20 <sup>bc</sup> | 1.64 <sup>a</sup>  | 1.92 <sup>ab</sup> | 6.66 <sup>bc</sup> | 5.86 <sup>b</sup>  | 6.26 <sup>bcd</sup> |
| Vermicompost+S      | 10.70 <sup>a</sup>  | 12.79 <sup>b</sup>  | 11.74 <sup>bc</sup> | 2.70 <sup>a</sup>  | 1.62 <sup>a</sup>  | 2.16 <sup>a</sup>  | 6.56 <sup>bc</sup> | 9.26 <sup>a</sup>  | 7.91 <sup>ab</sup>  |
| Vermicompost+V      | 9.70 <sup>ab</sup>  | 18.08 <sup>a</sup>  | 13.89 <sup>a</sup>  | 2.73 <sup>a</sup>  | 1.63 <sup>a</sup>  | 2.18 <sup>a</sup>  | 9.56 <sup>a</sup>  | 9.30 <sup>a</sup>  | 9.43 <sup>a</sup>   |
| Grand Mean          | 9.10                | 10.62               | 9.86                | 2.28               | 1.19               | 1.73               | 7.52               | 6.33               | 6.93                |
| SEM±                | 2.14                | 1.18                | 0.98                | 0.07               | 0.029              | 0.02               | 0.57               | 3.11               | 0.90                |
| LSD <sub>0.05</sub> | 2.53**              | 1.88***             | 1.71***             | 0.46***            | 0.29***            | 0.27***            | 1.31***            | 3.05*              | 1.64**              |
| CV%                 | 16.09               | 10.24               | 10.03               | 11.85              | 14.33              | 9.05               | 10.06              | 27.85              | 13.71               |

, \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001, CV; Coefficient of variation, LSD; Least significant difference, ±; standard error of mean, means within the column followed by the same letter are not significantly different at a 5% level of significance by DMRT.

The stem diameter of the seedling at 40 DAS were significant ( $P<0.001$ ) among the tested growing media (Table 7). The highest stem diameter recorded for Vermicompost+Vermiculite (2.18 mm) which was at par with Vermicompost+Soil (2.16 mm), FYM+Vermiculite (2.05 mm) and Vermicompost+Perlite (1.92 mm) followed by FYM+Soil (1.72 mm) and FYM+Cocopeat+Vermiculite (1.71). The smallest stem diameter was occurred in Cocopeat (1.04 mm) which was at par with FYM+Cocopeat+Perlite (1.2 mm).

There were found significant ( $P<0.01$ ) difference among the tested growing media for the root length (Table 6). The highest length of root was recorded for the growing media Vermicompost+Vermiculite (9.43 cm) which was at par with Vermicompost+Soil (7.91 cm) followed by FYM+Vermiculite (7.63), FYM+Cocopeat+Vermiculite (6.82 cm) and the shortest length of root was found for the growing media FYM+Cocopeat+Perlite (5.36 cm) which was at par with Cocopeat (6.01 cm), Vermicompost+Perlite (6.26 cm), FYM+Soil (6.33 cm).

### 3.4 Discussions

The number of germination and establishment of seedling during transplanting stage was found significantly higher in the cocopeat growing media than other growing media. Use of Cocopeat as growing media have higher water holding capacity and moisture supply as well as sufficient porosity which helps better seedling emergence. Similarly, using growing media Cocopeat (50): Vermicompost (50) has higher seedling emergence, shoot height and vigor of seedlings were also reported by Hazarika *et al.*, (2022); Zaller (2007); Hota and Arulmozhiselvan (2017). The difference in the number of seedling between two species might be the difference in the genetic capacity to the seedling emergence. Baiyeri and Mbah, (2006) reported that there is difference in growing media for seed germination and growth in a nursery. Growing media is a reservoir of moisture and plant nutrients (Grower, 1987). Jeevitha *et al.*, (2019) reported that among the different growing media 75% Vermicompost+25% FYM media was found significantly superior for seedling production of tomato in terms of seedling height, seedling girth, leaf area, shoot length and root length. He also found higher germination for media containing 75% vermicompost and 25% Cocopeat.

Many types of growing media or substrates such as rockwool, perlite, vermiculite and peat have been used to grow many kinds of crops (Yilmaz *et al.*, 2014). Higher

seedling height, number of leaves, stem diameter and root length were recorded in vermicompost and FYM during the seedling growing stage than cocopeat and use of soil. Our results are alike of Abadet *et al.*, (2002) which reported that a good growing medium provides sufficient support to the plant and provides sufficient nutrients and water; allow oxygen as well as gaseous exchange between the roots and atmosphere. More Seedling height observed in the use of vermicompost might be due to conducive environment as well as sufficient amount of nutrients presence in such type of media. Similar result was obtained by Hazarika *et al.* (2022) and Nissi (2018) in tomato.

The advantage of using Cocopeat has good physical properties, high total pore space, high water content, low shrinkage, low bulk density and slow biodegradation (Prasad, 1997). By using vermicompost, plant parameters like seed germination and growth, seedling girths were found significantly different with other treatments. Atiyeh *et al.*, (2001) stated that vermicompost has humic acids, plant growth promoters, N-fixing and P-solubilizing bacteria, enzymes and vitamins, which helps for increased growth, higher yield and better-quality plant products.

Vermiculite enhanced root and shoot growth and highest number of leaves in tomato and capsicum seedling growth. The result is supported by (Sihlongonyane *et al.*, 2018; Parameshwarareddy *et al.*, 2017; Wahome *et al.*, (2011). Meena *et al.*, (2017) also stated that Vermiculite and cocopeat provides adequate nutrients and enhances both the physical and biological properties and the water holding capacity of soil. Sihlongonyane *et al.*, (2018) also reported that among the four growing media sand, sawdust, vermiculite and soil, the shortest stem length and lower number of leaves of tomato were recorded for soil media.

Mathowa *et al.*, (2017) in sweet pepper also observed the poor performance of seedling in cocopeat only. Observation of higher stem diameter of Tomato and Capsicum by use of vermicompost with other substrate was found in similar finding of Sahni *et al.*, (2008). Joshi *et al.*, (2010) recorded the lowest stem diameter in tomato plants grown in soil.

### 4. CONCLUSION

In vegetative production, seedling stage is an important stage that has influences on growth and development, early yield, total yield and fruit per plant. The germination and seedling establishment was higher in

cocopeat growing media but other growth parameters are poor for the long period nursery until external fertilizer application was applied during growth. But use of vermicompost has great commercial potential as media for growing vegetable seedlings. Vermicompost helps in germination as well as for the plant growth for entire period in nursery without fertilizer addition. Application of perlite and vermiculite for growing media reduced the root plug during the transplanting in the main field for plastic trays. Therefore, in terms of germination and seedling health use of vermicompost or FYM in the mixed form with cocopeat or the soil

as a growing media for plastic seedling trays would be sustainable technology for the farmers for commercial seedling production of vegetables rather than use of single growing media cocopeat and vermicompost.

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# EVALUATION OF DIFFERENT WEED MANAGEMENT PRACTICES ON THE YIELD OF SPRING MAIZE IN GAURADAH, JHAPA

Rumita Limbu Sanwa<sup>1,3,\*</sup>, Karishma Khanal<sup>1,3</sup>, Shwastika Baral<sup>1,3</sup> and Goma Dhital<sup>2,3</sup>

<sup>1</sup> Gauradaha Agriculture Campus, Gauradaha, Jhapa

<sup>2</sup> Lamjung Campus, Sundar bazar, Lamjung

<sup>3</sup> Institute of Agriculture and Animal Science, Tribhuvan University, Nepal

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\*Correspondence:

rumitalimbu2@gmail.com

Tel: +977-9867991795

## ABSTRACT

Maize is more likely to have weed infestations due to its steady early growth rate and extensive row spacing, which favor weed development even before crop emergence. As a result, there prevails strong correlation between weed density and maize yield. An experiment was conducted at the Gauradaha Agriculture Campus, Jhapa, in the spring of 2022 to assess the effect of various weed control methods on maize yield. The experiment had three replications and eight treatments (control, cover crops, hand weeding at intervals of 15 days, botanical weedicides, inorganic weedicides, black plastic mulch, straw mulch, and small inter-row spacing). The number of cobs per hectare, sterility%, and shelling% were not significantly affected by various treatments. The use of black plastic mulching for weed management achieved significantly higher numbers of grain/cob (427.83), row/cob (13.40), weight of grain/cob (153.33gm), test weight, and yield (8.46t/ha). Similarly, the lowest test weight was recorded in T8 (111), i.e., the small inter-row spacing plot. While the lowest yield was observed in T4, i.e., botanical weedicides (3.20). This study found that plastic mulching had a positive impact on the majority of yield and yield-attributing indicators in spring maize, which could be helpful in weed-control strategies.

## 1. INTRODUCTION

Maize (*Zea mays*) belongs to the tribe Maydeae of the Poaceae family. Due to its greater tolerance to a variety of environmental conditions and higher productivity, maize is sometimes known as the “queen of grains” (Begam *et al.*, 2018). With the versatility to be used as food, feed, and fodder, maize is Nepal’s second-most significant cereal crop after rice (Karki *et al.*, 2015). The grains of the maize crop are very nutritious and include considerable levels of carbohydrates (44.60–69.60%), protein (9.87%), minerals (1.10–2.95%), fat (2.17–4.43%), and fiber (2.10–26.70%) (Enyisi *et al.*, 2014). The most adaptable crop, maize, is grown in more than 166 nations around the world, encompassing tropical, subtropical, and temperate zones (ANGRAU, 2021). In the world, it is grown on roughly 201.98 million ha, yielding 1162.35 million tons and averaging 5.75 tons/ha in productivity (FAOSTAT, 2020). It is estimated that weeds cause 37% of the global yield losses in maize (Sharma *et al.*, 2022). Asia provides 32% of the world’s total maize production and 34% of the world’s total maize-producing land (CGIAR, 2022).

Maize is a versatile crop and has remarkable adaptability under varied agro-climatic conditions (Yadav *et al.*, 2015). With a total yield of 2,997,733 MT and an average productivity of 3.05 MT per ha, maize is grown on 979,776 ha of land. About 6.83% of agricultural GDP comes from maize (MoAD, 2022). The country’s low maize productivity is a result of numerous production restrictions. Among them, weed infestation has been a serious problem limiting maize production globally. Any weed management method, whether cultural, mechanical, biological, or chemical, aims to make the environment unfavorable for weeds (Harker and O’Donovan, 2013). In Nepal, hybrid technology has been introduced to improve maize yield. Nepal, however, lacks a lot of site-specific hybrids (Kandel and Shrestha 2020). The grain production of spring maize was lowered by 37.17% due to weed infestation (Shrivastav *et al.*, 2015). Weeds are typically omnipresent, robust species with rapid growth and deep roots, and they are quite capable of competing with cultivated crops for the available resources, which on the other side actually

harms crop growth and productivity. Worldwide yield losses in maize due to weeds are estimated at around 37% (Sharma *et al.*, 2022).

An experiment at ARS Pakhribas (in Nepal's eastern mid-hill) found yield reductions of up to 70% in maize grown in weedy environments (Mishra, 2004). Since, maize has a steady early growth rate and wide row spacing in comparison to other cereals, weed encroachment is seen as a severe problem. Wide spacing between maize rows, frequent irrigation, and the intensive use of chemical fertilizers all promote yield loss by creating an atmosphere that is suitable for weed growth (Fanadzo *et al.*, 2007; Bajwa *et al.*, 2014). Herbicides are heavily utilized in maize weed control, although there are also a number of cultural and mechanical techniques. As a result, knowledge of the harmful effects of weed competition and contamination as well as their effective control strategies is equally crucial for successful maize production. Early weed interference in maize development would increase plant-to-plant variability in dry matter accumulation, lowering grain production at maturity (Cerrudo *et al.*, 2012). Between four and seven weeks after sowing, or the critical period of weed control (CPWC), maize plants are more susceptible to competition from nearby weedy plants (Shrestha *et al.*, 2019). For efficient maize production, it is also crucial to comprehend the negative effects of weed competition and contamination, as well as their appropriate control strategies. Therefore, in the present investigation, we carried out a study to evaluate the role of different weed management practices in improving the yield of maize and suppressing weed flora and select the best one for the Gauradaha, Jhapa. The objective of the study was to eliminate weed seed before germination, and develop the best technique for weed management that would increase maize to motivate farmers to continue maize farming and achieve its potential.

## 2. MATERIALS AND METHODS:

### 2.1. Experimental site

A field experiment was conducted at the Gauradaha Campus, Institute of Agriculture and Animal Science, Jhapa, during the spring season of 2022. Geographically, the area is located at 26.56° N latitude and 87.72° E longitude, with an elevation of 79 meters above sea level. It was conducted in a Randomized Complete Block Design (RCBD) with eight treatments and three replications.

### 2.2. Maize sample

The maize seed variety Arun-2 used in this experiment was provided by National Maize Research Program (NMRP), Bharatpur, Chitwan.

### 2.3. Land preparation and crop management

Land preparation was carried out three days before maize sowing using a power tiller and a spade to break up clods manually. Farm Yard Manure (FYM) was incorporated @ 6 tons per hectare during final land preparation. A series of shallow furrows were dug, dividing the entire field into flat beds of manageable length and width. The size of the individual plot was 4m×3.5m (14m<sup>2</sup>). The seed rate was 25–30kg per ha with row spacing of 70cm and plant-to-plant distance of 25cm, except for T8 (narrow inter-row spacing), where plant-to-plant distance of 20cm and row-to-row distance of 60cm were maintained. The plots were spaced apart by 0.5meters, while replications were separated by 1 meter. In rows, a single maize seed was sown 3–4cm deep and softly covered with soil. A fertilizer dose of 120kg N, 60kg P<sub>2</sub>O<sub>5</sub> and 40 kg K<sub>2</sub>O per hectare was applied. A half dose of urea, a full dose of phosphorous, and a full dose of potassium were sprayed during the last land preparation. Each plot received 182.5gm of urea, 525gm of SSP, and 93gm of MOP. A controlled irrigation system was used for the experiment. To help the seeds germinate better, irrigation was done prior to sowing. Throughout the duration of the crop, a total of five irrigations were performed. After emergence, irrigation was provided, and then irrigation was applied at the seedling, knee-height, tasseling, and silking stages. After 45 days after sowing (DAS), the side dressing of the remaining dose of urea and earthing up were done in all plots, with the exception of the ones with black plastic mulch. For plant protection measures, Emamectin benzoate was applied once at a rate of 10grams per sixteen liters during the infestation of Fall Army Worm (*Spodoptera frugiperda*). Harvesting was done manually by pulling the ears from the stalks of plants when black spots on the bottom (side attached to the cob) of the seed were seen in every plot.

## 2.4. Treatment details

**Table 1.** Treatment details

| Treatment | Treatment details                                                                                             |
|-----------|---------------------------------------------------------------------------------------------------------------|
| T1        | Control                                                                                                       |
| T2        | Cover crops (cowpea 312 variety)                                                                              |
| T3        | Hand weeding (30DAS,45DAS,-60DAS,75DAS)                                                                       |
| T4        | Botanical weedicides (5ml/15lit,-30DAS, 45DAS,60DAS, 75DAS)                                                   |
| T5        | Inorganic weedicides<br>Atrazine: 2.1gm/42m <sub>2</sub> (3DAS)<br>2-4D 58%Sl:7.26ml/42m <sub>2</sub> (20DAS) |
| T6        | Plastic mulch: black plastic of 45mm thickness and 18.4 m was used.                                           |
| T7        | Straw mulch: Dry and pest free straw was used, covering the whole plot.                                       |
| T8        | small inter row spacing<br>Plant-plant: 20cm<br>Row-row:60cm                                                  |

## 2.5. Parameters recorded

### 2.5.1. Yield and yield attributes

- Weight of individual cob with husk (gm):** Five plants were chosen at random from each net plot and their cobs were cut off. Fresh weight was obtained and the average was recorded.
- Weight of Individual cob without husk (gm):** The husk from five randomly selected cobs from each net plot were removed and weighted without husk.
- Number of grains per cob:** The number of grains per cob was calculated as given below. The total number of grains per cob = Number of grain rows per cob x Number of grains per row
- Cob length (cm):** The length of the cob was measured from five randomly selected plants each net plot from base to the tip of the cob without husk.
- Sterile length(cm):** The sterile length of cob was measured from five randomly selected plants each net plot from tip to the initiation of kernels.

- Sterility:** Sterility % was calculated by following formula.

$$\text{Sterility \%} = \frac{\text{length of unfilled portion of cob}}{\text{length of unfilled portion of cob}} \times 100\%$$

- Grain weight per plant (gm):** Five cobs from each net plot were randomly chosen, and the grains from those cobs were separated and air dried. Records were made on the grain weight.
- “1000 Grain” Weight (gm):** The weight of 1000 dried grains from five randomly selected cobs from each net plot was recorded.
- Shelling percentage:** It is the ratio of grains to ear and expressed in percentage. It was calculated as following method.

$$\text{Sterility \%} = \frac{\text{grain yield}}{\text{cob yield}} \times 100\%$$

- Grain yield (ton ha<sup>-1</sup>):** Cobs from each net plot were harvested at physiological maturity. Cobs were separated and air dried, shelled, cleaned and weighed.

$$\text{Grain yield (ton/ha) at 14\%} = \frac{\text{plot yield(kg)} * (100-\text{GMC})}{(100-14) * \text{net harvested area m}^2} \times 10000$$

(100-GMC)/ (100-14) =conversion factor for grain yield at 14 %

(10000/net harvested area (m<sup>2</sup>) =conversion factor for Actual harvested area into hectare basis

Where GMC= Grain Moisture Content

### 2.5.2. Economic analysis

- Cost of cultivation**

Based on the inputs required, such as manpower, fertilizer, compost, seed, and other research materials, the cost of cultivation was calculated. It was determined using the Jhapa district's prevailing market pricing.

- **Gross return**

Based on the current market pricing, total production (including grain and biomass yield) was converted into gross return (Rs/treatment).

- **Net return (Rs/treatment)**

It was calculated by subtracting the cost of cultivation from the gross return.

Net return = Gross return- cost of cultivation

- **B:C ratio: It was calculated by the formula**

$$B:C \text{ ratio} = \frac{\text{Gross return}}{\text{Total cost of cultivation}}$$

## 2.6. Data collection

The data on yield parameters were taken at the time of harvesting from net plot i.e., 3<sup>rd</sup> and 4<sup>th</sup> row of each plot.

## 2.7. Statistical analysis

Analysis of Variance (ANOVA) for all parameters was analyzed by using R Stat version 4.2.1 at 5% level of significance. All the analyzed data were subjected to Duncan's Multiple Range Test (DMRT) for mean comparison. R-statistics was used to calculate the mean and standard error, while the software Agricolae was used for mean separation.

## 3. RESULTS AND DISCUSSION

**Table 2.** Effect of different weed management practices on number of cobs per hectare, number of Grain, row and weight of grain per cob of spring maize in Gauradaha, Jhapa.

| Treatments | No. of Cob per ha | No. of Grain per cob        | Row per cob                | Weight of grain per cob      |
|------------|-------------------|-----------------------------|----------------------------|------------------------------|
| T1         | 15238.10 ± 1190   | 303.76 <sup>c</sup> ± 13    | 10.93 <sup>b</sup> ± 0.52  | 105.00 <sup>cd</sup> ± 4.36  |
| T2         | 13095.24 ± 2416   | 321.16 <sup>bc</sup> ± 7.17 | 11.26 <sup>b</sup> ± 0.4   | 105.66 <sup>cd</sup> ± 1.20  |
| T3         | 15476.19 ± 1448   | 337.03 <sup>bc</sup> ± 14.4 | 12.20 <sup>ab</sup> ± 0.2  | 122.20 <sup>bc</sup> ± 0.987 |
| T4         | 14524.81 ± 238    | 303.98 <sup>c</sup> ± 11.1  | 10.93 <sup>b</sup> ± 0.52  | 104.33 <sup>cd</sup> ± 8.41  |
| T5         | 15238.15 ± 1038   | 342.70 <sup>bc</sup> ± 15.7 | 12.06 <sup>ab</sup> ± 0.75 | 114.53 <sup>bcd</sup> ± 9.78 |
| T6         | 16429.57 ± 412    | 427.83 <sup>a</sup> ± 33.4  | 13.40 <sup>a</sup> ± 0.11  | 153.33 <sup>a</sup> ± 6.17   |
| T7         | 15952.38 ± 1717   | 355.58 <sup>b</sup> ± 9.33  | 12.20 <sup>ab</sup> ± 0.11 | 126.33 <sup>b</sup> ± 4.49   |
| T8         | 15476.19 ± 858    | 320.25 <sup>bc</sup> ± 16.1 | 10.12 <sup>b</sup> ± 0.57  | 98.00 <sup>d</sup> ± 6.11    |
| Grand Mean | 15178.57          | 339.28                      | 11.85                      | 116.17                       |
| C.V        | 13.42             | 7.40                        | 6.56                       | 8.52                         |
| MS Error   | 4151482           | 631                         | 0.60                       | 98                           |
| LSD        | 3568.125          | 43.98                       | 1.36                       | 17.33                        |
| F Value    | 0.734ns           | 7.5***                      | 3.39*                      | 9.74***                      |

C.V.: Coefficient of Variation, LSD: Least Significant Difference, ns: non-significant, (\*\*\*): Significant at 0.1% level of significance, (\*): Significant at 5% level of significance, Figure after ± indicate standard error, Same letter signify no significant difference between treatments.

**3.1. Number of cobs per hectare:** It was not significantly affected by different weed management practices in spring maize in Gauradaha, Jhapa but highest number of cobs per hectare was seen in T6 i.e., plastic mulch (16429.57) followed by T7 i.e., straw mulch

(15952.38), T3 i.e., i.e., hand weeding (15476.19), T8 i.e., Small inter row spacing (15476.19) and lowest number of cobs per hectare was seen in T2 i.e., cover crops (13095.24 ± 2416) is shown in Table 2.

### 3.2. Grain per cob:

The total number of grains per cob is an important yield component parameter of maize. The data on grain per cob as presented in “Table 2”, was significantly different for various weed management practices. Maximum grain per cob was recorded in T6 i.e., plastic mulch (427.83), followed by T7 i.e., straw mulch (355.58), T5 i.e., inorganic weedicide (342.70) and T3 i.e., hand

weeding (337.03). This is supported by a field experiment carried out by Hussain *et al.* (2022) affirmed that plastic mulching recorded highest grain per cob (403.67). However, the lowest grain per ear was observed in T1 i.e., control (303.76), which was also supported by (Sharma *et al.*, 2019). Number of grains per cob directly relate with the yield of the crop (M.S.I. *et al.*, 2012).

**Table 3.** Effect of different weed management practices on sterility %, shelling %, 1000 grain weight and yield of spring maize in Gauradaha, Jhapa.

| Treatments | Sterility%  | Shelling%    | 1000 grain weight         | Yield                      |
|------------|-------------|--------------|---------------------------|----------------------------|
| T1         | 24.1 ± 4.26 | 74.2 ± 2.58  | 112 <sup>bc</sup> ± 2.31  | 4.28 <sup>bc</sup> ± 0.562 |
| T2         | 20.8 ± 4.26 | 70.7 ± 3.61  | 117 <sup>bc</sup> ± 4.83  | 3.47 <sup>c</sup> ± 0.864  |
| T3         | 20 ± 1.62   | 72.5 ± 2.41  | 131 <sup>ab</sup> ± 3.84  | 5.71 <sup>b</sup> ± 0.712  |
| T4         | 24.4 ± 1.82 | 70.3 ± 2.91  | 119 <sup>bc</sup> ± 8.48  | 3.20 <sup>c</sup> ± 0.443  |
| T5         | 20.5 ± 2.77 | 73.1 ± 0.768 | 125 <sup>bc</sup> ± 6.58  | 4.62 <sup>bc</sup> ± 0.536 |
| T6         | 14.6 ± 2.64 | 78.0 ± 4.76  | 146 <sup>a</sup> ± 9.38   | 8.46 <sup>a</sup> ± 0.495  |
| T7         | 19 ± 3.64   | 70.8 ± 1.35  | 130 <sup>abc</sup> ± 0.69 | 5.65 <sup>b</sup> ± 0.085  |
| T8         | 26.4 ± 7.24 | 72.9 ± 0.511 | 111 <sup>c</sup> ± 3.64   | 5.37 <sup>b</sup> ± 0.168  |
| Grand Mean | 21.22       | 72.71        | 123.86                    | 5.09                       |
| C.V        | 30.70       | 6.65         | 8.32                      | 19.01                      |
| MS Error   | 42.49       | 23.45        | 106.3                     | 0.939                      |
| LSD        | 11.41       | 8.48         | 18.05                     | 1.69                       |
| F Value    | 0.96ns      | 0.866ns      | 3.83*                     | 8.784***                   |

C.V.: Coefficient of Variation, LSD: Least Significant Difference, ns: non-significant, (\*\*\*): Significant at 0.1% level of significance, (\*): Significant at 5% level of significance, Figure after ± indicate standard error, Same letter signify no significant difference between treatments.

### 3.3. Row per cob:

Number of grain rows per cob directly affects cob weight and ultimately grain yield of maize. Among various weed management practices, row per cob was significantly higher in T6 i.e., plastic mulch (13.40), which was at par with T3 i.e., hand weeding (12.20), T7 i.e., straw mulch (12.20), and T2 i.e., cover crops (12.06). However, the least row per comb was recorded in T4 i.e., botanical weedicide (10.93), which was also supported by (Sharma *et al.*, 2019).

### 3.4. Weight of grain per cob:

Different weed management techniques had a significant impact on grain weight per cob. Highest grain content per cob was discovered in T6 i.e., plastic mulch (153.33), followed by T7 i.e., straw mulch (126.33)

which was at par with T3 i.e., hand weeding (122.20).

### 3.5 Sterility%:

The various weed management techniques had no significant influence on the sterility %. However, the black polythene mulch treated plot (14.6) and the straw mulch treated plot (19) had the lowest sterility percent.

### 3.6. Shelling%:

The proportion of shelling did not significantly differ amongst the different weed management techniques. Nonetheless, T6 i.e., black plastic mulch treated plot (78%) had the largest percentage of shelling.

### 3.7. 1000 grain weight:

Apart from combined effect of all the other individual yield determining elements, the ultimate final grains yield of a cereal crop depends upon the 1000-grains

weight. Any variation in the 1000-grain weight will affect the grain yield. It is cleared from the data that the 1000-grain weight of maize grain was significantly influenced by different weed management practices as shown in Table 3. T6 i.e., black plastic mulch treated plot (146) recorded a significantly higher test weight which was at par with T3 i.e., hand weeding (131) and T7 i.e., straw mulch (130). However, the lowest test weight was recorded in T8 i.e., Small inter row spacing (111) small inter row spacing. Also, in a field experiment carried out by Hussain *et al.* (2022) recorded 1000-grain weight (318.33g) in plastic mulching. According to Hussein (1997), the length of weed competitions was correlated with a decline in the weight of 1,000 grains. In an experiment conducted by M.S.I. *et al.* (2012) found that the 1000-grain weight is the most significant of the several yield-related variables as it has a direct correlation with grain yield.

### 3.8. Yield:

Grain yield is the cumulative behavior of several yield-determining factors, including the number of cobs per plant, cob length, number of grains per cob, and 1000-grain weight, which demonstrated fluctuations owing to present growing conditions and various crop management practices. The yield of maize was highest in T6 i.e., plastic mulching (8.46) at par with T3 i.e., Hand weeding (5.71) and T7 i.e., Straw mulch (5.65) while lowest yield was observed in T4 i.e., Botanical weedicides (3.20). The average yield of Arun 2 variety 5.043 t/ha was recorded by (Koirala K.B., 2004) in Lumle, Nepal and 5.93 t/ha was observed by (Thapa *et al.*, 2022) in Itahari, Nepal. According to Zhang *et al.* (2007), plastic mulching significantly increased soil water content and temperature, which increased maize's photosynthetic rate primarily through enabling stomatal opening, possibly explaining why plastic mulch-treated plots produced greater yields. Zhang, F. (2022) reported significantly higher grain yield in maize along with increased nitrogen uptake efficiency and nitrogen fertilizer productivity on plastic mulch treated plot. The current findings are consistent with those of Xiukang *et al.* (2015), who claimed that plastic mulching reduces soil evaporation when water moves from a deeper soil layer to the topsoil by capillarity and by maintaining stability of the topsoil water content, which in turn increases transpiration. Higher production was favored by increased transpiration, a higher transpiration to evapotranspiration ratio, and less evaporation.

The result was in consistent with the findings of Hussain

*et al.* (2022) in an experiment carried out in Pakistan where highest yield was (9.61 t/ha) in plastic mulching. The reason of more grains yield of maize in plastic mulch might be due to lessened weed competition and improved usage of growth resources for higher crop performance which consequently improved photosynthetic efficiency. A major factor influencing maize yield are the photosynthetic rate, and decreasing net photosynthesis leads to less dry mass accumulation following silking (Yan *et al.*, 2021) and (Long *et al.*, 2015). Ren *et al.* (2016) further highlighted the fact that increased leaf photosynthetic capacity offers a fundamental path to enhancing maize output. According to Chaui *et al.* (2022), more vegetative growth improves area and net accumulation of photosynthesis, increasing availability of photosynthetic product for greater spike and kernel formation.

### 3.9. Economics

#### 3.9.1 Cost of cultivation

The cost of different treatments ranged between Rs 355 to Rs 2155 over production period. The treatment T1 (control) and T4(Botanical weedicides) had the lowest cost, whereas T6 (plastic mulch treated plot) had the highest cost. The maximum benefit of Rs. 476.05 was observed in T7 (Straw mulching) followed by Rs.417.72 on T3 (Hand weeding) whereas, loss of Rs.791.45 was recorded in T6 (plastic mulch treated plot).

#### 3.9.2 Benefit cost ratio (BC)

It ranged from -0.37 to 1.02 with highest BC ratio of 1.02 was obtained in T7 (Straw mulching) while the subsequent higher BC ratio of 0.99 was found in T8 (small inter row spacing). The lowest BC ratio was -0.37 in T6 (plastic mulch treated plot).

**Table 4.** Effect of different weed management practices on economics of spring maize in Gauradaha, Jhapa

| Treatments | Total cost<br>(NRs) | Total Benefit<br>(NRs) | Benefit Cost<br>Ratio (BCR) |
|------------|---------------------|------------------------|-----------------------------|
| T1         | 355                 | 302.2                  | 0.85                        |
| T2         | 455                 | 104.95                 | 0.23                        |
| T3         | 555                 | 417.72                 | 0.75                        |
| T4         | 355                 | 167.4                  | 0.47                        |
| T5         | 425                 | 308                    | 0.72                        |
| T6         | 2155                | -791.45                | -0.37                       |
| T7         | 475                 | 476.05                 | 1.02                        |
| T8         | 395                 | 394.25                 | 0.998                       |

#### 4. CONCLUSION

The various weed management practices did not significantly affect number of cobs per hectare, shelling % and sterility%. But was significantly affected by plastic mulching on grain per cob, row per cob, weight of grain per cob, 1000 grain weight and yield. The Benefit Cost Ratio (BCR) was found significantly higher in straw mulching. Overall, the use of plastic mulching can be an effective strategy for enhancing the yield of spring maize in Jhapa. However, taking into consideration the potential environmental impacts of plastic waste, straw mulching can be alternative

mulching materials that are locally available, cheaper, more sustainable and environment friendly.

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# EVALUATION OF *Trichoderma* spp. AGAINST WIRESTEM DISEASE OF CAULIFLOWER CAUSED BY *Rhizoctonia solani*

Chandra Bahadur Budha<sup>1,\*</sup>, Sundar Man Shrestha<sup>2</sup>, Hira Kaji Manandhar<sup>2</sup> and Suraj Baidya<sup>3</sup>

<sup>1</sup> Ministry of Agriculture and Livestock Development, Kathmandu, Nepal

<sup>2</sup> Agriculture and Forestry University, Chitwan, Nepal

<sup>3</sup> Nepal Agricultural Research Council, Lalitpur, Nepal

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\*Correspondence:

chandrabudha1988@gmail.com

Tel: +977-9858320220

## ABSTRACT

The biological effectiveness of twenty-eight native isolates of *Trichoderma* along with two known species (*Trichoderma viride* and *Trichoderma harzianum*) were tested against *Rhizoctonia solani* using dual culture technique. Of them, seven isolates and the two species of *Trichoderma* were tested in pot culture under net-house conditions against wirestem disease of cauliflower caused by *R. solani*. Inhibitory effects on dual culture were statistically very significant for all tested *Trichoderma*. Thirteen *Trichoderma* isolates exhibited more than 90% bio-control index and twenty-three isolates had more or at par with *T. viride* and *T. harzianum*. In an in-vitro trial, isolate from Dhankuta (Pakhribas) was most effective against *R. solani* as evident by the highest bio-control index at 6 days after incubation. Similar to this, in an in-vivo experiment, tested isolates of *Trichoderma* contributed more or less in the plant canopy area, root length and shoot circumference attributes. *Trichoderma* isolates significantly contributed to root dry matter and biomass while there was a highly significant difference in reduction of disease severity. *T. harzianum* and the isolate from Dhankuta (Pakhribas) were most effective for the control of wirestem disease.

## 1. INTRODUCTION

*Trichoderma* is among the most potential antagonists against a wide range of pathogens that causes diseases in various crops (Srivastava *et al.*, 2015, Pandey *et al.*, 2019). In 1932, Weinding first established the potential of *Trichoderma* as a bio-control agent and suggested that this genus of fungi has parasitic properties toward soil-borne plant pathogens like *R. solani* (Howell, 2003). *Trichoderma* is able to produce various volatile compounds like antibiotics that inhibit the growth of other fungi along with hyphal interactions (Dennis & Webster, 1971). *Trichoderma* spp. exert mycoparasitism, antibiosis, and competition against plant pathogens and induce resistance, and promote growth in plants (Naik & Rani, 2008, Mohiddin *et al.*, 2010). Biocontrol of different *Trichoderma* spp. are known to be species specific owing to the ability to parasitize different phytopathogenic fungi. Some efficient species are; *T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. pseudokoningii*, *T. virens*, *T. viride*, *T. polysporum*, *T. atroviride*, *T. brevicompactum*, *T. reesei* etc. (Abdelateif, 2017, Puyam, 2016, Srivastava *et al.*, 2014). This

article aims at evaluating native *Trichoderma* isolates isolated from the soils against soil borne pathogen, *R. solani*.

## 2. MATERIALS AND METHODS

In order to assess the native *Trichoderma* isolates/species, *in-vitro* and *in-vivo* studies were conducted. First, the native isolates/species were screened in the laboratory against the *R. solani* by dual culture method. Under net-house conditions, the effective isolates/species were tested for wirestem disease on cauliflower (inoculated with *R. solani*) in pot cultures.

### 2.1. Dual culture of isolates with *R. solani*

Twenty-eight native isolates of *Trichoderma* spp. isolated from the soils of various locations of Nepal and two known species (*T. viride* and *T. harzianum*) obtained from the laboratory of National Plant Pathology Research Centre (NPPRC), Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur were evaluated for their biocontrol efficacy against *R. solani* *in-vitro* by

dual culture method (Table 1). The test was performed in the Central Agricultural Laboratory, Hariharbhawan, Lalitpur from December, 2018 to January, 2019. The experiment was conducted in a completely randomized

design (CRD) with three replications (one plate as one replication) where different isolates/species were used as the treatments. The *R. solani* was provided by NPPRC, Khumaltar, Lalitpur.

**Table 1.** *Trichoderma* species and isolates used in the dual culture experiment

| Isolates/Species          | Location                   | Source/Fresh isolation from |
|---------------------------|----------------------------|-----------------------------|
| <i>Trichoderma</i> -TP 1  | Palpa 1                    | Maize field                 |
| <i>Trichoderma</i> -TRD 1 | Rajhena, Dang 1            | Maize field                 |
| <i>Trichoderma</i> -TSB   | Sangha, Bhaktapur          | Salla forest                |
| <i>Trichoderma</i> -TP 2  | Palpa 2                    | Maize field                 |
| <i>Trichoderma</i> -TRD 2 | Rajhena, Dang 2            | Maize field                 |
| <i>Trichoderma</i> -TNK   | Nagarjun, Kathmandu        | Fallow land                 |
| <i>Trichoderma</i> -TKB   | Khajura, Banke             | Rice field                  |
| <i>Trichoderma</i> -TPR   | Padariya, Rupandehi        | Vegetable field             |
| <i>Trichoderma</i> -TRK   | Raniban, Kathmandu         | Sal forest area             |
| <i>Trichoderma</i> -TI    | Illam                      | Vegetable field             |
| <i>Trichoderma</i> -TRS   | Rajbiraj, Saptari          | Legume field                |
| <i>Trichoderma</i> -TGL   | Godawari, Lalitpur         | Fallow grassland            |
| <i>Trichoderma</i> -TRJ   | Rajikot, Jumla             | Apple orchard               |
| <i>Trichoderma</i> -TKK   | Koteswor, Kathmandu        | Fallow land                 |
| <i>Trichoderma</i> -TKB   | Khala, Baglung             | Forest                      |
| <i>Trichoderma</i> -TKS   | Kapurkot, Salyan           | Ginger field                |
| <i>Trichoderma</i> -TRC 1 | Rampur, Chitwan 1          | Fallow grassland            |
| <i>Trichoderma</i> -TNK   | Naikap, Kathmandu          | Vegetable field             |
| <i>Trichoderma</i> -TTS   | Tarahara, Sunsari          | Rice field                  |
| <i>Trichoderma</i> -TRC 2 | Rampur, Chitwan (Sectoria) | Fallow grassland            |
| <i>Trichoderma</i> -TK    | Kabhrepalanchok            | Potato field                |
| <i>Trichoderma</i> -TLK   | Lumle, Kaski               | Potato field                |
| <i>Trichoderma</i> -TD    | Dhulikhel, Kabhrepalanchok | Vegetable field             |
| <i>Trichoderma</i> -TMP   | Malepatan, Kaski           | Vegetable field             |
| <i>Trichoderma</i> -TMC   | Mangalpur, Chitwan         | Rice field                  |
| <i>Trichoderma</i> -TGM   | Galeswor, Myagdi           | Maize field                 |
| <i>Trichoderma</i> -TSP   | Sarshadhara, Parbat        | Forest                      |
| <i>Trichoderma</i> -TPD   | Pakhribas, Dhankuta        | Vegetable field             |
| <i>T. viride</i>          | -                          | NPPRC                       |
| <i>T. harzianum</i>       | -                          | NPPRC                       |

Both the pathogen and the antagonists were cultured and maintained in Borosil Petri plate (9 cm diameter) each containing approximately 20 ml of potato dextrose agar (PDA) medium. A 5-mm sized mycelial disc of five-day-old *R. solani* was placed 3-cm apart from the periphery in Petri plates. After 24 hours, 5-mm sized mycelial discs of three-day-old *Trichoderma* were inoculated in the same way, 3-cm apart from the *R. solani* and 3-cm from the periphery.

The inoculated Petri plates were incubated at  $24 \pm 2^\circ\text{C}$ . Radial growth of the *Trichoderma* isolates and *R. solani*

were measured using a measuring scale and the area of each colony was measured by sketching on the plastic sheet and further processing was done to the final area recording using ImageJ 1.52a to study antagonist behavior at 6<sup>th</sup> day after inoculation (Figure 1).

*Trichoderma* as bio-control agents were evaluated by comparing bio-control index (BCI) which was computed using the colony area of *Trichoderma* isolates and test *R. solani* by the formula given by Szekeres *et al.* (2006).

$$\text{BCI (\%)} = \frac{\text{Area occupied by bio-control agent only}}{\text{Area occupied by colonies of both bio-control agent and test pathogen}} \times 100$$

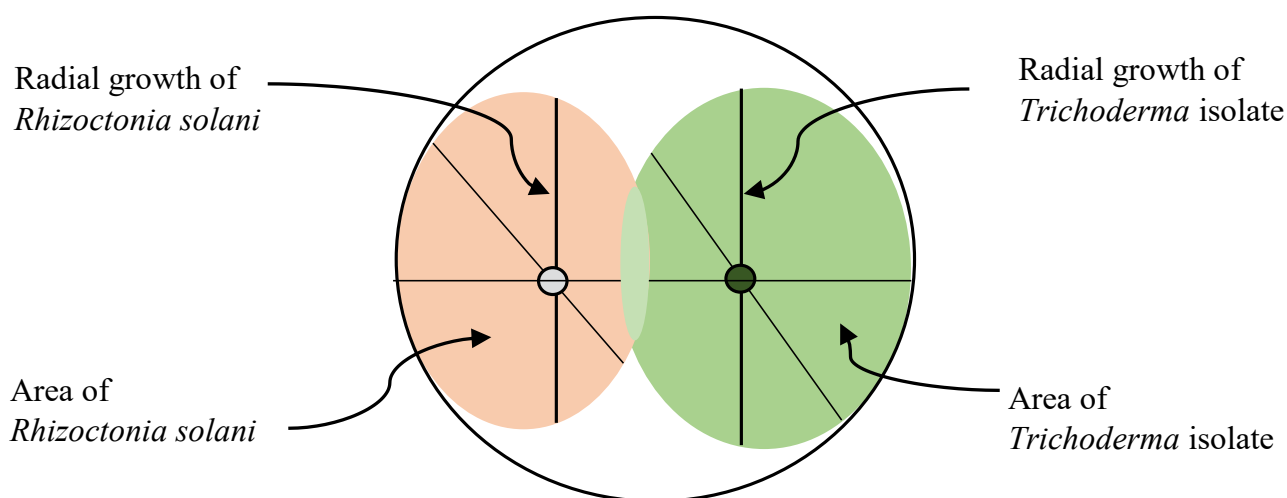


**Figure 1.** Measurement of growth of *R. solani* and *Trichoderma* isolate in dual culture Petri plate

## 2.2. Pot Culture under Net-House Conditions

Two known species (*T. viride* and *T. harzianum*) and seven native isolates, which were found effective in dual cultures, were evaluated for their effectiveness against wirestem disease on cauliflower (inoculated with *R. solani*) in earthen pot culture under net-house conditions (Table 2). The experiment was conducted at NPPRC, Khumaltar, Lalitpur from May, 2019 to September, 2019. The experiment laid out in CRD was replicated thrice and each experimental unit contained five plants.

**Table 2.** *Trichoderma* species and isolates (as treatment) used in the pot culture experiment

| Isolates/Species             | Location            | Source/Fresh isolation from |
|------------------------------|---------------------|-----------------------------|
| <i>Trichoderma</i> -TPD      | Pakhribas, Dhankuta | Vegetable field             |
| <i>Trichoderma</i> -TTS      | Tarahara, Sunsari   | Rice field                  |
| <i>Trichoderma</i> -TRK      | Raniban, Kathmandu  | Sal forest area             |
| <i>Trichoderma</i> -TRC 1    | Rampur, Chitwan     | Fallow grassland            |
| <i>Trichoderma</i> -TLK      | Lumle, Kaski        | Potato field                |
| <i>Trichoderma</i> -TRD 2    | Rajhena, Dang       | Maize field                 |
| <i>Trichoderma</i> -TRJ      | Rajkot, Jumla       | Apple orchard               |
| <i>T. viride</i>             | -                   | NPPRC                       |
| <i>T. harzianum</i>          | -                   | NPPRC                       |
| Control (Without antagonist) | -                   | -                           |

Approximately, 6.0-6.5 kg of sterilized pot mixture was filled in each earthen pot and inoculated with five mycelial discs (5-mm diameter grown on PDA) of *R. solani* in the upper layer of the soil 35 days before transplantation of cauliflower seedling. Twenty grams of *Trichoderma* mass culture prepared in finger-millet was inoculated in the upper layer of the soil 30 days before transplantation for colonization in soil. Moisture was maintained by proper irrigation. Twenty days after transplantation, 100 ml suspension of *Trichoderma* isolates ( $10^6$ ) grown on PDA Petri plates was drenched.

Canopy of the plant, shoot circumference, root length, root dry matter, biomass and disease severity were recorded at 80 days after transplanting. The disease assessment was carried out by scoring the lesions on collar region (Figure 2) of plants based on the standard

scoring scale of 0-4 given by Keinath & Farnham (2001) and disease severity percent as given:

- 0 = healthy, no symptoms of wire-stem
- 1 = superficial stem necrosis, longitudinal cracks  $\geq 1$  cm long and marginal discoloration along the crack
- 2 = stem partially girdled,  $\leq 75\%$  of the stem circumference girdled
- 3 = stem girdled,  $> 75\%$  to 100% of the stem circumference girdled
- 4 = stem completely girdled and plant stunted

$$\text{Disease Severity (\%)} = \frac{\text{Sum of all numeric rating i.e. scoring}}{\text{Total no. of plant observed} \times \text{Maximum rating}} \times 100$$

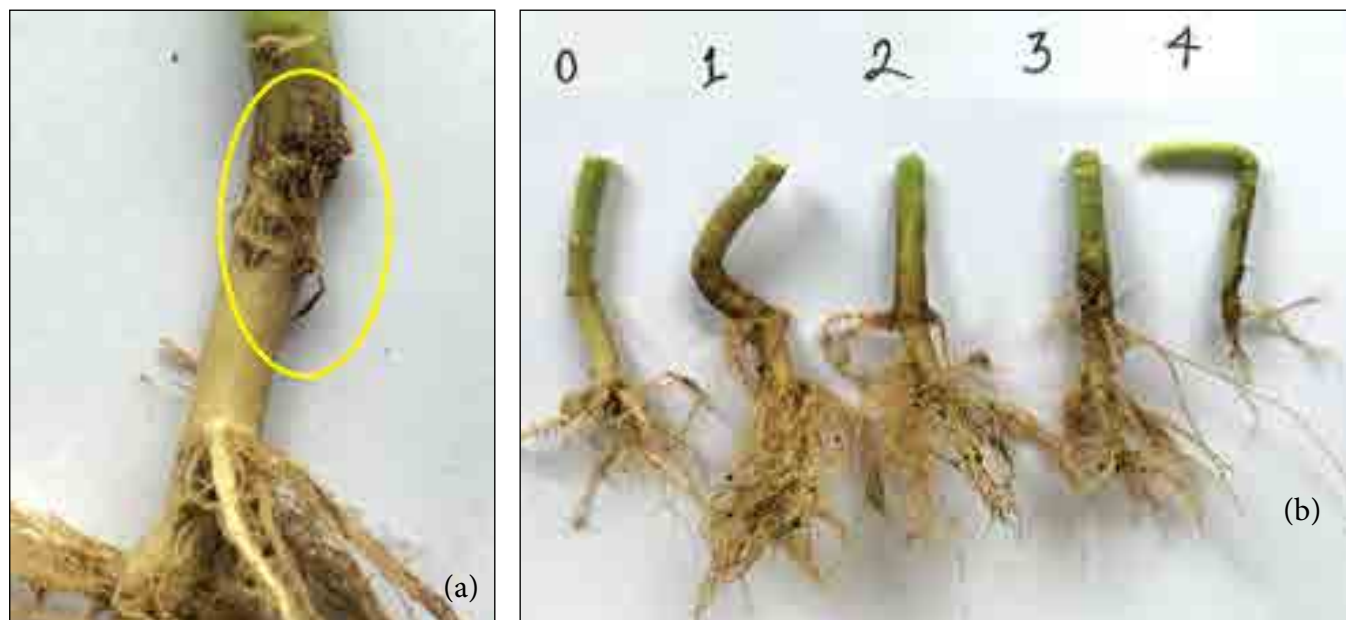


**Figure 2.** Wirestem disease assessment, (a) longitudinal cracks, and (b) scoring scale

### 2.3. Statistical Analysis

The data recorded were tabulated in Microsoft Excel 2013 data worksheets. R-Studio version 1.2.1335 was used for the statistical analysis of the data with agricolae 1.3-1 and lsmeans 2.30-0 for analysis of variance (ANOVA). The F-test was performed to compare the mean of treatment effects. For mean separation, Duncan's multiple range test (DMRT) was performed at 5% level of significance. The Microsoft Excel 2013 and SPSS were used for tabular and graphical representation.

## 3. RESULTS AND DISCUSSION

### 3.1 Antagonism *in-vitro*

In dual culture test for *in-vitro* antagonism, inhibition zones [Figure 3(a)] were formed between some of the *Trichoderma* isolates and the pathogen, *R. solani* while most of the *Trichoderma* isolates appeared to mask the pathogen i.e. overgrowth [Figure 3(b)] on the colonies of the *R. solani*.



**Figure 3.** Interaction of *R. solani* and *Trichoderma* isolate, (a) inhibition zone in between, and (b) *Trichoderma* isolate overgrowth on *R. solani*

All tested *Trichoderma* isolates significantly inhibited or overcame the growth of the *R. solani*. BCI was highly significant among the tested *Trichoderma* isolates (Table 3). *Trichoderma* isolate of Pakhribas, Dhankuta had the highest BCI (100%) followed by isolate Padariya, Rupandehi (98.32%) while isolate Palpa-1 (49.43%) had the least BCI. Thirteen tested isolates exhibited BCI more than 90% and twenty-three isolates had more or at par with *T. viride* (96.25%) and *T. harzianum* (88.37%). Those twenty-three isolates were found to overgrow on the colony of *R. solani* completely in 10 days after inoculation.

In dual culture, some *Trichoderma* isolates formed brownish inhibition zones when came in contact with *R. solani* while most *Trichoderma* isolates appeared to overgrow the colony of the pathogen. The browning zones formed as a result of mycelia being parasitized through the mechanism of coiling, encircling and lysis of *R. solani* hyphae by *Trichoderma* (Agrios, 2005, Askew & Laing, 1994). Timila *et al.* (2015) have identified and reported this mechanism by native

*Trichoderma* isolates from Nepal. In the present study, the tested *Trichoderma* isolates were found varied in their abilities to suppress *R. solani*. It demonstrates that not all *Trichoderma* are equally effective as biocontrol agents. Similar results have been explained by several authors (Amin *et al.*, 2010, Seema & Devaki, 2012, Gveroska, 2013, Bastakoti *et al.*, 2017, Manandhar *et al.*, 2019). The faster growth of *Trichoderma* during competition for resources and space, as well as mycotoxin production, and contribute to the pathogen's suppression (Howell, 2003, Timila *et al.*, 2015, Waghunde *et al.*, 2016). Further, *Trichoderma* causes hyphal lysis and inactivation of enzymes limiting the development and activity of the pathogen, *R. solani* (Naik & Rani, 2008). In antagonism confirmation, BCI considered the advantages of antagonism based on different mechanisms like competition for space and nutrition, production of antifungal metabolites and mycoparasitism, into consideration by enabling development during incubation time (Szekeres *et al.*, 2006).

**Table 3.** BCI in dual culture between *Trichoderma* isolates and *R. solani* at 6 days after inoculation

| Isolates/Species             | BCI (%)                           |
|------------------------------|-----------------------------------|
| <i>Trichoderma</i> -TP 1     | 49.43 <sup>n</sup> (44.65)        |
| <i>Trichoderma</i> -TRD 1    | 87.74 <sup>efghijk</sup> (69.68)  |
| <i>Trichoderma</i> -TSB      | 78.98 <sup>jklm</sup> (62.75)     |
| <i>Trichoderma</i> -TP 2     | 94.75 <sup>bcde</sup> (78.31)     |
| <i>Trichoderma</i> -TRD 2    | 94.75 <sup>bcdef</sup> (77.35)    |
| <i>Trichoderma</i> -TNK      | 69.02 <sup>m</sup> (56.29)        |
| <i>Trichoderma</i> -TKB      | 75.70 <sup>klm</sup> (60.50)      |
| <i>Trichoderma</i> -TPR      | 98.32 <sup>ab</sup> (82.71)       |
| <i>Trichoderma</i> -TRK      | 97.73 <sup>ab</sup> (82.39)       |
| <i>Trichoderma</i> -TI       | 92.11 <sup>bedefgh</sup> (73.92)  |
| <i>Trichoderma</i> -TRS      | 92.79 <sup>bc</sup> (79.78)       |
| <i>Trichoderma</i> -TGL      | 82.09 <sup>hijklm</sup> (65.10)   |
| <i>Trichoderma</i> -TRJ      | 89.60 <sup>edefghi</sup> (72.90)  |
| <i>Trichoderma</i> -TKK      | 86.03 <sup>fghijkl</sup> (68.17)  |
| <i>Trichoderma</i> -TKB      | 68.89 <sup>m</sup> (56.15)        |
| <i>Trichoderma</i> -TKS      | 90.58 <sup>bcdefgh</sup> (74.18)  |
| <i>Trichoderma</i> -TRC 1    | 91.47 <sup>bcdefg</sup> (75.42)   |
| <i>Trichoderma</i> -TNK      | 88.66 <sup>edefghij</sup> (70.64) |
| <i>Trichoderma</i> -TTS      | 93.60 <sup>bcdefg</sup> (75.48)   |
| <i>Trichoderma</i> -TRC 2    | 90.06 <sup>edefghij</sup> (72.02) |
| <i>Trichoderma</i> -TK       | 81.01 <sup>ijklm</sup> (64.24)    |
| <i>Trichoderma</i> -TLK      | 97.73 <sup>ab</sup> (83.16)       |
| <i>Trichoderma</i> -TD       | 86.66 <sup>fghijkl</sup> (68.78)  |
| <i>Trichoderma</i> -TMP      | 85.27 <sup>ghijkl</sup> (67.65)   |
| <i>Trichoderma</i> -TMC      | 76.18 <sup>klm</sup> (60.80)      |
| <i>Trichoderma</i> -TGM      | 84.47 <sup>ghijkl</sup> (66.85)   |
| <i>Trichoderma</i> -TSP      | 74.13 <sup>lm</sup> (59.45)       |
| <i>Trichoderma</i> -TPD      | 100.00 <sup>a</sup> (89.96)       |
| <i>T. viride</i>             | 96.25 <sup>bcd</sup> (79.45)      |
| <i>T. harzianum</i>          | 88.37 <sup>defghij</sup> (70.23)  |
| Control (Without antagonist) | 0.05 <sup>o</sup> (1.28)          |
| F-test                       | ***                               |
| S.Em±                        | 3.32                              |
| LSD (≤0.05)                  | 9.43                              |
| CV (%)                       | 8.49                              |

Value of BCI in the parentheses were arc sine transformed; Means in a column having same small letter(s) do not differ significantly at 5 percent probability by DMRT; \*\*\* = highly significant; S.Em = Standard error of means; LSD (≤0.05) = Least significant difference, showing 5% level of significance; CV (%) = Coefficient of variation percentage

### 3.2 Effect Of *Trichoderma* Isolates Against Wirestem Disease Of Cauliflower

Bio-control efficacy of *Trichoderma* isolates against *R. solani* was studied *in-vivo* on wirestem disease of cauliflower plants. The effect was significant on root dry matter, biomass and disease severity over the control while on plant canopy, root length, and shoot circumference, the effect was non-significant among the treatments (Table 4). All the *Trichoderma* isolates were found at par with known *T. viride* (17.54%) and *T. harzianum* (18.11%) for increase in root dry matter. *Trichoderma* isolate of Rajhena gave the highest (62.35%) increase in plant biomass followed by Tarahara (60.31%) and Pakhribas (55.38%). The reduction of disease severity was highest in *T. harzianum* (47.22%) treated plants followed by *Trichoderma* isolate of Pakhribas (38.88%), Rajikot (36.12%), Tarahara (33.33%) and *T. viride* (33.33%), which were not significantly different from each other. However, all *Trichoderma* isolates were found to have the potential to raise yield attributing character as revealed by increased canopy, root and shoot systems and biomass compared to control. Increased root growth and biomass were observed in pot culture study. This might be due to the enhanced nutrient utilization by plants, and thus more tolerance to pathogens reflected by their linear relationship as positively correlated (Figure 4). Considering the fact that *Trichoderma* was also found to solubilize macro- and micro-elements of plant nutrients along with a significant increase in biomass (Li *et al.*, 2015). Also, probably due to displacement and control of deleterious micro-flora (Harman, 2000) or production of secondary metabolites including, growth hormones, endochitinase, proteolytic enzymes gave symbiotic relationship with plants-microbes interactions (Ranveer *et al.*, 2018) which might have resulted for significant growth.

Similarly, *Trichoderma* contributed to reducing the *R. solani* disease significantly on cauliflower plants. Similar to this, Uddin *et al.* (2011) and Rehman *et al.* (2012) noted decreased damping-off incidence of *R. solani* incidence and significantly improved cauliflower growth vigor as compared to control. Dabbas *et al.* (2009) also reported lowest disease intensity and highest percent disease management over control. This could have been the effect of induced systemic resistance by the action of *Trichoderma* on plant (Harman *et al.*, 2004).

**Table 4.** Different attributes and disease severity of cauliflower treated with different *Trichoderma* isolates/species in soils inoculated with *R. solani* in pot culture

| Isolates/Species             | Plant canopy area (cm <sup>2</sup> ) | Root length (cm) | Root dry matter (%) | Shoot circumference (mm) | Plant biomass (g)    | Disease severity (%) |
|------------------------------|--------------------------------------|------------------|---------------------|--------------------------|----------------------|----------------------|
| <i>Trichoderma</i> -TPD      | 364.54                               | 14.58            | 24.88 <sup>a</sup>  | 30.6                     | 192.41 <sup>a</sup>  | 36.67 <sup>d</sup>   |
| <i>Trichoderma</i> -TTS      | 430.15                               | 16.09            | 25.88 <sup>a</sup>  | 30.67                    | 198.51 <sup>a</sup>  | 40.00 <sup>bcd</sup> |
| <i>Trichoderma</i> -TRK      | 381.39                               | 13.8             | 23.49 <sup>a</sup>  | 26.15                    | 144.75 <sup>bc</sup> | 48.33 <sup>bc</sup>  |
| <i>Trichoderma</i> -TRC 1    | 398.95                               | 14.13            | 23.52 <sup>a</sup>  | 29.25                    | 166.83 <sup>ab</sup> | 50.00 <sup>ab</sup>  |
| <i>Trichoderma</i> -TLK      | 452.98                               | 14.3             | 23.09 <sup>ab</sup> | 27.81                    | 181.65 <sup>ab</sup> | 50.00 <sup>ab</sup>  |
| <i>Trichoderma</i> -TRD 2    | 370.20                               | 14.61            | 25.79 <sup>a</sup>  | 30.08                    | 201.03 <sup>a</sup>  | 50.00 <sup>ab</sup>  |
| <i>Trichoderma</i> -TRJ      | 386.79                               | 14.69            | 25.88 <sup>a</sup>  | 27.77                    | 183.52 <sup>ab</sup> | 38.33 <sup>cd</sup>  |
| <i>T. viride</i>             | 410.33                               | 15.12            | 23.57 <sup>a</sup>  | 26.89                    | 191.83 <sup>a</sup>  | 40.00 <sup>bcd</sup> |
| <i>T. harzianum</i>          | 344.23                               | 15.63            | 23.68 <sup>a</sup>  | 28.06                    | 193.01 <sup>a</sup>  | 31.67 <sup>d</sup>   |
| Control (without antagonist) | 320.34                               | 13.55            | 20.05 <sup>b</sup>  | 24.62                    | 123.83 <sup>c</sup>  | 60.00 <sup>a</sup>   |
| F-test                       | ns                                   | ns               | *                   | ns                       | **                   | ***                  |
| S.Em±                        | 39.94                                | 0.82             | 1.08                | 1.97                     | 13.25                | 2.09                 |
| LSD (≤0.05)                  | -                                    | -                | 3.18                | -                        | 39.09                | 6.16                 |
| CV%                          | 17.92                                | 9.73             | 7.78                | 12.12                    | 12.91                | 8.66                 |

Means in a column having same small letter(s) do not differ significantly at 5% percent probability by DMRT, ns = not significant; \* = significant, \*\* = significant, \*\*\* = highly significant; S.Em = Standard error of means; LSD (≤0.05) = Least significant difference, showing 5% level of significance; CV (%) = Coefficient of variation percentage.

### 3.3. Correlation between disease severity percent and different attributes in pot culture

Plant canopy area, root length, root dry matter, shoot circumference and plant biomass were negatively correlated with disease severity (Table 5). Disease

severity was found contributing 18.71%, 17.33% and 13.92% reduction in plant biomass, root dry matter and root length, respectively (Figure 4). The results clearly demonstrate the effect of the disease on the crop plant, which can directly affect crop yield. Plant canopy area and shoot circumference were not affected.

**Table 5.** Correlations between different attributes of cauliflower and disease severity by *R. solani* in pot culture

|                     | Disease severity | Canopy area | Root length | Root dry matter | Shoot circumference | Plant biomass |
|---------------------|------------------|-------------|-------------|-----------------|---------------------|---------------|
| Disease severity    | -                |             |             |                 |                     |               |
| Canopy area         | -0.028           | -           |             |                 |                     |               |
| Root length         | -0.373*          | 0.100       | -           |                 |                     |               |
| Root dry matter     | -0.416*          | 0.184       | 0.379*      | -               |                     |               |
| Shoot circumference | -0.106           | 0.435*      | 0.541**     | 0.534**         | -                   |               |
| Plant biomass       | -0.433*          | 0.136       | 0.612**     | 0.688**         | 0.626**             | -             |

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\*. Correlation is significant at the 0.01 level (2-tailed).

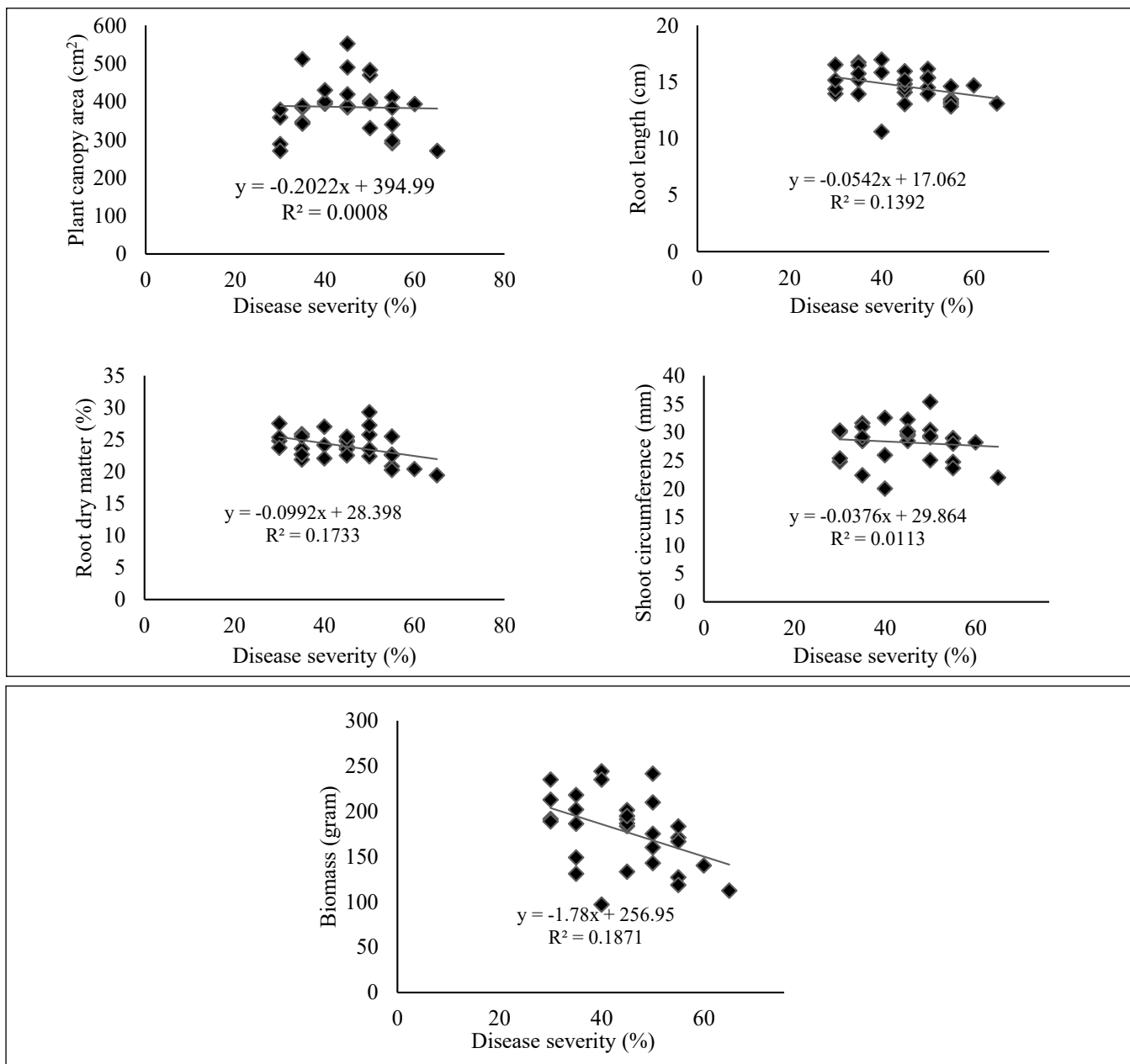


**Figure 4.** Simple linear relationship between disease severity by *R. solani* and different attributes of cauliflower in pot culture

#### 4. CONCLUSION

From the present experimental studies, it can be concluded that native isolates of *Trichoderma* spp. are potential for the management of diseases caused by *R. solani* on cauliflower. Of the isolates tested, we found *T. harzanium* and Dhankuta (Pakhribas) isolate the most effective in managing wirestem disease. These *Trichoderma* with proper identity need to be promoted for commercial production and use in farmers' field.

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## EXPLORATION OF SUSTAINABLE LIVE FISH BUSINESS IN NEPAL

Hareram Devkota<sup>1,2,3,\*</sup>, Dilip Kumar Jha<sup>1</sup>, Tista Prasai Joshi<sup>2</sup>, Shreemat Shrestha<sup>3</sup>, Mahendra Prasad Bhandari<sup>3</sup> and Nabaraj Poudel<sup>3</sup>

<sup>1</sup> Department of Aquaculture, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal

<sup>2</sup> Environmental Research Laboratory, Nepal Academy of Science and Technology (NAST), Lalitpur, Nepal

<sup>3</sup> Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur, Nepal

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\*Correspondence:

hdevkota6@gmail.com

Tel: +977-9856033580

### ABSTRACT

Live fish businesses are likely to become more popular in Nepal due to their reliable source of food. The live fish food survey data was collected from a Google Form questionnaire in the Nepalese language. This study investigates consumers, retailers, and wholesalers in Nepal's live fish market to identify significant concerns that could be addressed in the decision-making of live fish professionals. Nonparametric tests are used for non-normally distributed data, and parametric tests are used for normally distributed data. The study found high mortality and administrative obstacles of 68.60% and 48.60%, respectively. If the wrong choice of aeration method and equipment is made, more fish will survive in the retailer's shop and during transportation time. Fish Models II and III will be an alternative to the current method of transportation, which shows that increasing the tank capacity by one standard deviation increases fish survival by 0.7 and 0.65 standard deviations, respectively. To increase the dissolved gas, the survey suggests that transportation and aeration methods should be improved. This increases the volume of transportation while using the same resources, meeting market demands

### 1. INTRODUCTION

Nepal's abundant water resources make aquaculture promising. Nepal has 200 species of fish, 190 of which are native, and the rest are exotic. Nepal's piscatorial industry is vital to its economy. Aquaculture in Nepal is an emerging industry that has shown immense potential to contribute significantly to the country's economic growth and food security. Nepal is blessed with abundant aquatic resources that cover approximately 5.5% of the total land area, but only 2% of the estimated 826,818-hectare water surface area is used for aquaculture and capture fisheries. This industry provides jobs, food security, resource conservation, and foreign currency. Aquaculture accounts for 0.44% of Nepal's GDP and 1.83% of its agricultural GDP (CFPCC, 2022). The Nepalese fishery sector provides employment opportunities for the large population of Nepal (Gurung, 2016). Artisanal fishing supplies more than 70% of Nepal's fish demand. Many Nepalese eat live fish as their main food source. Nepal needs a sustainable aquaculture industry. Understanding the national live fish market is crucial. Nepal began commercial fish farming in 1947 (Rai *et al.*, 2008). The

Terai region now leads commercial carp production. Improving fish production: Nepal needs more fish. The piscatorial industry in Nepal employs a large portion of the population, reducing poverty and boosting the economy. Fisheries production in Nepal can boost food security and the economy. Furthermore, aquaculture growth has positively contributed to global food and nutrition security by mitigating reductions in fish production from capture fisheries to meet the increasing demand for fish. Minor and major 1947 producers are joining carp farming, proving its success. Due to its abundant water and rich soil, the Terai region of Nepal is ideal for carp farming. Water management in Terai's lowlands allows fish farming and productivity. Gurung (2003) stated that *Aristichthys nobilis* (bighead), *Cyprinus carpio* (common carp), *Labeo rohita* (rohu), and *Catla catla* (Bhukur) thrive in aquatic environments. Carp aquaculture, a lucrative and efficient alternative to conventional agriculture, has given residents of these regions many ways to make money. Kent (1997), Madeley (1981), and Rahman & Islam (2020) point out that sufficient supply of fish market demand boosts the

country's GDP. Due to little knowledge of production and supply, fish culture and consumption declined in Nepal during the 1980s (Madeley, 1981). Recently, healthy people have preferred fish (Ruffle *et al.*, 2019). Fish is essential to a healthy diet (Carlucci *et al.*, 2015; Rahman & Islam, 2020). Ahmed and Azra (2022) report that health-conscious consumers are eating more live fish. Ahmed and Azra (2022) mentioned that the live fish trade is profitable.

The Nepalese live fish market has other problems in addition to its demand-based distribution network. The trade in live fish, especially in rural areas, employs skilled and unskilled workers. Nepal imports 75% of its aquaculture products, mostly from India. Freezing, vacuum packaging, or ice storage preserve these products. This situation threatens Nepal's economy and food security. The Nepalese government is encouraging domestic aquaculture production to address this problem. Additionally, sustainable methods must be implemented to reduce the ecological footprint and ensure Nepal's long-term viability. Nepal can become self-sufficient with sustained investment and innovation. This study examined how Nepalese buyers, sellers, and retailers worked together to address live fish trade and consumption issues. This study should help stakeholders decide on live fish transport, animal welfare, and sales strategies. Researchers examined government policies, local governance, and other

stakeholders to identify gaps that could be filled to improve the welfare of fish market animals. It may also influence local and national food and nutrition policies. This article analyzes consumers and their preferences, suppliers, and consumer issues. The study begins with consumers' perceptions and behaviors when buying fish from markets, then moves on to suppliers and their methods. The final section evaluates the fish market.

## 2. MATERIALS AND METHOD

### 2.1. Survey site and data collection

The regions of Bharatpur and Ratnanagar, located in the Chitwan Valley, and Kathmandu and Lalitpur, located in the Kathmandu Valley, were selected based on their potential for the trade of live fish. The National Live Fish Traders Association (NLFTA) and the Central Fisheries Promotion and Conservation Center (CFPCC) in Balaju, Kathmandu, gave a list of live fish stores and suppliers at the study sites, as shown in Figure 1. The fish shop and supplies are chosen at random. Using a Google form, information was collected from face-to-face interviews, direct observations, and focus group discussion. We used supplier and retailer focus group discussions with the participatory method to gather information in the Kathmandu Valley and the Chitwan Valley. The fish shop provided information on customers who bought fish in the second month of 2022.

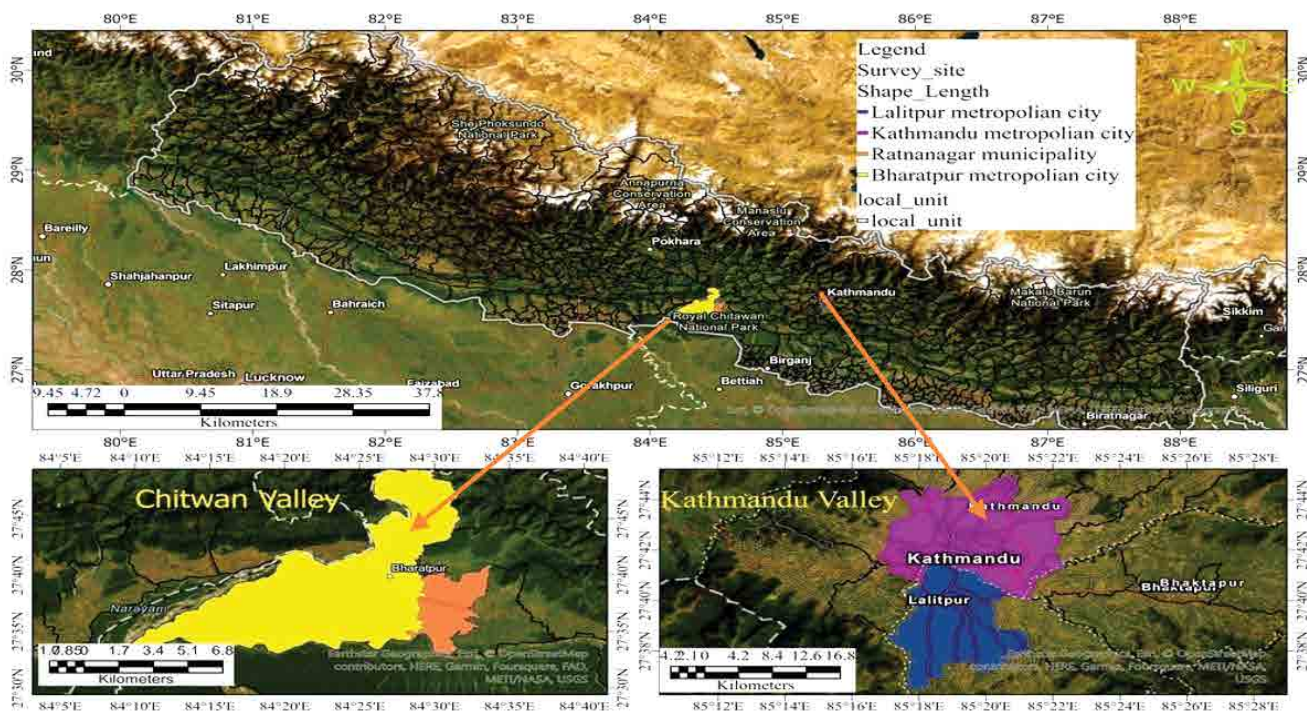


Figure 1. The study areas shown on the map in different colors

## 2.2. Processing of the response

Socioeconomic status, demographic trends, market conditions, consumer preferences, and marketing problems were recorded. Respondents in Chitwan and Kathmandu were sampled using a simple sampling method. The sample includes 500 consumers, suppliers, and retailers. Unfamiliar fish species and non-consumers were removed from the 407 responses. The analysis includes only 300 valid responses from consumers.

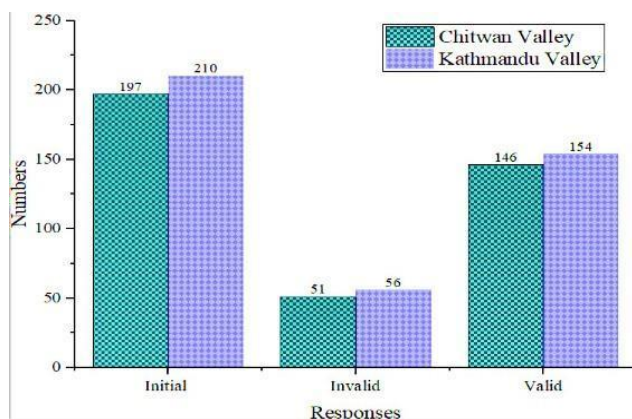


**Figure 2.** Processing of Consumer Response

## 2.3 Data analysis

The self-administered Google Forms questionnaires collected data on the building and content of relationships. Multiple choice and multiple response questions are well-designed questionnaires in native language (Annex 1). Before conducting the final survey, pre testing of the questionnaire was done by filling out the questionnaire form and taking 10% of the respondents. In the pilot study, Cronbach's alpha was calculated, which was 0.69. The questionnaire was modified according to the pretesting result of Cronbach's alpha. The information was entered into Microsoft Excel 2016, and the Excel data was edited and cross validated before being imported into SPSS version 25. The statistical methods employed for data analysis and interpretation were descriptive and inferential tools and techniques. The normality testing was done by the Kolmogorov-Smirnov test as the sample size was greater than 50 for applying a suitable parametric statistical method. The association between categorical variables was tested by the chi-square test. The interpretation and presentation of data were based on inferential statistics. The preference of fish species and problems ranking of live fish transportation were assigned by mean rank with Kruskal Wallis test.

## 2.4. Regression analysis

Regression analysis as part of the investigation, a multiple linear regression equation (Equation i) was made to explain the things that affect how people think about live fish and how they are used on the market. To perform an analysis of the relationships that exist between the dependent variables, consumer perception, and independent variables, the multiple linear regression equation (i) is used.

A population model for a multiple linear regression model that relates a y variable to k x variables

is written as

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_k x_k + e \dots (i)$$

Where,

Y is the dependent variable.

X is the independent variable.

K is the number of independent variables.

$\beta$  is the constant, and the subsequent  $\beta_1, \beta_2, \dots, \beta_n$  are the regression coefficients corresponding to the variables  $X_1, X_2, \dots, X_k$ . and e are the error terms.

"k" for predictor variables means k+1 regression parameters (coefficients).

However, the multiple regression model used is represented by regression line (i), and the inclusion of significant variables and exclusion of no significant variables criteria were used to fit the predicting equation of the four types of regression models.

## 2.5. Consumer perception

The questionnaire asks questions about age, sex, level of education, income, employment, and location, among others. Consumer preferences for fish preparation methods and the value placed on fresh, live fish were also considered. Simple descriptive statistics such as mean, maximum frequency, minimum frequency, and percent were used to look at live fish.

## 2.6. Socioeconomic and business status

Simple descriptive statistics such as mean, maximum frequency, minimum frequency, and percent were used to look at things such as the size of the families of the respondents, how much fish they ate, their work history, etc.

## 2.7. Profitability analysis

The discounted cost of production and the discounted gross return of the fish industry were used to calculate the benefit-cost ratio using the formula given below.

### 2.7.1 BC ratio

The discounted cost of production and the discounted gross return from fish farming were used to calculate the benefit-cost ratio using the formula given below (Gahatraj & Subedi, 2020). The purpose of this report is to analyze the financial health of a business. The BC ratio is a financial indicator that serves as a measure of a professional's revenue-generating potential relative to its expenses. Handling is involved in the calculations.

$$\text{BC ratio} = \frac{\text{Discounted gross return}}{\text{Discounted cost of live fish handling}}$$

### 2.7.2. Producer's Share ratio

This unique farmer-owned label, also known as the Agri Share Ratio, has deeper implications for farmers and their communities in terms of who supplies the products and who captures the profits (Wijaya *et al.*,

2022). The percentage of the consumer-paid price that the producers receive is known as the producer's share. It was calculated using the following formula:

$$\text{Producer share ratio} = \frac{\text{Retailer price}}{\text{Discounted cost of farm gate price}}$$

## 3. RESULTS AND DISCUSSION

### 3.1. Value and supply chain of the live fish market in nepal

Many Terai farmers raise fish for home delivery in this supply chain. The fish farmers in the Terai region breed and raise the fish middlemen, transport them to Nepalese cities, and deliver them on time. Pond-based aquaculture raises fish. Traders and processors prepare fish for shipping. Wholesalers and retailers buy fish from carriers on the market. Manufacturers, retailers, and retailers affect purchases. The distribution of live fish is complicated. Terai farmers supply Chitwan and Kathmandu wholesalers and retailers. Chitwan shops send small, live fish to Kathmandu. Nepal's aquaculture industry is now complex.

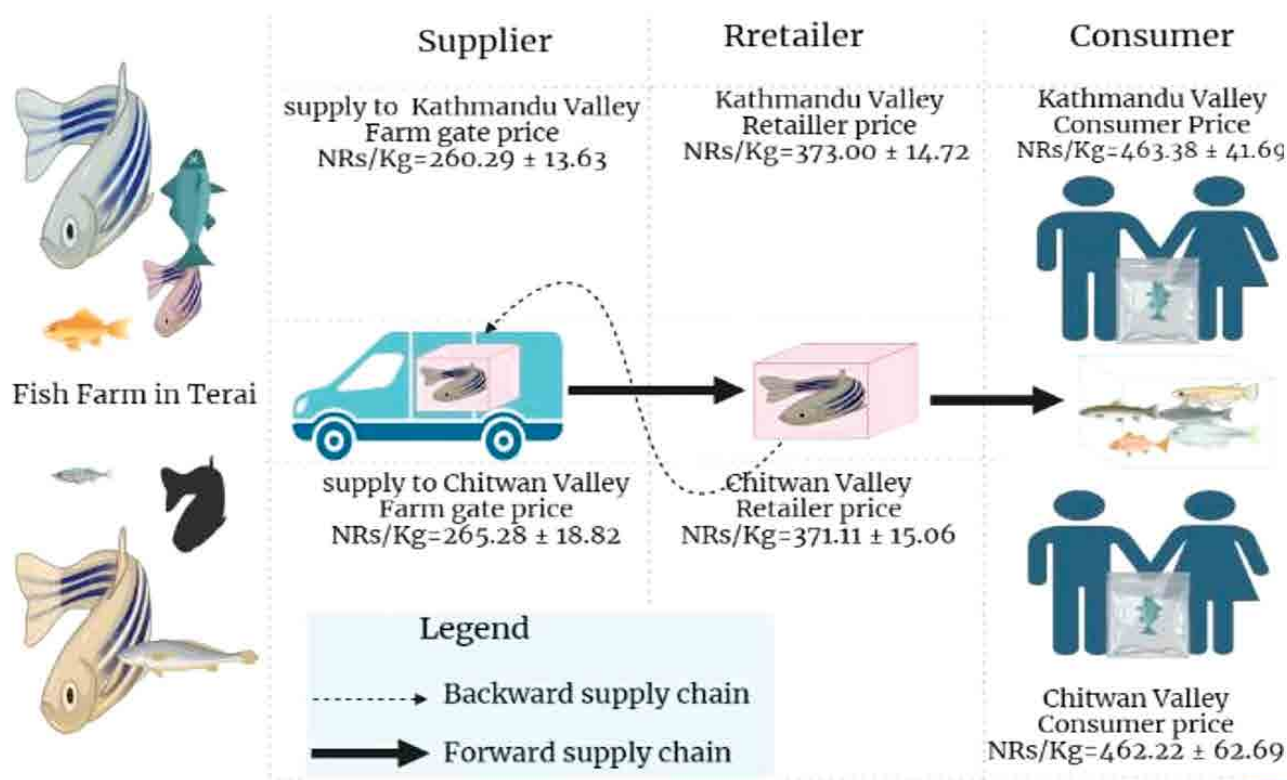


**Figure 3.** Status of the supply and value chain of the live fish marketing system in Nepal

### 3.2. Consumer behavior on live fish consumption

**Table 1.** Perception of the consumer about the consumption of different species of fish according to different factors (Kruskal-Willi test), n=300

| Fish species                                  | Consumer group  | n   | Rank   | p(0.05%) |
|-----------------------------------------------|-----------------|-----|--------|----------|
| Rohu ( <i>Labeo rohita</i> )                  | Diet            | 224 | 157.34 | 0.01*    |
|                                               | Health          | 76  | 130.34 |          |
| Rohu ( <i>Labeo rohita</i> )                  | Dead            | 137 | 165.31 | 0.000*** |
|                                               | Live            | 163 | 138.06 |          |
| Rohu ( <i>Labeo rohita</i> )                  | Chitwan Con.    | 146 | 160.11 | 0.03*    |
|                                               | Kathmandu, Con. | 154 | 141.39 |          |
| Rohu ( <i>Labeo rohita</i> )                  | Buy dead also   | 137 | 165.31 | 0.000*** |
|                                               | Return w/o buy  | 163 | 138.06 |          |
| Trout ( <i>Oncorhynchus mykiss</i> )          | Chitwan Con.    | 146 | 140.42 | 0.02*    |
|                                               | Kathmandu, Con. | 154 | 160.05 |          |
| Silver ( <i>Hypophthalmichthys molitrix</i> ) | Killing Method  | 139 | 144.4  | 0.01*    |
|                                               | preservative    | 161 | 155.77 |          |
| Silver ( <i>Hypophthalmichthys molitrix</i> ) | Dead            | 137 | 145.57 | 0.05*    |
|                                               | Live            | 163 | 154.64 |          |
| Silver ( <i>Hypophthalmichthys molitrix</i> ) | Buy Dead also   | 137 | 145.57 | 0.05*    |
|                                               | Return w/o buy  | 163 | 154.64 |          |
| Naini ( <i>Cirrhinus cirrhosis</i> )          | Killing Method  | 139 | 147.08 | 0.03*    |
|                                               | Preservative    | 161 | 153.45 |          |
| Naini ( <i>Cirrhinus cirrhosis</i> )          | Freshness       | 197 | 152.85 | 0.03*    |
|                                               | Price           | 103 | 146    |          |
| Common ( <i>Cyprinus carpio</i> )             | Nonemployee     | 121 | 138.71 | 0.01*    |
|                                               | Employee        | 179 | 158.47 |          |
| Rohu ( <i>Labeo rohita</i> )                  | Nonemployee     | 121 | 131.83 | <.001*** |
|                                               | Employee        | 179 | 163.12 |          |
| Grass ( <i>Ctenopharyngodon Idella</i> )      | Nonemployee     | 121 | 162.53 | <.001*** |
|                                               | Employee        | 179 | 142.37 |          |
| Pangas ( <i>Pangasius hypothalamus</i> )      | Nonemployee     | 121 | 170.17 | <.001*** |
|                                               | Employee        | 179 | 137.2  |          |
| Common ( <i>Cyprinus carpio</i> )             | Literacy        | 47  | 130.67 | 0.02*    |
|                                               | School          | 147 | 147.56 |          |
| Grass ( <i>Ctenopharyngodon Idella</i> )      | University      | 106 | 163.37 |          |
|                                               | Literacy        | 47  | 165.22 | 0.01*    |
| Pangas ( <i>Pangasius hypothalamus</i> )      | School          | 147 | 151.81 |          |
|                                               | University      | 106 | 142.16 |          |
| Pangas ( <i>Pangasius hypothalamus</i> )      | Literacy        | 47  | 165.61 | 0.03*    |
|                                               | School          | 147 | 151.93 |          |
| Pangas ( <i>Pangasius hypothalamus</i> )      | University      | 106 | 141.82 |          |

Significant at 0.05; \*\* Significant at 0.01;\*\*\* Significant at 0.001

**Table 2.** Fish species ranking based on consumer preference (Kruskal-Willi test), n = 300

| Preferable fish species                       | Market    | n   | Rank  | Cases (%) | p (0.05%) |
|-----------------------------------------------|-----------|-----|-------|-----------|-----------|
| Common ( <i>Cyprinus carpio</i> )             | Chitwan   | 108 | 156.2 | 70.7      | 0.161     |
|                                               | Kathmandu | 104 | 145.2 |           |           |
| Rohu ( <i>Labeo rohita</i> )                  | Chitwan   | 76  | 159.6 | 46.3      | 0.041*    |
|                                               | Kathmandu | 63  | 142   |           |           |
| Trout ( <i>Oncorhynchus mykiss</i> )          | Chitwan   | 52  | 140.8 | 42.3      | 0.028*    |
|                                               | Kathmandu | 75  | 159.6 |           |           |
| Pangas ( <i>Pangasius hypothalamus</i> )      | Chitwan   | 14  | 145   | 13.3      | 0.07      |
|                                               | Kathmandu | 26  | 155.7 |           |           |
| Grass ( <i>Ctenopharyngodon Idella</i> )      | Chitwan   | 13  | 150   | 9.3       | 0.833     |
|                                               | Kathmandu | 15  | 151   |           |           |
| Silver ( <i>Hypophthalmichthys molitrix</i> ) | Chitwan   | 13  | 152.5 | 7.7       | 0.414     |
|                                               | Kathmandu | 10  | 148.7 |           |           |
| Bhakur ( <i>Catla catla</i> )                 | Chitwan   | 7   | 150.7 | 4.7       | 0.898     |
|                                               | Kathmandu | 7   | 150.3 |           |           |
| Naini ( <i>Cirrhinus cirrhosis</i> )          | Chitwan   | 3   | 149.1 | 3         | 0.361     |
|                                               | Kathmandu | 6   | 151.8 |           |           |
| Bighead ( <i>Hypophthalmichthys nobilis</i> ) | Chitwan   | 4   | 150.6 | 2.7       | 0.924     |
|                                               | Kathmandu | 4   | 150.4 |           |           |
| Total                                         |           | 600 |       | 200       |           |

**Table 3.** Ranking of fish species based on Demographic factors on perception of fish consumption n=300

| Description                     | Groups         | Market  |      |           |       | X <sup>2</sup> | p(0.05%) |
|---------------------------------|----------------|---------|------|-----------|-------|----------------|----------|
|                                 |                | Chitwan |      | Kathmandu |       |                |          |
|                                 |                | n       | %    | n         | %     |                |          |
| Education level                 | Literacy       | 28      | 9.33 | 19        | 6.33  | 3.9            | 0.14     |
|                                 | School         | 73      | 24.3 | 74        | 24.67 |                |          |
|                                 | University     | 45      | 15   | 61        | 20.33 |                |          |
| Occupation                      | Nonemployee    | 51      | 17   | 70        | 23.33 | 3.5            | 0.063    |
|                                 | Employee       | 95      | 31.7 | 84        | 28    |                |          |
|                                 |                |         |      |           |       |                |          |
| Preferable fish culture region  | Hills          | 118     | 39.3 | 112       | 37.33 | 2.8            | 0.098    |
|                                 | Terai          | 28      | 9.33 | 42        | 14    |                |          |
|                                 |                |         |      |           |       |                |          |
| Benefits of fish consumption    | Diet           | 121     | 40.3 | 103       | 34.33 | 10             | 0.001*** |
|                                 | Health         | 25      | 8.33 | 51        | 17    |                |          |
|                                 |                |         |      |           |       |                |          |
| Familiar to live fish transport | Unseen         | 55      | 18.3 | 53        | 17.67 | 0.3            | 0.56     |
|                                 | Seen           | 91      | 30.3 | 101       | 33.67 |                |          |
|                                 |                |         |      |           |       |                |          |
| Shortage of live fish           | Buy dead also  | 60      | 20   | 77        | 25.67 | 2.4            | 0.12     |
|                                 | Return w/o buy | 86      | 28.7 | 77        | 25.67 |                |          |
|                                 |                |         |      |           |       |                |          |
| Purchasing fish conditions      | Dead           | 60      | 20   | 77        | 25.67 | 2.4            | 0.12     |
|                                 | Live           | 86      | 28.7 | 77        | 25.67 |                |          |
|                                 |                |         |      |           |       |                |          |
| Live fish prefer reason         | Freshness      | 92      | 30.7 | 105       | 35    | 0.9            | 0.35     |
|                                 | Price          | 54      | 18   | 49        | 16.33 |                |          |
|                                 |                |         |      |           |       |                |          |
| Dead fish discard Reason        | Killing method | 66      | 22   | 73        | 24.33 | 0.2            | 0.7      |
|                                 | Preservative   | 80      | 26.7 | 81        | 27    |                |          |
|                                 |                |         |      |           |       |                |          |
| Opinion on Live Fish Price      | Optimum        | 120     | 40   | 122       | 40.67 | 0.4            | 0.52     |
|                                 | Expensive      | 26      | 8.67 | 32        | 10.67 |                |          |
|                                 |                |         |      |           |       |                |          |

Significant at 0.05; \*\* Significant at 0.01;\*\*\* Significant at 0.001

### 3.3. Live fish business

**Table 4.** The correlation between different parameters of the live fish business

| <b>A. Retailer of live fish (n = 58)</b> |         |         |         |         |         |           |
|------------------------------------------|---------|---------|---------|---------|---------|-----------|
| Parameters                               | A       | B       | C       | D       | E       | F         |
| A Purchase amount (Kg / lot)             | 1       |         |         |         |         |           |
| B Survival (%)                           | 0.69*** | 1       |         |         |         |           |
| C Tank capacity(x1000L)                  | 0.99*** | 0.7***  | 1       |         |         |           |
| D Total expense (NRs/day)                | 0.6***  | 0.43**  | 0.62*** | 1       |         |           |
| E Total income (NRs/day)                 | 0.61*** | 0.44*** | 0.63*** | 0.96*** | 1       |           |
| F BC Ratio                               | 0.47    | 0.23    | 0.46    | 0.28    | 0.46    | 1         |
| <b>B. Live fish suppliers(n=35)</b>      |         |         |         |         |         |           |
| Parameters                               | A       | B       | C       | D       | E       | F         |
| A Purchase amount (Kg / lot)             | 1       |         |         |         |         |           |
| B Survival (%)                           | 0.52*** | 1       |         |         |         |           |
| C Tank capacity(yx1000L)                 | 0.80*** | 0.35**  | 1       |         |         |           |
| D Total expense (NRs/day)                | 0.67*** | 0.37**  | 0.52*** | 1       |         |           |
| E Total income (NRs/day)                 | 0.57*** | 0.28*   | 0.42*** | 0.89*** | 1       |           |
| F BC ratio                               | 0.15    | 0.05    | 0.06    | 0.02    | 0.40*** | 1         |
| G Farmers share ratio                    | 0.08    | -0.02   | 0.02    | -0.06   | 0.32**  | 1 0.99*** |

**Note:** Strength of the correlation (r) = 0.0- 0.1 = no correlation; 0.1-0. 3 = low correlation; 0.3-0. 5 = medium correlation; 0.5- 0.7 = high correlation; 0.7- 1 = very high correlation; \* significant at 0.05; \*\* significant at 0.01; \*\*\* significant at 0.001

**Table 5.** Comparison of different factors in the business of live fish of two major cities of Nepal

| Description                | Markets        |                | Mann-Whitney U |          |
|----------------------------|----------------|----------------|----------------|----------|
|                            | Chitwan (18)   | Kathmandu (40) | Statistics     | p(0.05%) |
| <b>A. Retailers</b>        |                |                |                |          |
| Business Duration (Year)   | 3.78 ± 0.94    | 4.63 ± 1.48    | 234            | 0.03*    |
| Purchase amount (Kg / lot) | 98.51 ± 11.02  | 95.08 ± 14.81  | 295.5          | 0.28     |
| Survival (%)               | 92.13 ± 1.25   | 89.42 ± 1.73   | 77.5           | <.001*** |
| Purchase rate (NRs / kg)   | 371.11 ± 15.06 | 373 ± 14.72    | 339            | 0.72     |
| Sale rate (NRs/g)          | 462.22 ± 62.69 | 463.38 ± 41.69 | 329            | 0.6      |
| Tank capacity(x1000L)      | 1.58 ± 0.35    | 1.68 ± 0.51    | 315            | 0.42     |
| Rent (x100(NRs/day))       | 6.57 ± 0.39    | 8.16 ± 0.42    | 0              | <.001*** |
| Total expense (NRs/day)    | 20.75 ± 5.08   | 21.22 ± 7.18   | 347.5          | 0.83     |
| Total income (NRs/day)     | 25.1 ± 7.70    | 25.27 ± 8.80   | 356            | 0.95     |
| BC Ratio                   | 1.21 ± 0.16    | 1.19 ± 0.12    | 319            | 0.49     |
| <b>B. Suppliers</b>        | Chitwan (18)   | Kathmandu (17) | Statistics     | p(0.05%) |
| Business Duration (Year)   | 5.17 ± 1.25    | 5.82 ± 1.74    | 113.5          | 0.06     |
| Purchase amount (Kg / lot) | 133.33 ± 54.23 | 125.88 ± 30.83 | 136            | 0.55     |
| Survival (%)               | 89.76 ± 4.15   | 88.88 ± 2.82   | 135.5          | 0.56     |
| Purchase rate (NRs/ kg)    | 265.28 ± 18.82 | 260.29 ± 13.63 | 147            | 0.84     |
| Sale rate (NRs/ kg)        | 371.39 ± 18.13 | 374.71 ± 9.02  | 151.5          | 0.96     |
| Tank capacity(x 1000L)     | 1.33 ± 0.54    | 1.26 ± 0.31    | 138.5          | 0.61     |
| Rent (X100(NRs/day))       | 56.67 ± 10.43. | 111.76 ± 15.90 | 1              | <.001**  |
| Total expense (NRs/day)    | 41.06 ± 14.95  | 68.79 ± 16.14  | 36             | <.001**  |
| Total income (NRs/day)     | 49.21 ± 19.67  | 82.54 ± 20.22  | 37             | <.001**  |
| BC Ratio                   | 1.21 ± 0.16    | 1.17 ± 0.13    | 116            | 0.22     |
| Farmers share ratio        | 1.41 ± 0.1     | 1.44 ± 0.06    | 117.5          | 0.24     |

**Table 6.** Comparison of different factors in the business of live fish in two major cities, n=35

| Problems                | Market    | Number | Mean rank | Cases (%) | p-Value  |
|-------------------------|-----------|--------|-----------|-----------|----------|
| High Mortality          | Chitwan   | 18     | 14.75     | 68.6      | 0.016*   |
|                         | Kathmandu | 17     | 21.44     |           |          |
| Administrative Obstacle | Chitwan   | 18     | 26.03     | 48.6      | 0.000*** |
|                         | Kathmandu | 17     | 9.5       |           |          |
| High Cost of Aeration   | Chitwan   | 18     | 19.28     | 37.1      | 0.365    |
|                         | Kathmandu | 17     | 16.65     |           |          |
| Low- amount transport   | Chitwan   | 18     | 18.36     | 25.7      | 0.777    |
|                         | Kathmandu | 17     | 17.62     |           |          |
| Total                   |           |        |           | 180       |          |

Significant at 0.05; \*\* Significant at 0.01; \*\*\* Significant at 0.001

A study of the Nepalese live fish market found that common carp accounted for 70.7% of sales. Rohu (46.3%) is more popular in Chitwan, and trout (42.3%) is more popular in Kathmandu. This shows that socioeconomic status and location do not affect people's consumption habits of live fish. Common carp and Rohu, two of the largest commercial fish, are easy to find in the market. Therefore, Nepalese people choose live fish based on the availability of species. Both cities eat 62.2% live fish, as does the rest of the region. The demand for live fish depends on species availability; it is not affected by income or location. In addition to the benefits, peo-

ple of all ages and backgrounds agreed to eat live fish. This shows that the preferences of live fish are based on available species, not socioeconomic status or the location of the study site. Chitwanians give more priority on live fish more than dead fish for their nutritional value. Nutritional value, freshness, socioeconomic status, and location all have an impact on the demand for live fish in Chitwan Valley. Dead Rohu was healthy for the diet group, non-employee consumers, and employees. Live Rohu fish are considered local, so importing them is difficult.

**Table 7.** Factors affecting the survival of live fish in the live fish business

| A. Suppliers(n=35)                 | Model I              | Model II             | Model III            | Model-IV              |
|------------------------------------|----------------------|----------------------|----------------------|-----------------------|
| Constant                           | 82.36<br>(-35.2) *** | 84.16<br>(47.35) *** | 81.79<br>(56.4) ***  | 90.48<br>(81.08) ***  |
| Purchase amount (Kg / lot)         | -0.24(-0.07)         |                      |                      |                       |
| Tank capacity(L)                   | 0.95(0.28)           | 0.7(5.63) ***        | 0.65(4.87) ***       | 0.2(-0.66)            |
| Blower with coarse-air stone       | 0.35(2.33) *         | 0.27(2.01) *         | 0.16(1.07)           | -0.17(-1.26)          |
| Coarse air stone vs. tank capacity |                      |                      |                      | 0.52(-1.7)            |
| 12-hour transport duration         | -0.7(-3.06) **       | -0.42(-2.65) **      |                      |                       |
| 8 hours of transport duration      | -0.33(-1.79)         | -0.17(-1.21)         |                      |                       |
| To remove waste                    | -0.34(-2.46) *       | -0.26(-2) *          |                      |                       |
| F-value                            | 5.13***              | 7.51***              | 8.09***              | 9.81***               |
| R 2                                | 0.61                 | 0.56                 | 0.44                 | 0.49                  |
| Adjusted R2                        | 0.49                 | 0.49                 | 0.38                 | 0.44                  |
| B. Retailer(n=58)                  |                      |                      |                      |                       |
| Constant                           | 85.58<br>(18.08) *** | 85.58<br>(18.1) ***  | 87.62<br>(95.15) *** | 89.93<br>(-69.13) *** |
| Purchase amount (Kg/lot)           | 0.55(2.6) ***        | 0.67(3.14) **        |                      |                       |
| Tank capacity(L)                   | -0.06(-0.32)         | -0.21(-1.01)         | 0.37(2.97) **        | 0.09(-0.54)           |
| Coarse air stone vs. tank capacity |                      |                      |                      | -2.08(-2.16) *        |
| Blower with coarse-air stone       | -0.02(-0.2)          |                      |                      | 2.28(2.31) *          |
| Total income (Nrs/day)             | 1.61(1.53)           |                      |                      |                       |
| Total expenditure (Nrs/day)        | -0.09(-0.1)          | -0.07(-0.6)          |                      |                       |
| BC ratio                           | -0.3(-0.7)           |                      |                      |                       |
| F-value                            | 6.17***              | 7.29***              | 8.83**               | 5.25**                |
| R 2                                | 0.42                 | 0.29                 | 0.14                 | 0.48                  |
| Adjusted R2                        | 0.35                 | 0.25                 | 0.12                 | 0.23                  |

Significant at 0.05; \*\* Significant at 0.01;\*\*\* Significant at 0.001

From the regression analysis, the following four types of regression lines were estimated for the survival of fish.

#### Model 1

$$\text{Survivability} = 82.36 + -0.24\text{PA} + 0.95\text{TC} + 0.35\text{BWCAS} + (-0.7)\text{THTD} + (-0.34)\text{WR} \quad (\text{i})$$

#### Model 2

$$\text{Fish survival} = 84.16 + 0.7\text{TC} + 0.27\text{BWCAS} + (-0.42)\text{THTD} + (-0.17)\text{EHTD} + (-0.26)\text{WR} \dots\dots\dots(\text{ii})$$

#### Model 3

$$\text{Survivability} = 81.79 + 0.65\text{PA} + 0.16(\text{TC}) \dots\dots\dots(\text{iii})$$

#### Model 4

$$\text{Survivability} = 90.48 + 0.2\text{PA} + -0.17\text{BWCAS} + 0.52(\text{CAS} * \text{TC}) \dots\dots\dots(\text{iv})$$

Where,

PA = Purchase amount (kg/lot)

TC = tank capacity (L)

BWCAS= Blower with coarse-air stone

THTD = 12 -hours of transport duration

EHTD = 8 -hours transport duration

WR= Frequency of waste removal

Live fish have a shorter supply chain than dead fish due to transport issues. Live fish shipping supply chains were similar (Adhikari *et al.*, 2018). Nepal's complicated aquaculture supply chain includes farmers, transporters, retailers, and customers. Rohu controls 25% of Nepal's fish market. Chitwan has more Rohu options than Kathmandu. Trout outsells Rohu. Consumers prefer Rohu over the dead or living version in surveys in western Nepal (Gurung *et al.*, 2016) and Bangladesh (Rahman & Islam, 2020). Rohu and Terai's observations are similar. Pangas were preferred to Magur (*Clarius sps*) (Gurung *et al.*, 2016). These studies found that Rohu is the best for Nepal's Terai region and trout for its hilly area. The survey found that Rohu is preferred to other fish due to its affordability and taste. Its quality and taste are unquestionable. Preserved Rohu is used when fresh Rohu is not available. Therefore, the consumer responded that fish was preferred for health, as the same result of Rahman, unlike Kathmandu residents, who value the health benefits of eating live

fish, do Chitwan residents value its nutritional value? That is why Kresic *et al.* (2022) wanted to spread the word about fish's health and nutritional benefits. Like Carlucci D. *et al.* (2015), the local consumer values fresh, locally caught, frozen fish. Responsible fishing improves fish nutrition (Lee & Sadovy, 1998), consumers prefer local products (Carlucci *et al.*, 2015), and Koreans place value on fish quality (Kim & Lee, 2018). Because domestic fish products are healthier (Lee & Sadovy, 1998). Due to concerns about synthetic contamination, silver carp and Naini fans prefer to buy them alive and fresh. Consumer income and family size affect price, freshness, and taste (Lee & Sadovy, 1998; Quagrainie *et al.*, 2008; Uddin *et al.*, 2019) (Gurung *et al.*, 2016). Chitwan consumers prefer Rohu to Kathmandu consumers, which is just the opposite in the case of trout. Therefore, local farms in these markets sell fresh versions of both species. (Table 1). A separate study in the US found that fish eaters had lower education and income (Burger, 2002). "Grass carp" and "Pangas" are used by people who cannot read or write and do not work, while "common carp" is used by those who can read, write, and work. Therefore, reliable live fish transport technology is needed so that people can buy fish to meet their nutritional needs. Kathmandu businessmen understand live fish better than Chitwan. Tank size has a large impact on fish survival but a small impact on fish numbers. According to Berka (1986), the price depends on the tank size needed to safely transport the fish. Supplier income rises proportionally to the farmer's share. But when there are many dead fish and the supplier's margin is lower than usual, farmers must give some of their profit to the supplier. Nepalese consumers of live fish consider that the price of live fish is not expensive. If live fish move in a small amount of water using traditional methods, they will die. Adhikari *et al.* (2018) believe that transporting live fish is dangerous, expensive, and energy-intensive, and that carp must be kept alive longer.

Chitwan has far more bureaucratic problems than Kathmandu. Most of the respondents said that many fish died in Kathmandu (68.50%) and that there were many rules at the Chitwan site (48.50%) during transit. Zhang (2019) also mentioned the mortality of fish transport. Table 4 shows the mean rank comparison of multiple responses, showing that Kathmandu's high mortality (68.50%) and Chitwan's administrative problems (48.50%) are Nepal's biggest live fish transport challenges. Moving live fish in small amounts of water is expensive due to high mortality (Zhang, 2019). Adhikari *et al.* (2018) suggest a change in the process

to ensure that carp arrive safely. The biggest problem is fish mortality (Table 4). It is a claim that passive aeration methods, such as microporous tubes (2%), porous stones (5%), and ceramic diffusers (44%), have much lower gas solubility than active methods, such as the venturi nozzle (60%) and the oxygenation cone (80%). The lack of aeration equipment poses two main risks to fish survival. First, the causes of fish mortality are being investigated. Thus, ordinary least squares (OLS) regression was used to determine the many factors that affect fish survival during marketing and transportation and how many fish survive. According to Timsina *et al.* (2018), multiple linear regression was used to examine interrelationships and estimate their predictive value. Model III of transport and marketing uses the SD of factors that affect the survival of live fish. The four regression models for shipping live fish are highly significant. The blower's coarse air stones share Model III's SD of 0.16. 44% of live fish transport success rates are due to constants. Most fish tanks use coarse air stones for aeration, which only release 3 to 5 mg/L of oxygen into the water and stress most fish. Small bubble sizes (Oliveira *et al.*, 2018) and DO increases to 25 from 6.5 mg/L (Mahasri, 2018) can increase fish survival by 44% compared to current practice. To improve the survival of live fish in transport, switch from coarse air stones to small bubbles.

## 5. CONCLUSION

Live Rohu (70.7%) and common carp (46.3%) are sold at the Nepalese fish market that each person eats. With the growing popularity of fish consumption in Nepal, the demand for live Rohu and common carp increases,

which is increasing prices and makes these two species more popular. Geographic and population factors that have not changed in the consumption pattern beside the concern for the benefit of the fish consumer groups. The high mortality rates and administrative obstacles present problems with the live fish supply chain, which is the biggest concern for both people who work in the business and customers. The survey found that the high death rate is due to the small size of the transport tank with aeration of the rough air stone. Because of these things, a lot of air or oxygen diffuses out through the water rather than dissolving in it. This research aims to find a link between cause and effect that explains how the aeration instruments and the transport tank affect each other. The results of this study show that the aeration system needs to be reexamined so that the maximum amount of gas dissolution that can occur in the transport tank can be increased to meet the demand for live fish in the supply and retailer market.

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## Annex: 1

### 1. Survey form of live fish consumer's perception

| Parameters                                                 | Options                |               |                      |         |       |
|------------------------------------------------------------|------------------------|---------------|----------------------|---------|-------|
| 1. Your residential location                               | Bagmati Terai          | Bagmati hills |                      |         |       |
| 2. education level                                         | literate               | school        | university           |         |       |
| 3. profession                                              | employed               | unemployed    |                      |         |       |
| 4. Do You Eat Fish?                                        | Yes                    | No            |                      |         |       |
| 5. Benefits of fish consumption                            | Diet                   | Health        |                      |         |       |
| 6. Can You Identify Fish species?                          | Yes                    | No            |                      |         |       |
| 7. Favorite Fish species (Choose At Least Two)             | Common carp            | Pangas        | Like Rohu            | Tilapia | Trout |
| 8. What Will You Do If You don't Find Live Fish In Market? | Return without buy     |               | Buy dead also        |         |       |
| 9. Which Factor Do You Prioritize When While Buying Fish?  | Freshness              | Price         |                      |         |       |
| 10. What Type of Favorite Fish Do You Prefer?              | Live                   | Dead          |                      |         |       |
| 11. Favorite Source of Fish                                | Hills                  | Terai         |                      |         |       |
| 12. Difference In Price of Dead and Live Fish              | Optimum                | Expensive     |                      |         |       |
| 13. Why Don't You Prefer to buy dead fish?                 | Unknown killing method |               | Unknown preservative |         |       |
| 14. Have You Ever Seen Transporting Live Fishes?           | Yes                    | No            |                      |         |       |

### 2. Survey form on the status of live fish suppliers

| Parameters                                | Option/amount                    |                              |                                              |
|-------------------------------------------|----------------------------------|------------------------------|----------------------------------------------|
| 1. Your Business address?                 |                                  |                              |                                              |
| 2. Business duration (year)?              |                                  |                              |                                              |
| 3. Farm gate price?                       |                                  |                              |                                              |
| 4. Purchase amount (kg/lot)?              |                                  |                              |                                              |
| 5. Purchase rate of live fish Nrs/kg)?    |                                  |                              |                                              |
| 6. Retailer price (Nrs/kg)?               |                                  |                              |                                              |
| 7. Transported tank capacity (L)?         |                                  |                              |                                              |
| 8. Transport duration (hr)?               |                                  |                              |                                              |
| 9. Rent of vehicle (NRS/Day)?             |                                  |                              |                                              |
| 10. Aeration Instruments?                 | Oxygen cylinder coarse air stone | Blower with coarse air stone |                                              |
| 11. Reason for of washing?                | To maintain Temperature          | To remove waste              |                                              |
| 12. Problems in Live Fish Transportation? | High Cost of Aeration            | High Mortality               | Low amount Transport Administrative obstacle |

### 3. Survey form on the status of live fish retailer's retailer

| Parameters                                 | Option/amount                    |                              |
|--------------------------------------------|----------------------------------|------------------------------|
| 1. Your business address?                  |                                  |                              |
| 2. Business duration (year)?               |                                  |                              |
| 3. Purchase price from suppliers (Nrs/kg)? |                                  |                              |
| 4. Purchase amount (kg/ lots)?             |                                  |                              |
| 5. Dead within purchase (kg)?              |                                  |                              |
| 6. Consumer price (Nrs/kg)?                |                                  |                              |
| 7. Sales Sale duration (days)?             |                                  |                              |
| 8. Daily sales sale amount (kg)?           |                                  |                              |
| 9. Installation tank capacity (L)?         |                                  |                              |
| 10. Rent of shop (NRS/Day)?                |                                  |                              |
| 11. Aeration Instruments?                  | Oxygen cylinder coarse air stone | Blower with coarse air stone |
| 12. Energy sources source for aeration?    | Domestic electricity             | Installed Backup power       |

# GROWTH PERFORMANCE OF LIVE FISH FEED: *Artemia salina* IN DIFFERENT SUPPLEMENTAL FEEDS IN AQUARIUM CULTURE

Nabin Khadka<sup>1,2,\*</sup>, Raksha Khadka<sup>3,4</sup>, Ram Bhajan Mandal<sup>5</sup> and Ashok Adhikari<sup>6</sup>

<sup>1</sup> Department of Aquaculture, Tribhuvan University, Nepal

<sup>2</sup> Nimbus International Company Pvt. Limited

<sup>3</sup> Sahakarmi Samaj, Kohalpur, Banke

<sup>4</sup> Graduate School, University of Maryland Eastern Shore, Maryland, U.S.

<sup>5</sup> Department of Aquaculture, Tribhuvan University, Nepal

<sup>6</sup> Ministry of Agriculture and Livestock Development, Singha Durbar, Kathmandu, Nepal

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\*Correspondence:

nabin.khadka.@nimbusnepal.com

Tel: +977-9867279407

## ABSTRACT

*Artemia* is an excellent live feed for newly hatched fish and crustaceans gained a unique position in the aquaculture system. Brine shrimp is a non-selective filter feeder, so different locally available source feeds should be evaluated as potential food source for their control mass culturing. The experiment was carried out to study the effect of different supplement feeds on the growth performance of *Artemia* at Central Fisheries Promotion and Conservation Centre, Baalaju, Kathmandu from December 2021 to February 2022. The experiment was carried out in complete randomized design (CRD) with four treatments, each treatment consisting four replications. Four treatments consist different combination of supplement feed i.e., T1: (spirulina + rice bran), T2: (soyabean + rice bran), T3: (Mustard oilcake + rice bran), T4: (Yeast + rice bran). All supplement feed contains 25% CP and feed diet was supplied by 2% of body weight. In terms of growth and yield performance parameters, significant effect was recorded on average length, individual weight and total biomass. Also, significant effect was recorded on survivability and density. However, no significant effect was recorded on CP content of *Artemia* biomass. T1 recorded the maximum average length ( $8.7 \pm 0.21$  mm), individual weight ( $0.0028 \pm 0.0001$  gm), and biomass ( $1.79 \pm 0.069$  gm/L) whereas lowest average length ( $7 \pm 0.08$  mm), individual weight ( $0.0013 \pm 0.0002$  gm) and biomass ( $0.61 \pm 0.005$  gm) was recorded in T4. Also, T1 recorded the maximum density ( $633.8 \pm 3.62$ ), and survivability ( $70.8 \pm 0.5$ ) whereas lowest density ( $467.9 \pm 4.26$ ) and survivability ( $58.0 \pm 1.71$ ) found in T4. Hence it can be concluded that the combination of spirulina and rice bran was best supplement diet and can be applied in the cultivation of *Artemia* for best growth performance and biomass Production in aquarium condition.

## 1. INTRODUCTION

*Artemia* is a genus of aquatic crustaceans also known as brine shrimp. *Artemia* populations are found worldwide in inland saltwater lakes, but not in oceans. *Artemia* are able to avoid cohabiting with most types of predators, such as fish, by their ability to live in waters of very high salinity up to 25% (Daintith, 1996). *Artemia* is a typical primitive arthropod with a segmented body to which is attached broad leaf-like appendages.

*Artemia* is widely used by aqua-culturist as an excellent live food for crustacean and fish (Azad *et al.*, 2018). It constitutes the principal ration and frequently the only food for the larvae and juveniles of many cultured species, such as freshwater prawns (*Macrobrachium* sp.), shrimp (penaeids), and lobsters (*Homarus* sp.), crabs and various finfish (Bardach *et al.*, 1972). *Artemia* Nauplii constitutes the most widely used food item among the live diets used in the larviculture of fish and shell fish (Van Stappen, 1996). *Artemia* considered as

an aquaculture live food, has a high nutritional content (Wache and Laufer, 1997). *Artemia* was found by several investigators to be an excellent food for newly hatched fish and shrimp larvae (Sorgeloos *et al.*, 1998). Being live feed, the larvae of *Artemia* are readily taken by aquatic animals under nursery conditions without fouling aquarium water (Islam *et al.*, 2019).

Inadequacy, and absence of live feed increase the chances of mortality of fish seed in hatcheries. Besides this, the inconsistent supply of the live feed in Nepalese market has limited the access to live feed for hatcheries. In Nepal, there is trend of feeding fish seed with *Artemia* at its juvenile stage with no any instances of feeding *Artemia* in its adult stage. Culturing *Artemia* up to its adult stage helps to feed fish seed with the different stages of *Artemia* i.e., nauplii, juvenile, and adult. This can address the constraints of inconsistent supply in the market and ensure its constant availability

in the hatchery. So, better combination is important for culturing *Artemia* to boost its growth and survivability.

Thus, the objective of this research is to compare the growth performances of live feed *Artemia salina* under the different locally available feed sources to find best combination.

## 2. MATERIALS AND METHODS

### 2.1 Location of experimental set up, period, design and unit

The experiment was carried out in the research laboratory of Central Fisheries Promotion and Conservation Center (CFPCC), Baalaju, Kathmandu which is located at Bagmati province of Nepal. Geographically the site is located at 27°44'0" N latitude, 85°18'0" E longitude and an altitude of 1400 meters above sea level. The experiment was conducted for 90 days from December 1, 2021 to February 30, 2022. The experiment was conducted in completely randomized design (CRD) having four treatments and four replications. In total

there were 16 experimental units. The randomization of treatments was done by creating random numbers in the MS Excel sheet using (RAND) command.

### 2.2 Treatments details

The total of four treatments were included in this experiment. The treatment consists of different combination of supplement feeds as Spirulina and rice bran, Soyabean meal and rice bran, Mustard oil cake and rice bran, Yeast and rice bran. The feed consists of both carbohydrate and protein source. The 25 % supplement feed was formulated by two feed sources according to the Protein requirement of *Artemia*. The 25% CP feed was formulated according to Pearson square method and feed was supplied according to the body weight of *Artemia* nauplii.

### 2.3 Proximate analysis

Proximate composition of different supplement feeds is given in Table 1.

**Table 1.** Proximate composition of different supplement feeds.

| Treatment (Mean) | Moisture(M) % | Crude Protein (CP)% | Fat(F)% | Total Ash (TA)% | Fiber (Fb) % | Method of analysis |
|------------------|---------------|---------------------|---------|-----------------|--------------|--------------------|
| T1               | 11.82         | 25                  | 10.27   | 7.74            | 6.255        | NIRS               |
| T2               | 12.225        | 25                  | 7.45    | 7.94            | 8.36         | NIRS               |
| T3               | 12.625        | 25                  | 7.32    | 7.59            | 8.505        | NIRS               |
| T4               | 12.125        | 25                  | 7.52    | 7.84            | 8.255        | NIRS               |

\*T1= Spirulina + rice bran, T2 = Soyabean meal + rice bran, T3 = Mustard oil cake + rice bran, T4 = yeast +rice bran, NIRS- Near Infra Red Spectroscopy

### 2.4 Sample collection

The crude salt and different supplement feeds were collected from market. The saline water was made 30 ppt and proximate analysis of feeds was analyzed by NIRS and Kjeldahl's method at Animals feeds and quality control laboratory at Harihar Bhawan, Lalitpur Nepal. *Artemia* cyst used in this experiment was collected from laboratory of Fisheries promotion and conservation Centre, Kathmandu Nepal.

### 2.5 Experiment set up

Four V-shaped containers made of plastic material were set up in the laboratory for hatching cyst. For culturing

*Artemia* sixteen aquaria were set up for rearing *Artemia nauplii*. The necessary equipment (aerators, heaters, thermometer, refractometer, Hanna pH meter, microbalance, burette, pipette etc.) were collected and set in the laboratory for measuring the water quality parameters and biomass of *Artemia* at different concentrations. The containers were kept at a position of the laboratory where sunlight could not bring any deleterious effects. Heaters were used to maintained temperature. Artificial aerator was used for maintaining the optimum level of oxygen. Florescent lamp was fitted to provide sufficient light during hatching. The aquaria were filled with 20-liter saline water having

concentrations 30 ppt. 3 gm/ liter *Artemia* cyst was provided in four containers containing 3-liter saline water having concentration of 30 ppt. Total biomass was measured by using microbalance (electrical analytical balance, OSK 11325A).

## 2.6 Hatching

Four 'V' shaped plastic containers containing 3-liter of saline water (30 ppt) were used for hatching. The decapsulated *Artemia* cyst 3 gm/ liter was provided to each tank with continuous vigorous aeration. Heater was provided to maintain temperature 25 degree centigrade. Constant florescent lamp was provided. After 48 hours hatching nauplii were harvested by turning off the air flow of the aerator. Then, nauplii were attracted by light, they were concentrated at the bottom of the 'V' shaped container where sufficient light was ensured. The hatched nauplii was extracted from the container onto a beaker with 100 ml distilled water. The content was mixed well and 1-ml sample was taken by pipet and put into Rafter Counter for counting. The counted nauplii @1 individual/ ml was transferred into culture tank by pipette.

## 2.7 Inoculation and feeding

The counted *Artemia nauplii* were inoculated in the rearing aquarium after hatching. The water of the aquarium was previously enriched with different supplement feeds. The different supplement feed was provided twice a day by 2 % of nauplii body weight. Feeding was done early morning and evening time. Nauplii became pre- adult after 5-8 days of hatching. Moderate and continuous aeration was provided by aerators to keep the food in suspension and supply of oxygen to *Artemia*. The growth rate and weight of *Artemia* was measured every 3<sup>rd</sup> day from 9<sup>th</sup> day. The experiment lasted for 21 days.

## 2.8 Harvesting

Adult *Artemia* was completely collected on 21 days manually by using 120µm size net. Harvested adult *Artemia* was dried for proximate analysis (CP %) by NIRS method.

## 2.9 Determination of water quality

The important water quality parameters such as temperature, dissolved oxygen and pH were measured periodically. A laboratory thermometer, electrical pH-meter and DO meter were used to measure temperature,

pH and dissolved oxygen respectively.

## 2.10 Measuring technique

A homogenous distribution of *Artemia* in water of the tanks was ensured by giving continuous vigorous aeration before sampling. The 100 ml water sample was taken from each aquarium to estimate the density, individual weight, average length, survivability, CP % and biomass of *Artemia* by different technique.

The body length was measured by using a microscope equipped with a drawing mirror and calculated using a digitizer. Adult *Artemia* was measured by measuring scale. The randomly selected individual weight was measured by micro weighing machine. The survivability was calculated by Counting and comparing individuals with initial stocking. For density, the individuals were counted manually from a randomly selected sample from each treatment. The harvested individuals were Measured by micro weighing machine. The total biomass was measured by digital electrical balance (electrical analytical balance, OSK,11325A).

The harvested adult artemia was taken from each treatment and dried. The crude protein was calculated by NIRS method on dry basis at Central, Fisheries promotion and conservation center, laboratory which is located at Baalaju, Kathmandu Nepal.

## 2.11 Statistical analysis

All the collected data were calculated and tabulated according to replication and treatments. MS excel was used for tables and SPSS version 20 and Gen stat 15<sup>th</sup> edition for statistical analysis. The growth Parameters of *Artemia* during experimental period in same temperature and salinity with different feed was tested using one way Analysis of Variance. ANOVA was done to test the significance of difference for each parameter. Calculation of the significant difference at 5% level of significance made by the mean comparison. The comparison of mean was done by DUNCAN at 5% level of significance.

## 3. RESULTS AND DISCUSSION

The experimental finding obtained during the investigation entitled "Growth performance of *Artemia Salina* in different supplemental feeds in aquarium condition at Balaju Kathmandu" were analysed. The results are presented and discussed below;

### 3.1 Water quality parameters

For an adequate production of *Artemia* in controlled conditions, water quality parameters should be maintained within an optimal range (salinity between 30-65 ppt, oxygen above 2 mg/L, temperature between 19-25 °C, and pH between 6.5-8.0). No significant effect was obtained on temperature, dissolved oxygen and pH of water with different supplements feeds (Table 2).

**Table 2.** Water quality parameters during experiment period.

| Treatments   | Water quality parameters |                              |         |
|--------------|--------------------------|------------------------------|---------|
|              | Dissolved Oxygen (mg/l)  | Temperature (Degree Celsius) | pH      |
| T1           | 5.525                    | 26.40                        | 6.425   |
| T2           | 5.850                    | 26.70                        | 6.450   |
| T3           | 5.050                    | 26.80                        | 6.775   |
| T4           | 5.975                    | 27.80                        | 6.950   |
| Significance | NS                       | NS                           | NS      |
| CV           | 4.3%                     | 6.4%                         | 6.9%    |
| SE           | ± 0.2574                 | ± 1.713                      | ± 0.460 |
| Df           | 12                       | 12                           | 12      |
| L.S.D.       | 0.3965                   | 2.639                        | 0.708   |

### 3.2 Effect of different supplement feed on survivability, density and crude protein content of artemia.

The effect of different supplement feed on Survivability was found highly significant ( $p < 0.05$ ) as shown in Table 3. The highest survivability rate ( $70.8 \pm 0.57\%$ ) was found in supplement feed with spirulina and rice bran which is statistically significant with other supplement feeds whereas lowest survivability rate ( $58 \pm 1.71$ ) was found in yeast and rice bran which is statistically at par with the supplement feeds mustard oil cake with rice bran and soyabean meal with rice bran. Similarly, the effect of different supplement feed on density was found highly significant ( $p < 0.05$ ) as shown in table 3. The highest density ( $633.8 \pm 3.62$ ) was found in supplement feed spirulina and rice bran which is highly significant with others supplement feeds whereas lowest density ( $467.9 \pm 4.26$ ) was found in supplement feed yeast with rice bran which is statistically at par with mustard oil cake with rice bran. Also, there is no significant ( $p > 0.05$ ) effect among the treatments with crude protein content in *Artemia* biomass.

**Table 3.** Mean value of survivability, density, CP % content of Artemia by feeding different supplement feeds

| Treatment                   | Parameters $\pm$ SE |                   |                 |
|-----------------------------|---------------------|-------------------|-----------------|
|                             | Survivability%      | Density ind./L    | C.P%            |
| Spirulina +Rice bran        | 70.8a $\pm$ 0.57    | 633.8a $\pm$ 3.62 | 66.7 $\pm$ 0.17 |
| Soyabean meal +Rice bran    | 61.1b $\pm$ 1.20    | 514.9b $\pm$ 4.41 | 66.5 $\pm$ 0.09 |
| Mustard oil cake +Rice bran | 58.5b $\pm$ 0.78    | 473.6c $\pm$ 5.04 | 66.5 $\pm$ 0.11 |
| Yeast + Rice bran           | 58.0b $\pm$ 1.71    | 467.9c $\pm$ 4.26 | 66.5 $\pm$ 0.10 |
| Sig.                        | **                  | **                | Ns              |
| LSD (0.005)                 | 3.559               | 13.46             | 0.3875          |
| F (3,15) Value              | <0.001              | <0.001            | 0.39            |
| P value                     | 26.92               | 311.30            | 0.761           |
| CV (%)                      | 3.7                 | 1.7               | 0.4             |
| Grand mean                  | 62.14               | 522.6             | 66.59           |

### 3.3 Effect of different supplement feed on average length, individual weight, biomass and total biomass of artemia.

The effect of different supplement feed on average length was found highly significant ( $P < 0.05$ ) as shown in table 3. The highest average length ( $8.7 \pm 0.21$ mm) was found in supplement feed Spirulina and rice bran which is followed by supplement feeds soyabean meal with rice bran whereas lowest average length ( $7.0 \pm 0.08$  mm) was found in the supplement feed yeast and rice bran which was also statistically at par with mustard oil cake. Similarly, the effect of different supplement feed on individual weight was found highly significant ( $P < 0.05$ )) as shown in table 3. The highest individual weight ( $0.0028 \pm 0.0001$  gm) was found in Supplement feed spirulina and rice bran which is statistically significant with other supplement feeds. Similarly, the lowest individual's weight ( $0.0013 \pm 0.002$ gm) was found in supplement feed yeast and rice bran which is statistically significant with other supplement feeds. Also, the effect of different supplement feed on total biomass was found highly significant ( $p < 0.05$ ) as shown in table 3. The highest biomass concentration ( $66.7 \pm 0.17$ ) was found in Spirulina and rice bran which is statistically significant with other supplement feed. Similarly, the lowest biomass concentration ( $12.2 \pm 0.11$ ) was found in yeast and rice bran which is statistically at par with supplement feed mustard oilcake and rice bran.

The purpose of the present study was to analyses four

**Table 4.** Mean value of average length, individual weight, biomass, total biomass, of *Artemia* in different supplement feeds.

| Treatment                    | Parameters<br>Mean± SE) |                       |              |                       |
|------------------------------|-------------------------|-----------------------|--------------|-----------------------|
|                              | Average length(mm)      | Individual weight(gm) | Biomass gm/L | Total biomass gm/20 L |
| Spirulina + Rice bran        | 8.7a ± 0.21             | 0.0028a±0.0001        | 1.79a±0.09   | 35.7a±1.38            |
| Soyabean meal + Rice bran    | 8.2a ±0.40              | 0.0021b±0.0009        | 1.11b±0.05   | 22.2b±1.02            |
| Mustard oil cake + Rice bran | 7.1b ±0.06              | 0.0016c±0.0056        | 0.79c±0.03   | 15.8c±0.60            |
| Yeast + Rice bran            | 7.0b±0.08               | 0.0013d±0.0020        | 0.61c±0.05   | 12.2c±0.11            |
| Sig.                         | **                      | **                    | **           | **                    |
| LSD (0.005)                  | 0.719                   | 0.0002320             | 0.1412       | 2.824                 |
| F (3,15) Value               | <.001                   | <.001                 | <.001        | <.001                 |
| P value                      | 12.41                   | 75.62                 | 127.29       | 127.29                |
| CV (%)                       | 6                       | 7.6                   | 8.5          | 8.5                   |
| Grand mean                   | 7.81                    | 0.001992              | 1.077        | 21.45                 |

different types of supplement diet and their effects on the growth performance, survivability, density and total biomass yields and also which of these feeds yields best results for the production of adult brine shrimp. The different supplement feed used in these experiments have very different chemical composition, the quantity of food required for maximum growth, survival rate, density and total biomass yields of *Artemia* varied from one type of feed to another feed.

Survivability depends on many factors like nature of feeds and environment of culture medium. The optimum temperature, dissolved oxygen and pH remained constant. So, survival rate directly depends on nature, quality and source of supplement feeds. This result possibly due to high protein content (57%) of diet. The protein content in spirulina is complete since it contains all essential amino acids forming 47% of protein weight (Bujard-E *et al.*, 1970). Also, Spirulina is a rich source of vitamins, minerals, poly unsaturated fatty acids, enzymes and pigments. It also enhances the nonspecific immune system, increasing the state of readiness of natural defenses. Beside this Spirulina protein is highly digestible because of absence of cellulose walls (Dillon and phan, 1993). The combination of nutrients, pigments and immunostimulants explains reports of dietary spirulina yielding better growth, appearance, reduction of stress and high survival rate. Similarly, rice bran is very cheap products available worldwide

which supports better growth in *Artemia* and is very suitable for batch culturing because it's contents of water-soluble components are minimal (Sorgeloos *et al.*, 1980). As a result of its fibrous structure, less than 50% of the initial crude product can be processed into *Artemia* food by homogenizing and sieving treatments. Similarly, in soyabean meal high ammonia levels were build up and high mortality were noted. This is due to fact that, soyabean powder appears to contain high amounts of soluble proteins which cannot be ingested by *Artemia* but provokes water pollution by bacterial development. (Sorgeloos *et al.*, 1980). This clearly indicates the suitability of spirulina with rice bran for best diet of *Artemia* in terms of growth performance and survivability.

The best yield found in spirulina containing feed is due to spirulina is nutritionally significant because of high concentrations of natural nutrients, bio-modulatory and immune-modulatory functions (Khan *et al.*, 2005). It contains various B-vitamins and minerals in high amount of calcium, iron, magnesium, manganese, potassium and zinc (Binkava *et al.*, 2002; Gireesh *et al.*, 2004). Spirulina contains ten times more proteins of dry weight than soyabean and three times more than that of beef proteins (Dillon *et al.*, 1995).

Khan *et al.* (2005) reported that spirulina species have antibacterial and antiparasitic activity. According to Bujard-E *et al.* (1970) protein content in spirulina is

complete since it contains all essential amino acids, poly unsaturated fatty acids (PUFA), and forming 47 % of total protein weight. Spirulina has several pharmacological activities such as anticancer, antiviral, antibacterial, metallo-protective, and immunostimulant effects. Thus, spirulina have both therapeutic and nutritional application. Mechanisms of anticancer, antiviral and antimicrobial effects of spirulina is due to its endonuclease which repair damaged DNA. Calcium sulfated polysaccharides which inhibit the invitro replication of viruses and fatty acids especially high content of  $\gamma$  linolenic acid which have a crucial role in growth and individual mass. Also, the metallo-protective role of spirulina may be attributed to the presence of beta-carotene, vitamins C, E, enzymes, superoxide dismutase, selenium and brilliant blue polypeptide pigment phycocyanin. Cyanobacteria have been found to be a good source of protein for fish (Nandesha *et al.*, 2001).

In addition, spirulina have no cell walls which results better digestion and absorption (Desikachary, 1959). This clearly shows that spirulina have positive effect on growth and yield of *Artemia*. From the above result, it is concluded that spirulina is a rich source of protein, vitamins, minerals and pigments. It also enhances the nonspecific immune system, increasing the state of readiness of natural defenses. The combination of nutrients, pigments and immunostimulants explains

reports of content dietary spirulina yielding better growth, reduction of stress and better appearance. Hence, spirulina gave the best result in growth performance and total biomass. Also, this result reveals that spirulina could be used as protein and lipid source diet for *Artemia* growth and production to have a better culturing and gain market acceptance. The extremely poor survival and growth of *Artemia* cultured on fresh baker's yeast clearly showed that, this diet is an inadequate food for brine shrimp. The observation of intact yeast cells in the fecal materials of *Artemia* fed fresh baker's yeast reveal a problem with digestibility of yeast by *Artemia*.

#### 4. CONCLUSION

The result indicated that different Supplemental feeds had a significant effect on growth parameters and total yields but did not affect on crude protein content in biomass of *Artemia* which are crucial parameters for Production purposes. Hence it can be concluded that the combination of spirulina and rice bran was best supplement diet and can be applied in the cultivation of *Artemia* in aquarium condition for best growth performance and biomass Production in controlled condition.

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# JOB SATISFACTION OF GOVERNMENT AGRICULTURE OFFICERS WORKING IN THE EXTENSION SERVICE OF NEPAL

Min Bahadur Pun <sup>1,\*</sup>, Ram Hari Timilsina <sup>2</sup>, Narayan Raj Joshi <sup>2</sup> and Udit Prasad Sigdel <sup>2</sup>

<sup>1</sup> Agriculture Knowledge Center, Chitwan, MoALD, Bagmati Province, Nepal

<sup>2</sup> Agriculture and Forestry University, Rampur, Chitwan, Nepal

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\*Correspondence:  
minraj2008@gmail.com  
Tel.: +977-9863937179

## ABSTRACT

*A study was conducted to assess the job satisfaction status of government agriculture officers working in the extension service of Nepal and to find out the significant satisfaction determining factors as perceived by them. Semi-structured questionnaire was applied via online Google forms and findings were drawn from 112 responses. 64.29% respondents perceived their overall job satisfaction to be neutral. Regarding the individual factors, they were found to be satisfied with four factors, namely; Physical facilities at the office, Guidance of/and relationship with supervisors/office chief, Cooperation of/and relationship with co-workers and welfare/retirement facility, but dissatisfied with capacity building and job promotion opportunity while neutral to pay factor. Regarding the most preferred attribute of job satisfaction, capacity building and job promotion opportunity ranked first. Guidance of/and relationship with supervisors/office chief and Pay factor ranked second and third respectively. 52.63% of the respondents working in central or policy level offices were found to be with less workload than they expect. 54.5% of respondents expressed having unfair practices in case of various opportunities within the organization. There should be enough and effective capacity enhancement opportunities and the concerned organization should foster a fair system to make them happy and productive manpower of the country.*

## 1. INTRODUCTION

Agricultural development is one of the most powerful tools to end extreme poverty, boost shared prosperity and feed a projected 9.7 billion people by 2050 (World Bank, 2020). Nepalese economy is considerably dependent on agriculture, which provides employment opportunities to around 65 % of the total population and contributes about 27 % in Gross Domestic Product (GDP) (MoALD, 2022). Despite the government of Nepal's (GON's) priority and policy supports for more than two decades, the growth of agriculture has been prolonged (less than 3.0 percent), mainly due to inadequate access to demand-driven technologies and extension services and inputs, credits, markets and incentives (Sah & Babu, 2019). Agricultural extension service is the foundation for any meaningful development in the agricultural sector (Dessalegn, 2014). Agricultural extension work becomes effective when the extension professionals are qualified, motivated, committed, and responsive to the ever-changing social, economic and political environment (Gebrehiwot, Kinfu & Deribe, 2012).

The administrative structure of the agriculture extension service in Nepal has been changing with the changing policy environment in the country. Before the country's restructuring, most agricultural extension functions were under the purview of Ministry of Agriculture and Livestock Development (MoALD) and its central and district level units. But with the promulgation of a new constitution in 2015 and a successful election of federal, provincial, and local levels of government in 2017, agriculture has been under the concurrent right of all levels of government – federal, provincial, and local, as per the constitutional provision. However, most of the extension functions have been vested on the local and provincial level government (Jaishi, Nepali & Shahi, 2018). For government officials, satisfaction with their job has a strong implication for upgrading the quality of government services and directly impacting the quality of services given to the citizen. Agriculture officers play a dynamic role in their respective fields. They are involved in various activities as Subject Matter Specialists (SMS) to solve farmers' problems and plan, implement, and report agricultural programs assigned

to them. As such, to achieve sustained agricultural productivity growth, agriculture officers' competence and their level of job satisfaction count crucial.

Job satisfaction refers to the degree to which employees have pleasurable or positive emotional state towards the job (Locke, 1976). It is an important area from an organizational perspective because it correlates with other variables such as job motivation, organizational commitment, performance, turnover, and absenteeism that directly impact an organization's effectiveness (Seid, Muluken & Mulugeta, 2013). So, employee satisfaction has been momentous in recent times to develop and accomplish organizations' goals and objectives. There are a variety of factors that can influence a person's level of job satisfaction. According to Kaya (1995), job satisfaction is the sum of all negative and positive aspects of the individuals' salary, physical and emotional working conditions, authority, and autonomous usage of this authority. A study conducted by Ellickson and Logsdon (2002) reflected that job satisfaction of public sector employees was significantly influenced by perceptions of employee satisfaction in terms of pay, promotional opportunities, relationships with supervisors, employees' performance management systems, and fringe benefits.

It is often criticized that government organizations are not doing enough to provide reliable and quality services (Subedi & Chaudhary, 2014). According to Sattar (2014), shortage of economic resources (low salary, poor working conditions), corruption, nepotism and favoritism, political interference and instability, dysfunctions of bureaucracy are the leading issues of developing states regarding job satisfaction of their employees. Subedi & Chaudhary (2014) found that civil servants of Nepal were with a low level of job satisfaction regarding the salary and facilities. However, they had average satisfaction levels in other dimensions of satisfaction as supervisor, promotion, work opportunities, and human relationship characteristics. Despite its importance, issue of job satisfaction has not still received proper research attention neither from researchers nor managers in any sector of Nepal. In such context, this study aimed to assess the job satisfaction level and the significant factors associated with it as perceived by the government agriculture officers working in extension service of Nepal.

## 2. MATERIALS AND METHODS

### 2.1. Population sampling and data collection

Though the study was mainly based on a quantitative research approach, qualitative research approach was

also adopted to draw views about related issues in general. The population chosen consisted of 6<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> level agriculture officers of the provincial government and Class III officers of the federal government of Nepal. The data was collected in the period of May to August, 2020. The researcher purposively requested maximum of agriculture officers of each province via online Google form, until required sample size was reached. Out of 120 respondents who submitted the questionnaire, 112 responses were taken into account with at least 5 representations from every province, based on the trueness of the response.

### 2.2 Survey instrument

The job satisfaction assessment questionnaire comprised of six factors of job, namely; Physical Facilities at office, Guidance of/and relationship with Supervisors/ Office Chief, Cooperation of and relationship with Co-workers, Pay (Salary, Allowances, and Reward), Welfare/Retirement facilities, Capacity Building and Job Promotion opportunity, each of which consisted 3 to 5 statements related to various aspects of those factors, as such 25 indicators in total. Statements in each factor were designed so that respondents had to choose their degree of agreement or disagreement next to each statement, which was the measure of the extent to which they were satisfied or dissatisfied with their current jobs.

### 2.3 Measurement of level of job satisfaction

A five-point Likert interval scale measurement was taken to measure the level of job satisfaction. Statements in the questionnaire were designed in such a way that respondents had to choose their degree of agreement or disagreement next to each statement of various factors of job satisfaction, which was the measure of the extent to which they were satisfied or dissatisfied with that factor in their current jobs, as shown in Table 1.

**Table 1.** Measures of five-point Likert interval scale

| Measure                    | Likert scale | Meaning                            |
|----------------------------|--------------|------------------------------------|
| Strongly disagree          | 1            | Strongly dissatisfied              |
| Disagree                   | 2            | Moderately dissatisfied            |
| Neither agree nor disagree | 3            | Neither satisfied nor dissatisfied |
| Agree                      | 4            | Moderately satisfied               |
| Strongly agree             | 5            | Strongly satisfied                 |

### 2.3.1 Overall satisfaction

Overall satisfaction of the respondents towards their job was assessed from mean value of the scale values assigned to 25 indicators/statements related to 6 factors.

#### Facet satisfaction

Facet satisfaction of the respondents towards particular factor was assessed based on the mean value of the scale values for that factor assigned by 112 respondents on 3 to 5 indicators/statements related to that factor.

### 2.3.2 Category of satisfaction level

Job satisfaction level was grouped into three categories, which was based on the mean value of the scale values assigned to indicators on each factor as shown in the Table 2.

**Table 2.** Measures of satisfaction level

| Mean value      | Satisfaction level                              |
|-----------------|-------------------------------------------------|
| >3+S.D.         | Satisfied                                       |
| 3-S.D. - 3+S.D. | Neither satisfied nor dissatisfied<br>(Neutral) |
| <3 - S.D.       | Dissatisfied                                    |

#### Measurement of Most Significant Factors of Job Satisfaction

The respondents were asked to choose only 3 in order, out of given six factors of job satisfaction which they think more important than others and rank them as first, second and third. Rank value of 3, 2, and 1 was given for the first, second, and third important factors, and the index value was calculated to finalize the rank using following formula.

Estimated index value to finalize the ranks

$$I_{\text{prob}} = \sum \frac{S_i F_i}{N}$$

where,

$I_{\text{prob}}$  = Index value for intensity ( $0 < I < 1$ )

$S_i$  = Scale of value of  $i^{\text{th}}$  intensity

$F_i$  = Frequency of  $i^{\text{th}}$  response

$N$  = Maximum probable score of  $\sum S_i F_i$  (336)

## 3. RESULTS AND DISCUSSION

### 3.1. Demographic characteristics of respondents

Table 3 and 4 show the demographic characteristics of the respondents. Almost half of the respondents were from Bagmati province. This was because the maximum number of central offices of federal government is located in this province and also there are relatively less vacant seats of agriculture officers in the offices of either government. Lesser number of participations from province 1, Karnali and Sudharpachchim province was due to high number of vacant seats of agriculture officers in the offices of provincial government in those provinces. Lesser number of participations from the province 2 was mainly due to the ignorance to respond. Lesser percentage of response (only 6) from the officer level 6<sup>th</sup> was mainly due to lesser number of them in the provincial government and also their ignorance to respond. Primary reason behind the variation in the number of participations from various discipline was the variation in available officers of respective discipline, for example, soil officers are in comparatively lesser number in the agricultural extension system of Nepal.

**Table 3.** Demographic characteristics of the respondents

| Demographics                  | Number | Percentage |
|-------------------------------|--------|------------|
| Working office                |        |            |
| Federal office                | 54     | 48.2       |
| Provincial office             | 58     | 51.8       |
| Central/policy level office   | 38     | 33.9       |
| Implementation office         | 74     | 66.1       |
| Provincial participation      |        |            |
| Koshi Province                | 10     | 8.9        |
| Madhesh Province              | 7      | 6.3        |
| Bagmati province              | 50     | 44.6       |
| Gandaki province              | 19     | 17         |
| Lumbini province              | 14     | 12.5       |
| Karnali province              | 5      | 4.5        |
| Sudurpachchhim province       | 7      | 6.3        |
| Designation level             |        |            |
| Officer level 6 <sup>th</sup> | 5      | 4.46       |
| Officer level 7 <sup>th</sup> | 37     | 33.03      |
| Officer level 8 <sup>th</sup> | 16     | 14.28      |
| Officer class III             | 54     | 48.21      |

| Working discipline |    |      |
|--------------------|----|------|
| Extension          | 41 | 36.6 |
| Horticulture       | 20 | 17.9 |
| Agronomy           | 11 | 9.8  |
| Plant protection   | 15 | 13.4 |
| Agri-Economics     | 19 | 17   |
| Soil Science       | 6  | 5.4  |

As shown in the Table 4, female respondents were only 18.75%, which clearly shows that government agriculture extension system of Nepal is male dominated. The demographics of respondents shows increase in female (15.28%) extension advisors in Nepalese Extension System, which is higher than the percentage of female extension workers in Nepal- 6.9% (Ghimire *et al.*, 2016), -7.7% (worldwide Extension, 2011). Almost two third of respondents had completed master's degree which indicates that maximum of agriculture officers come to the government section come after post-graduation. Less number of respondents having intermediate education tells that officer level 6<sup>th</sup>, who was the promoted technicians in the provincial government, responded less. More than two third of respondents were Brahmin/Chhetri and only 2 out 112 were from Dalit caste. This clearly reflects that civil service employment is dominated primarily by so called high castes. Only 8.9 % of respondents from the urban family background might reflect that maximum of those who study agriculture subject come from rural and semi-urban areas.

**Table 4.** Demographic characteristics of the respondents

| Demographics             | Number | Percentage |
|--------------------------|--------|------------|
| Gender                   |        |            |
| Male                     | 91     | 81.2       |
| Female                   | 21     | 18.8       |
| Age                      |        |            |
| Up to 30 years           | 59     | 52.7       |
| More than 30 to 40 years | 46     | 41.1       |
| More than 40 years       | 7      | 6.3        |
| Job duration             |        |            |
| Less than 5 years        | 57     | 50.9       |
| 5 to 10 years            | 45     | 40.2       |
| More than 10 to 20 years | 6      | 5.4        |
| More than 20 years       | 4      | 3.6        |

| Education level   |    |      |
|-------------------|----|------|
| Intermediate      | 5  | 4.5  |
| Bachelor          | 24 | 21.4 |
| Master            | 83 | 74.1 |
| Caste group       |    |      |
| Brahmin/Chhetri   | 79 | 70.5 |
| Janajati          | 20 | 17.9 |
| Dalit             | 2  | 1.8  |
| Madhesi           | 11 | 9.8  |
| Family background |    |      |
| Rural             | 52 | 46.4 |
| Semi-Urban        | 50 | 44.6 |
| Urban             | 10 | 8.9  |

### 3.2 Status of job satisfaction

#### 3.2.1 Overall satisfaction status

As discussed in the methods, overall satisfaction level of respondents was drawn, which was as presented in Table 5. The result showed that only 30.36% of the agriculture officers were satisfied with their job, 64.29% of them were indifferent (neither satisfied nor dissatisfied), and the remaining 5.36% were dissatisfied with their job.

**Table 5.** Overall satisfaction level of respondents

| Category                                         | Number | Percentage |
|--------------------------------------------------|--------|------------|
| Satisfied                                        | 34     | 30.36      |
| Neither satisfied nor dissatisfied (Indifferent) | 72     | 64.29      |
| Dissatisfied                                     | 6      | 5.36       |

#### 3.2.2 Satisfaction status for various factors of job

Satisfaction level for each of factors taken as a measure of job satisfaction was also measured, which was found as shown in Table 6. Mean value of assigned values for all indicative statements attached to each factor was calculated across all 112 respondents and the satisfaction level was categorized into three groups as discussed in the methodology.

**Table 6.** Satisfaction status for various factors of job

| S.N. | Factors                                                    | Mean | S. D. | Category     | Remarks         |
|------|------------------------------------------------------------|------|-------|--------------|-----------------|
| 1    | Physical facility at the office                            | 3.57 | 0.29  | Satisfied    | >3+S.D.         |
| 2    | Guidance of and relationship with supervisors/office chief | 3.69 | 0.11  | Satisfied    | >3+S.D.         |
| 3    | Cooperation of and relationship with co-workers            | 3.82 | 0.18  | Satisfied    | >3+S.D.         |
| 4    | Pay (salary, allowances, and reward)                       | 2.86 | 0.27  | Indifferent  | 3-S.D. - 3+S.D. |
| 5    | Welfare and retirement facility                            | 3.41 | 0.34  | Satisfied    | >3+S.D.         |
| 6    | Capacity building and job promotion opportunity            | 2.41 | 0.26  | Dissatisfied | <3-S.D.         |

As observed in Table 6, the six factors taken as a measure of job satisfaction in the study possessed variation in the mean values, which ranged between (2.4-3.82). Agriculture officers were found to be at a satisfied level for four factors, namely; physical facility at the office, Guidance of and relationship with supervisors/office chief, Cooperation of and relationship with co-workers and Welfare and retirement facility with mean value 3.57, 3.69, 3.82 and 3.41 respectively while they were at indifferent level for the pay factor with mean value 2.86. However, they were found to be dissatisfied with the factor “Capacity building and job promotion opportunity” with a minimum mean score (2.41). This illustrates that this factor contributes the most significant proportion for overall dissatisfaction of agriculture officers under study. The findings are consistent with Subedi and Chaudhary (2014) in case of Guidance of and relationship with Supervisors/office chief and that with coworkers as well as pay factor, but in contrast in case of capacity building and job promotion opportunity. Subedi and Chaudhary (2014) found the government employees to be at average level of satisfaction

### 3.2.3 Comparing the average job satisfaction level for various demographic variables

Mann-Whitney test was run for variables; working office, gender and marital status. As shown in the Table 7, the p-value of the test was found to be higher than 0.05 for the variables; gender and whether working in central/policy level or implementation level office, saying that there is no significant difference in the job satisfaction level for them. However, lesser p-value

(0.012<0.05) and higher mean value for provincial respondents concluded that satisfaction level is significantly higher in provincial agriculture officers than that in federal agriculture officers.

**Table 7.** Mean comparison: Mann-Whitney U Test

| Variable       | Category       | N=112 | Mean | Mean Rank | z- value | p-value |
|----------------|----------------|-------|------|-----------|----------|---------|
| Working office | Federal        | 54    | 3.13 | 48.54     | -2.505   | .012    |
|                | Provincial     | 58    | 3.37 | 63.91     |          |         |
|                | Central/Policy | 38    | 3.20 | 52.68     |          |         |
| Working Office | Implementation | 74    | 3.27 | 58.46     | -.891    | .373    |
|                |                |       |      |           |          |         |
| Gender         | Male           | 91    | 3.28 | 58.37     | -1.272   | .204    |
|                | Female         | 21    | 3.14 | 48.38     |          |         |

Kruskal-Wallis test was run to test the satisfaction level against variables; designation level, working discipline, age, job duration. As shown in Table 8, the p-value of the test was found to be higher than 0.05 for all these variables, concluding that satisfaction level of agriculture officers does not differ significantly among various categories of these variables.

**Table 8.** Mean comparison: Kruskal-Wallis test

| Variable           | Category                | N=112 | Mean | Mean Rank | p-value |
|--------------------|-------------------------|-------|------|-----------|---------|
| Designation level  | Officer 6 <sup>th</sup> | 5     | 3.57 | 78.20     | .062    |
|                    | Officer 7 <sup>th</sup> | 37    | 3.32 | 59.29     |         |
|                    | Officer 8 <sup>th</sup> | 16    | 3.39 | 67.94     |         |
|                    | Class III               | 54    | 3.13 | 49.00     |         |
| Working discipline | Extension               | 41    | 3.27 | 58.54     | .371    |
|                    | Horticulture            | 20    | 3.26 | 56.60     |         |
|                    | Agronomy                | 11    | 3.30 | 57.41     |         |
|                    | Plant Protection        | 15    | 3.36 | 64.03     |         |
|                    | Planning                | 19    | 3.22 | 54.21     |         |
|                    | Soil                    | 6     | 2.86 | 29.00     |         |
| Age                | Up to 30                | 59    | 3.19 | 51.50     | .195    |
|                    | >30-40                  | 46    | 3.34 | 63.04     |         |
|                    | >40                     | 7     | 3.20 | 55.64     |         |
| Job duration       | <5                      | 57    | 3.22 | 52.86     | .083    |
|                    | 5-10                    | 45    | 3.33 | 63.14     |         |
|                    | >10-20                  | 6     | 2.92 | 32.17     |         |
|                    | >20                     | 4     | 3.41 | 70.13     |         |

Based on the difference in mean value, some variables were subjected to ANOVA test and then post hoc test

if necessary. As reflected in the Table 9, for different categories of education level, the p-value was found too small compared to 0.05, saying that there is sufficient evidence that agriculture officers having a different level of education differ significantly regarding their levels of job satisfaction.

**Table 9.** Mean comparison for education level: ANOVA test

|                | Sum of squares | df  | Mean square | F     | Sig. |
|----------------|----------------|-----|-------------|-------|------|
| Between groups | 1.971          | 2   | .985        | 5.208 | .007 |
| Within groups  | 20.621         | 109 | .189        |       |      |
| Total          | 22.592         | 111 |             |       |      |

**Table 10.** Mean comparison for education level: Post hoc test (multiple comparisons)

| Category     | Mean  | Comparison   | Mean Difference | Std. Error | Sig. |
|--------------|-------|--------------|-----------------|------------|------|
| Intermediate | 3.628 | Bachelor     | 0.191           | 0.21       | 0.65 |
|              |       | Master       | 0.449           | 0.20       | 0.07 |
| Bachelor     | 3.437 | Intermediate | -0.191          | 0.21       | 0.65 |
|              |       | Master       | .258*           | 0.10       | 0.03 |
| Master       | 3.179 | Intermediate | -0.449          | 0.20       | 0.07 |
|              |       | Bachelor     | -.258*          | 0.10       | 0.03 |

\*. The mean difference is significant at the 0.05 level.

The post hoc test, as presented in Table 10, suggested that satisfaction level differs just between Bachelor and Master level. However, there was no significant difference between the two pairs (bachelor and intermediate, master and intermediate).

### 3.3 Significant factors of job satisfaction

The study also found out which job factors the respondents feel most important by forced ranking, as described in methods. They were asked to choose the three most important factors out of the given six factors of job satisfaction in order. The rank value of 3, 2, and 1 was given for the first, second, and third essential factors, and the composite rank value for each factor was calculated as the sum of the product of the response number and the rank value across all six factors of job satisfaction and dividing that with maximum possible

value that could be, which is 336 i.e. if all respondents had chosen any factor first important, the maximum value would be 336. As such, the index value ranged between 0 to 1. For example, the index value for the first row, i.e., “physical facilities at the office,” was calculated as follows:  $21 \times 3 + 12 \times 2 + 19 \times 1 = 106/336 = 0.32$ . Table 11 below is the detail of ranking of job satisfaction factors in order of respondents’ preference.

**Table 11.** Ranking of job satisfaction factors in order of respondents’ preference

| Factors                                                    | 1 <sup>st</sup> Important<br>(N=112) | 2 <sup>nd</sup> Important<br>(N=112) | 3 <sup>rd</sup> Important<br>(N=112) | Composite rank value | Rank |
|------------------------------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|----------------------|------|
| Physical facilities at office                              | 21                                   | 12                                   | 19                                   | 0.32                 | IV   |
| Guidance of and relationship with Supervisors/office chief | 31                                   | 28                                   | 15                                   | 0.49                 | II   |
| Cooperation of and relationship with co-workers            | 4                                    | 14                                   | 26                                   | 0.20                 | V    |
| Pay (Salary, allowances, and reward)                       | 30                                   | 11                                   | 21                                   | 0.40                 | III  |
| Welfare/retirement facility                                | 2                                    | 3                                    | 14                                   | 0.08                 | VI   |
| Capacity building and job Promotion opportunity            | 24                                   | 44                                   | 17                                   | 0.53                 | I    |
| Total                                                      | 112                                  | 112                                  | 112                                  |                      |      |

As shown in the table above, capacity building and job promotion opportunity was the first essential factor, with a composite rank value of 0.53. Next to this, the Guidance of/and relationship with supervisors/office chief was most important, with a 0.49 rank value. Based on the rank value, pay (salary, allowances, and reward), with 0.40, was the third most important factor of job satisfaction. Finally, welfare/retirement facilities were the least important factor among the six factors, with a rank value of only 0.08. The findings are consistent with findings of Pathak (2015) in case of employees in commercial banks.

### 3.4 Workload status of agriculture officers

Too much workload and a lesser workload may also be one of the job dissatisfying factors. More workload gives physical and mental exhaustion, which might bring problems in health and family matters. On the

other hand, a lesser workload may cause inactiveness and may hinder capacity enhancement. The study collected information about the status of workload at the respondents' current office. As shown in Table 12, only 42 percent of agriculture officers feel that they have optimum workload. Thirty-three percent are with less workload than expected, indicating one third of agriculture officers are not being efficiently utilized.

**Table 12.** Feeling about work load for respondents

| S.N. | Feeling             | Number | Percentage |
|------|---------------------|--------|------------|
| 1    | Less than I expect  | 37     | 33.0       |
| 2    | As I expect/optimum | 47     | 42.0       |
| 3    | More than I expect  | 28     | 25.0       |

To find out whether there is any significant difference in the workload situation for provincial or federal officers and whether they work in central/policy level office or implementation office and for male and female officers, chi-square test was run via SPSS. The p-value of the test was found to be much higher (0.555) than 0.05 for gender, slightly higher (0.056) for working office (federal versus provincial), but lower (0.007) for working office (central/policy level versus implementation level). This indicated that there was no significant difference in the workload situation for male and female officers and federal and provincial officers, but the workload situation differs significantly for those who work in the central/policy level office and implementation office at a 5 percent level of significance. 52.63 % of officers working in the central or policy level office expressed that they had less workload while only 22.97% of officers working in the implementation level said that. This indicated that more than half of the agriculture officers of central/policy level office are not efficiently utilized. So, number of agriculture officers should be made lesser in the central or policy level office or they should be given extra job responsibilities.

**Table 13.** Workload comparison: Chi-Square Test

| Variable       | Category   | Number | Less than I expect (%) | Optimum (%) | More than I expect (%) | Total  | p-value |
|----------------|------------|--------|------------------------|-------------|------------------------|--------|---------|
| Working office | Federal    | 54     | 37.04                  | 48.15       | 14.81                  | 100.00 | .056    |
|                | Provincial | 58     | 29.31                  | 36.21       | 34.48                  | 100.00 |         |
|                | Total      | 112    | 33.04                  | 41.96       | 25.00                  | 100.00 |         |

|                |                      |     |       |       |       |        |      |
|----------------|----------------------|-----|-------|-------|-------|--------|------|
| Working office | Central/Policy level | 38  | 52.63 | 28.95 | 18.42 | 100.00 | .007 |
|                | Implementation level | 74  | 22.97 | 48.65 | 28.38 | 100.00 |      |
|                | Total                | 112 | 33.04 | 41.96 | 25.00 | 100.00 |      |
| Gender         | Female               | 21  | 33.33 | 33.33 | 33.33 | 100.00 | .555 |
|                | Male                 | 91  | 32.97 | 43.96 | 23.08 | 100.00 |      |
|                | Total                | 112 | 33.04 | 41.96 | 25.00 | 100.00 |      |

### 3.5 Feeling about unfair practices within the organization

Every employee expects fair practices within the organization in which he/she works. If there is a feeling of any unfair practices that might be the cause of dissatisfaction and lower the efficiency of the employee, which finally affects the productivity and output of the organization. The study collected the information if there is any feeling about unfair practices among the respondents. As presented in Table 14, only 10.7% of officers don't feel any unfair practices. 54.5% of officers feel unfair practices moderately to strongly while 34.8% feel that partially.

**Table 14.** Feeling about unfair practices within the organization

| S.N. | Feeling                                      | Number | Percentage |
|------|----------------------------------------------|--------|------------|
| 1    | No, I don't feel any unfair practices at all | 12     | 10.7       |
| 2    | I partially feel so                          | 39     | 34.8       |
| 3    | I moderately feel so                         | 33     | 29.5       |
| 4    | I strongly feel so                           | 28     | 25.0       |

The Chi-Square test was applied to find out whether there is any significant difference in the feeling about unfair practices among some variables; working office, gender, designation level, and working period. The p-value of the test was found to be higher than 0.05 for these variables; indicating that there was not any significant difference.

### 3.6 Dissatisfied issues

The study also collected some qualitative information if the respondents feel any particular issues in which they are unsatisfied with. As expressed by majority of the respondents, there is bias in opportunity of exposure visit, international training and study, rewarding, posting and transfer on the basis of political affection

or personal connection. Besides, most common unsatisfied issues indicated by the respondents include low salary, late and no fixed or guaranteed system of promotion, political pressure in selection of beneficiary and execution of programs. Some of the respondents expected well health insurance and loan facilities for government employees and education facility for their children. As expressed by many of the officers, there is also some sort of dissatisfaction due to difficulty in implementation of programs most of which are often designed by the central level without well understanding of ground realities also because of poor implementation of policies and programs and poor monitoring from higher level. Considering this, policies and programs should be formulated so to execute effectively and efficiently as well as there should be sound monitoring activity from the authorities of higher level which is lacking or not in best version.

As suggested by some of the officers, there should be paid visiting leave every year for exposure and refreshments. Most of the officers are unsatisfied due to the rule that they are allowed for further study only in the subject of related discipline in which they are working in. So, that would be better if there is an opportunity to study any subject on leave irrespective of in which discipline the employee is working for. This is desirable because this not only provides an opportunity to develop knowledge and skills in his/her interested field but also the organization have manpower with additional competence which could be utilized as per need.

Most of the respondents working at the implementation level feel less competent to deliver quality technical support to farmers due to not having enough training and capacity development opportunities. Further, they don't get enough time to enhance their technical knowledge by studying and field experience because their major responsibility is to distribute subsidies, due

to which their maximum time is spent in creating files of the applicant of subsidized programs, monitoring of them and assisting subsidiaries for payment. Therefore, they suggest that Field-based knowledge enhancement should be encouraged for extension officers by frequent visits to farmers and by researching with farmers to verify the latest technologies. For that official load should be minimized for them.

#### 4. CONCLUSION

To achieve a sustained growth in agricultural productivity, agriculture officers' competence and their level of job satisfaction count crucial. Considering the most important determinant of job satisfaction and dissatisfied with, there should be enough and effective capacity enhancement opportunities and the concerned organization should foster a fair system to make them happy and productive manpower of the country. The agriculture officers should be assigned with new dimension of extension prevailing in the world like digital services, IT based delivery etc. Offering an opportunity to be promoted to any other discipline if one fulfills the certain qualifications could be one of the ways out to dissatisfaction with respect to the promotion of agriculture officers. The officers at the provincial and federal level office should be developed as resource persons for facilitating the major concerns of the respective provinces and assisting local level agriculture technicians. In addition, they should develop professional skills and knowledge by themselves to deliver to the farmers as a key responsibility of their own, instead of expecting all from the government. Further, government should focus more on effective implementation of indirect ways of subsidizing farmers like insurance premium, interest subsidy through banks, output based subsidy, smooth marketing system development etc. rather than direct and production based subsidy programs.

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# MOLECULAR IDENTIFICATION OF *Coffea arabica* VARIETIES IN NEPAL: INSIGHTS FROM PHYLOGENETIC ANALYSIS

Shreejan Pokharel<sup>1</sup>, Samsher Basnet<sup>2</sup>, Anish Basnet<sup>3</sup>, Ashmita Mainali<sup>3</sup>, Sadikshya Rijal<sup>3</sup>, Asmita Shrestha<sup>3</sup>, Bignya Chandra Khanal<sup>1</sup> and Gyanu Raj Pandey<sup>3,\*</sup>

<sup>1</sup> National Biotechnology Research Center, Nepal Agricultural Research Council, Lalitpur, 44700, Nepal

<sup>2</sup> National Entomology Research Center, Nepal Agricultural Research Council, Lalitpur, 44700, Nepal

<sup>3</sup> Shubham Biotech Nepal Pvt. Ltd., Bharatpur-29, Chitwan, 44200, Nepal

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\*Correspondence:  
gyanupandey9@gmail.com  
Tel: +977-9860470938

## ABSTRACT

This study aimed to identify and characterize the coffee varieties cultivated in Nepal using molecular phylogenetic analysis. The molecular identification and genetic relationship of twenty five coffee varieties were collected from the Nepal Coffee Development Center, Gulmi, Nepal. DNA was isolated from leaf tissue, and Internal Transcribed Spacers Region (ITS)-specific PCR was performed, followed by sequencing and phylogenetic tree construction. BLASTN was performed to identify the similarities with the sequences of the National Center for Biotechnology Information (NCBI) Database. Evolutionary divergence between the sequences was computed using Maximum Composite Likelihood Model. Sequences were analyzed using Maximum Likelihood Model and Tamura-Nei model to construct molecular phylogeny. BLASTN and molecular phylogeny confirm all the samples to be *Coffea arabica*. Evolutionary divergence in pairwise comparison was found to be 0% to 4.3%. Divergence of 4.3% was detected between CDC-S21 and CDC-S73. With this, we identified the coffee samples to be *C. arabica* and we also computed relatedness among our varieties.

## 1. INTRODUCTION

*Coffea arabica* L. and *Coffea canephora*, commonly known as Arabica and Robusta, are the most popular genus, famous for their consumption as popular drinks globally. Coffee belongs to the genus *Coffea* and the family Rubiaceae comprising almost 124 species (Razafinarivo *et al.*, 2013). Coffee is mainly grown in tropical and subtropical regions (Berthaud & Charrier, 1988). The three species of coffee with primary importance for worldwide commercial production are *Coffea arabica*, *C. canephora*, and *C. liberica* (Farah & Dos Santos, 2015), (Ferreira *et al.*, 2019). *C. arabica* is the only tetraploid ( $2n = 4x = 44$ ) and self-fertile species in the genus, whilst the others are diploid ( $2n = 2x = 22$ ) and genetically self-incompatible (Clarindo & Carvalho, 2008). Among the popular ones, *C. arabica* is highly cultivated, as it has superior taste, rich aroma, and low caffeine content is responsible for 70% of the world's coffee production. On the other hand, *C. canephora* accounts for 24% and *C. liberica* for 1% of the world's coffee production (Rohwer, 2002), (Kiran *et al.*, 2019).

In Nepal, coffee is commercially grown in around 44 districts, while Illam, Kavre, Nuwakot, Gulmi, Arghakhanchi, Palpa, Syangja, Kaski, Sindhupalchok, Sankhuwasabha, Parbat are the largest producers (MoALD, 2022). The Prime Minister Agriculture Modernization Project (PMAMP) has begun laying the groundwork for coffee plants in five districts designated as coffee-super zones, with the goal of increasing domestic coffee output at a time when domestic production is insignificant compared to surging demand (Prasain K, 2019). Nepali coffee is recognized for its distinctive aroma and flavor, being cultivated at higher altitudes (800-1600m), away from the usual coffee-growing region in other parts of the world. As a result, Coffee produced in Nepal is labeled as "Specialty Coffee" in several foreign markets and also noted for its organic certification and fair trade practices (NTCDB, 2020/21).

Despite the long history of Coffee plantations in Nepal, the taxonomical study has not been conducted to date, and genetic diversity is unknown. This study aims at

molecular identification of the coffee species found in Nepal and extrapolate genetic relationship among them. The implication of this study will be useful in crop improvement, along with unraveling taxonomical ambiguity. The conventional method of identification based on morphological characteristics like growth habitat, leaf type, floral characteristics, and fruit morphology is insufficient to explain the enormous variety found in allogamous wild coffee communities (Charrier & Berthaud, 1985).

On the other hand, a molecular method involving short sequences known as barcodes that are independent of the environment and life stage of the coffee plant is more efficient in coffee species identification (MoALD, 2022). ITS (Internal Transcribed Spacer) region of nuclear ribosomal cistron shows a high level of intra-specific variability and, thus, is often used for intra-specific discrimination in angiosperms (Kress *et al.*, 2005). Two spacers, ITS1 and ITS2, are located in between the small subunit 18S ribosomal gene and the large subunit 28S ribosomal gene and are separated by the 5.8S gene (Álvarez & Wendel, 2003). As ITS rDNA is often a target in metagenomic studies, an array of resources is available to facilitate sequence-based identifications. Nuclear ribosomal DNA (rDNA) is composed of the 18s, 5.8s, and 36s regions separated by intergenic spacers. As, the ribosomal DNA is ubiquitous throughout plants, nuclear rDNA has proven to be a powerful tool for phylogenetic analysis, techniques for determining the primary nucleotide sequence and the diversity of rates of evolution of the subunits and spacers within the molecule have been

developed (Jansen, 1994). Whereas the 18s and 26s coding regions have been used to address phylogenetic questions at the family level or higher taxonomic levels, the internal transcribed spacers (ITS 1 and ITS 2) appear to be useful for assessing relationships at lower taxonomic levels because the sequences of spacer regions generally evolve more rapidly than the coding regions. Recently, the ITS region has been shown to be useful for resolving phylogenetic relationships among several plant genera, including *Antennaria*, *calycodonia* (Hsaio *et al.*, 1994) and *Krigio* (Tamura & Nei, 1993). In the present study, we have sequenced the internal transcribed spacer (ITS 2) region of 25 *Coffea* taxa. The main purpose was to use the ITS sequences to attempt to resolve phylogenetic relationships among the closely related but highly diversified taxa associated within the genus *Coffea* found in Nepal.

## 2. MATERIALS AND METHODS

### 2.1 Sample collection

Twenty five young coffee leaves samples (Table 1) were harvested from Coffee Nepal Coffee Development Center, Aanpchaaur, Gulmi, Nepal (Latitude: 27°56'17.85" N to 27°5'44.87" N; Longitude: 83°25'29.2" E to 83°25'30.20" E) (Figure 1) stored in poly bags with zipper. The leaves surfaces were cleaned with running water and stored at -80 °C for DNA extraction.

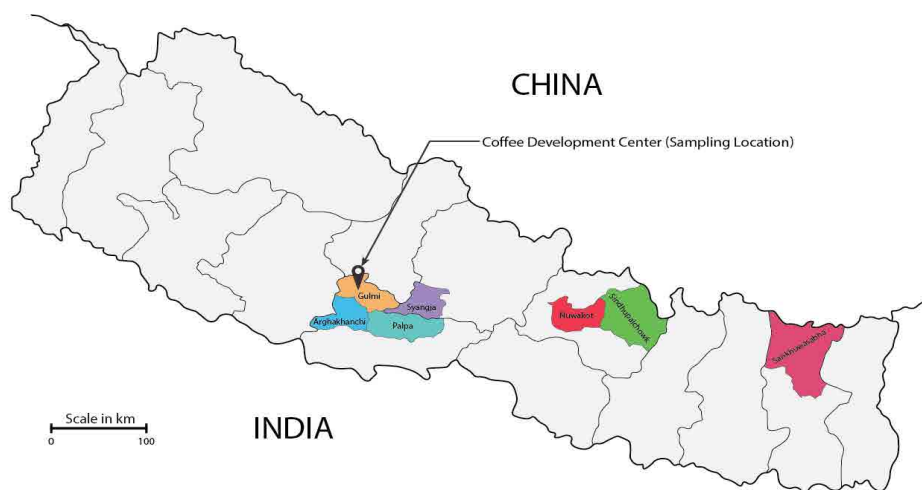


**Figure 1:** Map of Nepal showing districts with commercial coffee productions and sampling site.

(G = Green, Y = Yellow and R = Red; NA = Not Available)

**Table 1.** List of Coffee accessions with its features used in this study.

| Code    | Sample Type      | Year of Introduction (A.D.) | Young Leaf Color | Fruit Color         |
|---------|------------------|-----------------------------|------------------|---------------------|
| CDC-S1  | Brazillian       | 2002                        | Light green      | Maroon red (G to R) |
| CDC-S2  | Caturra Amarillo | 2002                        | Light green      | Yellow (G to Y)     |
| CDC-S3  | Brazillian       | 2002                        | Light green      | NA                  |
| CDC-S4  | Bourbon Amarelo  | 2002                        | Light green      | Red (G to Y to R)   |
| CDC-S5  | Brazillian       | 2002                        | Light bronze     | Red (G to Y to R)   |
| CDC-S6  | Bourbon Vermelo  | 2022                        | Light bronze     | Red (G to R)        |
| CDC-S21 | Local            | 1998                        | Light green      | Red (G to R)        |
| CDC-S23 | Tekisic/Catisic  | 2022                        | Green            | Red (G to R)        |
| CDC-S36 | Local            | 1998                        | Green            | Red (G to R)        |
| CDC-S48 | Tekisic/Catisic  | 2022                        | Light bronze     | Maroon red (G to R) |
| CDC-S51 | Local            | 1989                        | Green            | Maroon red (G to R) |
| CDC-S54 | Local            | 1989                        | Green            | NA                  |
| CDC-S58 | Local            | 1989                        | Light bronze     | Maroon red (G to R) |
| CDC-S65 | Local            | 1989                        | Green            | Maroon red (G to R) |
| CDC-S66 | Local            | 1989                        | Green            | Red (G to R)        |
| CDC-S70 | Local            | 1989                        | Green            | NA                  |
| CDC-S73 | Local            | 1989                        | Green            | Red (G to R)        |
| CDC-S75 | Local            | 1989                        | Light green      | Red (G to R)        |
| CDC-S78 | Local            | 1989                        | Bronze           | Maroon red (G to R) |
| CDC-S79 | Local            | 1989                        | Green            | Red (G to R)        |
| CDC-S82 | Local            | 1989                        | Green            | Red (G to R)        |
| CDC-S85 | Local            | 1989                        | Green            | Red (G to R)        |
| CDC-S88 | Local            | 1989                        | Green            | Red (G to R)        |
| CDC-S91 | Local            | 1993                        | Light green      | Red (G to R)        |
| CDC-S92 | Phirphire        | 1995                        | Light bronze     | Red (G to R)        |

## 2.2 DNA extraction

DNA samples were extracted using Doyle and Doyle method (Doyle & Doyle, 1987) with some optimizations. Leaf samples (1g) were taken and grounded in liquid nitrogen using a mortar and pestle. The pulverized paste was immediately transferred to micro-centrifuge tubes and 700µl extraction buffer (2% CTAB (w/v), Tris HCL pH 8.0 (0.1M), EDTA pH 8.0 (20mM), NaCl (1.4M), 2% PVP (w/v), 1% -mercaptoethanol) was added. The tubes were incubated at 65 0C in a water bath for 1 hour, and then centrifuged at 15,000 rpm for 15 minutes. The aqueous phase was carefully transferred to fresh tubes, and an equal volume of Chloroform: Isoamyl

alcohol (24:1) was added and mixed properly by inversion for a couple of minutes. The mixture was centrifuged at 15,000 rpm for 15 min at 25 0C. After the phase separation, the supernatant was transferred into a new tube. To the supernatant, 0.2ml sodium acetate was added in order to enhance the quality of DNA. An equal volume of isopropanol was added to each tube. The tubes were kept at -20 0C for 30 minutes and centrifuged at 15000 rpm for 7 minutes for precipitation. Then, the supernatant was removed, and the pellets were washed with 96% and 70% ethanol twice, respectively. The pellets were air-dried and resuspended in 1X TE buffer (Tris-HCl 10 mM, EDTA 1 mM, pH 8.0).

## 2.3 Gel electrophoresis

The quality of extracted DNA was also assessed by using 0.8% agarose gel electrophoresis (Cleaver Scientific, UK) in 1X TAE (50X TAE; 242gm Tris-base, 57.1 ml acetic acid (or 100% glacial acid) and 100ml of 0.5 M EDTA (pH-8.0) at 70V for 1 hr. The PCR amplification products were analyzed using 1.5% agarose gel at 70V for 1 hr. using the same buffer system. The gel was stained with ethidium bromide and photographed using a Gel Documentation system (VWR@Genosmart 2, UK). 100 bp ladder (Thermo scientific), was used as a molecular marker for the size comparison of the visible fragments.

## 2.4 PCR amplification and sequencing

Primer Pairs ITS (ITS1-5.8s-ITS2); ITS1 TCGTAACAAGGTTTCCGTAGGTG, ITS2 TATGCTTAAAYTCAGCGGG; designed by (Hsaio *et al.*, 1994) were used to amplify the ITS region of the extracted DNAs. The optimized PCR mixture contained 25 of the following; 2.5 10x Taq Buffer, 1.5 25 mM Mgcl<sub>2</sub>, 0.5 0.2mM dNTPs, 0.5 0.2U of Taq Polymerase, 2 DNA, 1mg/ml BSA and 7% DMSO. The PCR amplification was performed using MiniAmp Thermal cycler (AppliedBiosystems by Thermo Fisher Scientific) as follows, initial denaturation at 94 0C for 3 minutes, followed by 35 cycles of annealing at 53.8 0C for 1.15 minutes, followed by a final extension at 72 0C for 15 minutes.

The PCR products were sent to Macrogen Inc., Seoul, Korea, and sequencing was performed using sequencing primer ITS1 (5'-TCGTAACAAGGTTTCCGTAGGTG – 3') and ITS2 (5'- TATGCTTAAAYTCAGCGGG-3').

## 2.5 Sequence analysis

Raw sequences were assembled and trimmed using Codon Code Aligner. Contig sequences generated were subjected to BLASTN, and the database “Standard databases (nr etc.)” was selected. Highly similar sequences were taken in FASTA format for phylogenetic analysis. All sequences were deposited to the NCBI genebank, and accession numbers were received as listed in Table 3. Pairwise comparison of these ITS-2 sequence was performed using the Maximum Composite Likelihood model (Tamura *et al.*, 2004) to estimate the evolutionary divergence between the sequence.

The evolutionary history was inferred by using the

Maximum Likelihood method and Tamura-Nei model (Tamura & Nei, 1993). The bootstrap consensus tree inferred from 2000 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed. Initial tree(s) for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with a superior log-likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.2731)). This analysis involved 75 nucleotide sequences. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

## 2.6 Accession numbers of the nucleotide sequence used:

### 2.6.1 Accession numbers of the samples of our study:

MZ678246, MZ734310, MZ734319, MZ734320, MZ734321, MZ734322, MZ734325, MZ734324, MZ734326, MZ734331, MZ734332, MZ734333, MZ734334, MZ734336, MZ734335, MZ734337, MZ734338, MZ734339, MZ734344, MZ734345, MZ734346, MZ734347, MZ734348, MZ734323 and MZ734349.

### 2.6.2 Accession numbers used from the NCBI database:

|             |                 |             |
|-------------|-----------------|-------------|
| AY853406.1, | MF417757.1,     | MF417758.1, |
| MK615726.1, | MK611791.1,     | MK615727.1, |
| MK615729.1, | MN719947.1,     | MK615731.1, |
| MK615728.1, | DQ153609.1,     | MK611792.1, |
| MN719952.1, | MK615737.1,     | MK615732.1, |
| AY780425.1, | MK615738.1,     | MK615730.1, |
| MK615733.1, | DQ153593.1,     | MF417756.1, |
| MF417755.1, | DQ153591.1,     | MK615734.1, |
| DQ153594.1, | DQ153638.1,     | MN719950.1, |
| DQ153632.1, | DQ153592.1,     | DQ153616.1, |
| DQ153614.1, | DQ153596.1,     | DQ153615.1, |
| DQ153626.1, | DQ153563.1,     | DQ153617.1, |
| MN719972.1, | MT250045.1,     | MN719966.1, |
| AF542982.1, | AF543006.1,     | AF543002.1, |
| AF542985.1, | AF542983.1,     | AF542981.1, |
| AF543004.1, | AF542988.1,     | AF542992.1, |
| AF543007.1  | and AF543003.1. |             |

### 3. RESULTS AND DISCUSSION

The DNAs of all the twenty five coffee samples from Coffee Development Center from Gulmi, Nepal, taken in this study were successfully extracted. The presence of DNA for 25 samples from extraction was verified by gel electrophoresis at 0.8% agarose (Figure

2). Subsequently, the ITS-based PCR mixture was optimized, modifying suitably the concentration of the sample DNAs, dNTPs, MgCl<sub>2</sub>, and ITS. Following that, ITS-based PCR was performed for each coffee sample, and the completion of PCR was verified by conducting 1.5 % agarose gel electrophoresis against 100bp ladder, as shown in Figure 3 with a size of range of 600-700 bp.

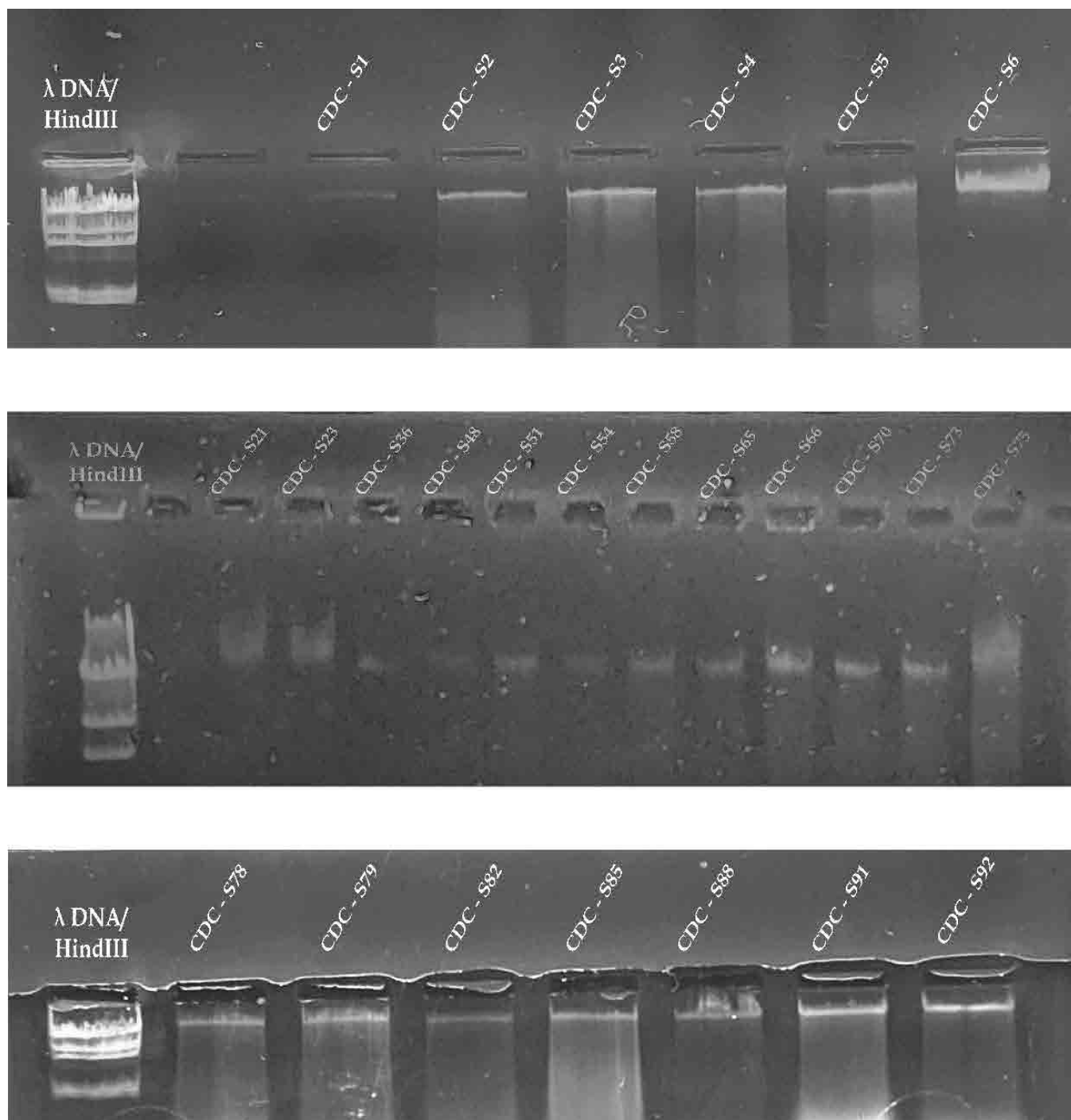


**Figure 2.** Gel electrophoresis of extracted 25 DNA samples with 0.8% Agarose ran against HindIII digested λDNA.

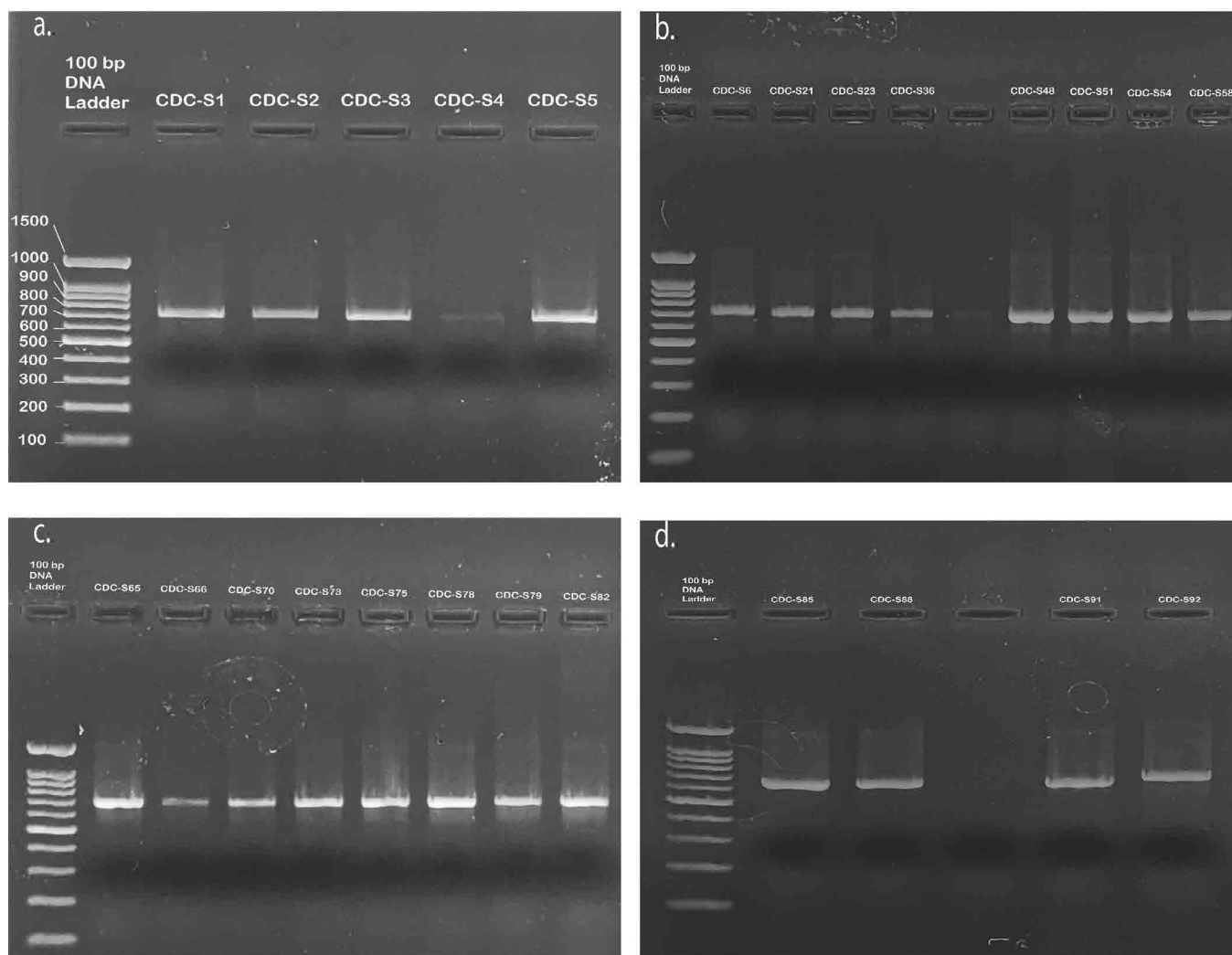


**Figure 3.** Gel electrophoresis of PCR with amplification of ITS region of the 25 coffee samples used in the study. The rightmost lane in each figure a, b, c, and d represents the reference of 100bp ladder. Where the subsequent samples read from right to left. PCR Amplification products were fractionated in 1.5% agarose gel. Amplified from genomic DNA using ITS region (ITS1-5.8s-ITS7) was amplified with primers ITS1 (5'- TCGTAACAAGGTTTCCGTAGGTG-3') and ITS2 (5'- TATGCTTAAAYTCAGCGGG-3') (Hsiao *et al.*, 1994). From right to left Figure a represents the samples serially as CDC-S1, CDC-S2, CDC-S3, CDC-S4, and CDC-S5. Similarly, Figure b represents the samples as CDC-S6, CDC-S21, CDC-S23, CDC-S36, CDC-S48, CDC-S51, CDC-S54, and CDC-S58, while the Figure c reads the sample as CDC-S66, CDC-S70, CDC-S73, CDC-S75, CDC-S78, CDC-S79, and CDC-S82. Lastly, the samples of Figure d precedes as CDC-S85, CDC-S88, CDC-S91, and CDC-S92 from right to left. The length of ITS regions of our 25 samples ranges between 600bps to 700bps.

### 3.1 ITS-based sequencing and BLASTN analysis of 25 coffee samples

The sequencing results of the ITS-PCR amplifications product of 25 coffee samples are presented in Table 2. The ITS regions of the coffee samples were approximately 100% amplified and sequenced. The nucleic acid sequence of samples was analyzed using the BLASTN

program to determine the identity of the coffee samples. The BLASTN analysis showed that the coffee samples had 100% similarity to *C. arabica*. The cultivar of *C. arabica* mainly comprised of Typica, Bourbon, and near to Java and Typica. The sequences are deposited in the GenBank with the accession numbers, as shown in Table 2. The length of ITS regions of our 25 samples ranged from 650 bp to 704 bp.

**Table 2.** Sequencing data after BLAST in NCBI showing Organism, Similarity percentage, Cultivar.

| Code    | Organism              | Query Coverage (%) | Similarity Percentage | Genbank Accession Number | Sequence Lengths (bp) | Cultivar                |
|---------|-----------------------|--------------------|-----------------------|--------------------------|-----------------------|-------------------------|
| CDC-S1  | <i>Coffea arabica</i> | 99                 | 99.08                 | MZ678246                 | 650                   | Typica                  |
| CDC-S2  | <i>Coffea arabica</i> | 97                 | 99.41                 | MZ734310                 | 699                   | Typica                  |
| CDC-S3  | <i>Coffea arabica</i> | 99                 | 99.42                 | MZ734319                 | 694                   | NA                      |
| CDC-S4  | <i>Coffea arabica</i> | 100                | 99.26                 | MZ734320                 | 678                   | Bourbon                 |
| CDC-S5  | <i>Coffea arabica</i> | 98                 | 99.13                 | MZ734321                 | 703                   | Bourbon                 |
| CDC-S6  | <i>Coffea arabica</i> | 96                 | 100                   | MZ734322                 | 690                   | Bourbon                 |
| CDC-S21 | <i>Coffea arabica</i> | 96                 | 99.41                 | MZ734325                 | 701                   | Bourbon                 |
| CDC-S23 | <i>Coffea arabica</i> | 97                 | 99.58                 | MZ734324                 | 695                   | Bourbon                 |
| CDC-S36 | <i>Coffea arabica</i> | 96                 | 100                   | MZ734326                 | 687                   | Bourbon                 |
| CDC-S48 | <i>Coffea arabica</i> | 97                 | 100                   | MZ734331                 | 669                   | Bourbon                 |
| CDC-S51 | <i>Coffea arabica</i> | 97                 | 99.88                 | MZ734332                 | 686                   | Typica                  |
| CDC-S54 | <i>Coffea arabica</i> | 96                 | 100                   | MZ734333                 | 690                   | Bourbon                 |
| CDC-S58 | <i>Coffea arabica</i> | 96                 | 99.88                 | MZ734334                 | 682                   | Bourbon                 |
| CDC-S65 | <i>Coffea arabica</i> | 96                 | 100                   | MZ734336                 | 680                   | Bourbon                 |
| CDC-S66 | <i>Coffea arabica</i> | 96                 | 99.85                 | MZ734335                 | 702                   | Bourbon                 |
| CDC-S70 | <i>Coffea arabica</i> | 99                 | 99.81                 | MZ734337                 | 686                   | Near to Java and Typica |
| CDC-S73 | <i>Coffea arabica</i> | 96                 | 99.7                  | MZ734338                 | 698                   | Bourbon                 |
| CDC-S75 | <i>Coffea arabica</i> | 94                 | 99.71                 | MZ734339                 | 691                   | Bourbon                 |
| CDC-S78 | <i>Coffea arabica</i> | 97                 | 100                   | MZ734344                 | 681                   | Bourbon                 |
| CDC-S79 | <i>Coffea arabica</i> | 98                 | 98.49                 | MZ734345                 | 704                   | Bourbon                 |
| CDC-S82 | <i>Coffea arabica</i> | 98                 | 99.7                  | MZ734346                 | 687                   | Bourbon                 |
| CDC-S85 | <i>Coffea arabica</i> | 97                 | 99.85                 | MZ734347                 | 692                   | Bourbon                 |
| CDC-S88 | <i>Coffea arabica</i> | 96                 | 99.85                 | MZ734348                 | 701                   | Bourbon                 |
| CDC-S91 | <i>Coffea arabica</i> | 96                 | 99.56                 | MZ734323                 | 702                   | Bourbon                 |
| CDC-S92 | <i>Coffea arabica</i> | 97                 | 99.41                 | MZ734349                 | 691                   | Bourbon                 |

**Table 3.** Pairwise comparisons of ITS-2 sequences obtained from 25 coffee-tree accession listed in Table 2. The numbers of base substitutions per site from between sequences are shown. Analyses were conducted using the Maximum Composite Likelihood model (Tamura *et al.*, 2004)

|    |               | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    |
|----|---------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1  | <b>CDC-S1</b> |       |       |       |       |       |       |       |       |       |       |       |       |
| 2  | CDC-S2        | 0.011 |       |       |       |       |       |       |       |       |       |       |       |
| 3  | CDC-S3        | 0.008 | 0.010 |       |       |       |       |       |       |       |       |       |       |
| 4  | CDC-S4        | 0.011 | 0.007 | 0.007 |       |       |       |       |       |       |       |       |       |
| 5  | CDC-S5        | 0.014 | 0.013 | 0.010 | 0.006 |       |       |       |       |       |       |       |       |
| 6  | CDC-S6        | 0.006 | 0.023 | 0.016 | 0.003 | 0.016 |       |       |       |       |       |       |       |
| 7  | CDC-S91       | 0.014 | 0.018 | 0.015 | 0.007 | 0.012 | 0.019 |       |       |       |       |       |       |
| 8  | CDC-S23       | 0.011 | 0.015 | 0.015 | 0.004 | 0.010 | 0.009 | 0.012 |       |       |       |       |       |
| 9  | CDC-S21       | 0.013 | 0.009 | 0.012 | 0.006 | 0.012 | 0.021 | 0.015 | 0.012 |       |       |       |       |
| 10 | CDC-S36       | 0.017 | 0.016 | 0.012 | 0.003 | 0.012 | 0.011 | 0.013 | 0.012 | 0.012 |       |       |       |
| 11 | CDC-S48       | 0.000 | 0.008 | 0.006 | 0.000 | 0.006 | 0.003 | 0.011 | 0.005 | 0.006 | 0.005 |       |       |
| 12 | CDC-S51       | 0.013 | 0.013 | 0.009 | 0.004 | 0.009 | 0.001 | 0.010 | 0.006 | 0.012 | 0.010 | 0.000 |       |
| 13 | CDC-S54       | 0.013 | 0.018 | 0.012 | 0.004 | 0.015 | 0.004 | 0.015 | 0.012 | 0.013 | 0.013 | 0.002 | 0.006 |
| 14 | CDC-S58       | 0.013 | 0.012 | 0.009 | 0.004 | 0.006 | 0.008 | 0.007 | 0.007 | 0.010 | 0.010 | 0.003 | 0.009 |
| 15 | CDC-S66       | 0.009 | 0.015 | 0.016 | 0.004 | 0.015 | 0.018 | 0.018 | 0.013 | 0.009 | 0.013 | 0.005 | 0.009 |
| 16 | CDC-S65       | 0.000 | 0.007 | 0.009 | 0.000 | 0.006 | 0.013 | 0.009 | 0.004 | 0.000 | 0.005 | 0.006 | 0.002 |
| 17 | CDC-S70       | 0.005 | 0.013 | 0.012 | 0.000 | 0.012 | 0.009 | 0.013 | 0.007 | 0.013 | 0.005 | 0.003 | 0.002 |
| 18 | CDC-S73       | 0.013 | 0.041 | 0.038 | 0.023 | 0.039 | 0.038 | 0.036 | 0.032 | 0.043 | 0.035 | 0.033 | 0.029 |
| 19 | CDC-S75       | 0.006 | 0.013 | 0.012 | 0.000 | 0.013 | 0.023 | 0.007 | 0.010 | 0.010 | 0.007 | 0.011 | 0.006 |
| 20 | CDC-S78       | 0.011 | 0.013 | 0.010 | 0.006 | 0.009 | 0.003 | 0.012 | 0.009 | 0.010 | 0.010 | 0.002 | 0.004 |
| 21 | CDC-S79       | 0.011 | 0.018 | 0.016 | 0.004 | 0.017 | 0.021 | 0.012 | 0.012 | 0.016 | 0.013 | 0.008 | 0.006 |
| 22 | CDC-S82       | 0.008 | 0.016 | 0.010 | 0.006 | 0.015 | 0.004 | 0.015 | 0.009 | 0.016 | 0.015 | 0.002 | 0.004 |
| 23 | CDC-S85       | 0.013 | 0.013 | 0.009 | 0.006 | 0.010 | 0.006 | 0.010 | 0.007 | 0.012 | 0.012 | 0.002 | 0.003 |
| 24 | CDC-S88       | 0.009 | 0.019 | 0.016 | 0.004 | 0.016 | 0.021 | 0.007 | 0.012 | 0.015 | 0.013 | 0.011 | 0.007 |
| 25 | CDC-S92       | 0.014 | 0.018 | 0.013 | 0.003 | 0.010 | 0.012 | 0.015 | 0.010 | 0.016 | 0.012 | 0.005 | 0.010 |
|    |               | 13    | 14    | 15    | 16    | 17    | 18    | 19    | 20    | 21    | 22    | 23    | 24    |
| 14 | CDC-S58       | 0.012 |       |       |       |       |       |       |       |       |       |       |       |
| 15 | CDC-S66       | 0.012 | 0.007 |       |       |       |       |       |       |       |       |       |       |
| 16 | CDC-S65       | 0.003 | 0.003 | 0.001 |       |       |       |       |       |       |       |       |       |
| 17 | CDC-S70       | 0.004 | 0.002 | 0.010 | 0.010 |       |       |       |       |       |       |       |       |
| 18 | CDC-S73       | 0.037 | 0.027 | 0.041 | 0.032 | 0.031 |       |       |       |       |       |       |       |
| 19 | CDC-S75       | 0.007 | 0.003 | 0.015 | 0.009 | 0.015 | 0.034 |       |       |       |       |       |       |
| 20 | CDC-S78       | 0.006 | 0.009 | 0.009 | 0.003 | 0.001 | 0.035 | 0.006 |       |       |       |       |       |
| 21 | CDC-S79       | 0.010 | 0.009 | 0.022 | 0.010 | 0.015 | 0.035 | 0.012 | 0.010 |       |       |       |       |
| 22 | CDC-S82       | 0.006 | 0.010 | 0.012 | 0.005 | 0.003 | 0.030 | 0.011 | 0.007 | 0.010 |       |       |       |
| 23 | CDC-S85       | 0.006 | 0.009 | 0.009 | 0.003 | 0.004 | 0.037 | 0.006 | 0.006 | 0.009 | 0.006 |       |       |
| 24 | CDC-S88       | 0.012 | 0.009 | 0.016 | 0.007 | 0.013 | 0.035 | 0.003 | 0.010 | 0.015 | 0.012 | 0.010 |       |
| 25 | CDC-S92       | 0.013 | 0.004 | 0.010 | 0.006 | 0.004 | 0.035 | 0.007 | 0.010 | 0.013 | 0.013 | 0.010 | 0.013 |

### 3.2 ITS-2 sequence divergence

The pairwise nucleotide-sequence divergence (Maximum Composite Likelihood Model) among the coffee taxa (Table 3) ranged from 0% to 4.3% within different taxa of *C. arabica*. Divergence of 4.3% was detected between CDC-S21 and CDC-S73. CDC-S48 and CDC-S65 were detected with no divergence with CDC-S1; CDC-S48, CDC-S65 and CDC-S70 were also detected with no divergence with CDC-S4. No divergence was observed between CDC-S21 and CDC-S65; and CDC-S48 and CDC-S51. These divergence were attributable to deletion and insertion events, and gaps were introduced to align the sequence (Charrier, 1997). Previously, intraspecific divergence of 4.3% was observed in *Calycadenia truncate* using ITS 2 sequences (Balwain, 1993). However, this phenomenon appeared particularly important within coffee-tree species (T. A. Charrier, 1997). Such results are expected when the rate of nucleotide divergence exceeds the homogenization rate of the gene copies within a multigene family, a situation that could arise in cases of explosive radiation or interspecific hybridization (Hillis & Davis, 1988).

### 3.3 Validation of molecular identification of 25 coffee samples by ITS phylogeny

The entire ITS sequence of individual sequenced results was analyzed and a phylogenetic tree (Figure 4) was constructed by the Maximum Likelihood method to validate the molecular identity of the 25 coffee samples as implied by the BLASTN. The similarities were assessed based on the genetic distances among the sample sequences. The molecular identity was corroborated through phylogenetic analysis by comparing the results of alignments of nucleotide sequences of coffee samples with some standard sequences selected such as *C. arabica*, *C. canephora*, *C. congensis*, *C. dewevrei*, *C. liberica*, *C. stenophylla*, *C. dubardii*, *C. affinis*, *C. kapakata*, *C. brevipes*, *C. arabica\**, *C. canephora*, *C. mayombensis*, *C. heterocalyx*, *Coffea sp.*, and *C. stenophylla\**

*C. liberica*. Moreover, the coffee species of Gulmi, Nepal, were assumed to be *C. arabica* until now. This study helped prove and document this speculation.

The distinct clad separation of *C. arabica*, along with our sample species, and the remaining species in the Neighbor-Joining phylogenetic tree, (Figure 4), validates that all of the samples are *C. arabica*. This paper documents the *Arabica* coffee for the first time in Nepal with validation. Phylogenetic analysis also demonstrated the relatedness among the coffee varieties, indicating origin of varieties.

The overall sequence homogeneity among members of a gene family such as the nuclear rDNA is assumed to be maintained by homogenization mechanisms associated with concerted evolution (Arnheim, 1983). As a result, rDNA repeats are usually very similar within individuals and species, although differences may accumulate between species (Hillis & Dixon, 1991) as we observed in pairwise comparison. An unusual feature of the ITS 2 sequence in *Coffea* was the importance of intraspecific variation. Even the presence of ITS variants within individuals was observed. Similar intraspecific variation has already been reported. The generation times of coffee-trees have been estimated as between 20 and 30 years. The observed deficiency in the homogenization mechanisms may be related to the long life cycles of coffee-trees, in the same manner that nucleotide-substitution rates have been reported to be related to the length of the reproductive cycle (Li & Graur, 1991). Moreover, it is most likely that spontaneous interspecific hybridization occurs between taxa and has been involved in speciation. Given their important role in post-translational processing, ITS regions are considered to be quite conserved (Hamby & Zimmer, 1992). However, in this study, ITS phylogeny has been successful in discriminating the coffee species. This finding adds more credibility to the use of ITS as a phylogeny reconstruction tool for intra-species discrimination in coffee species.

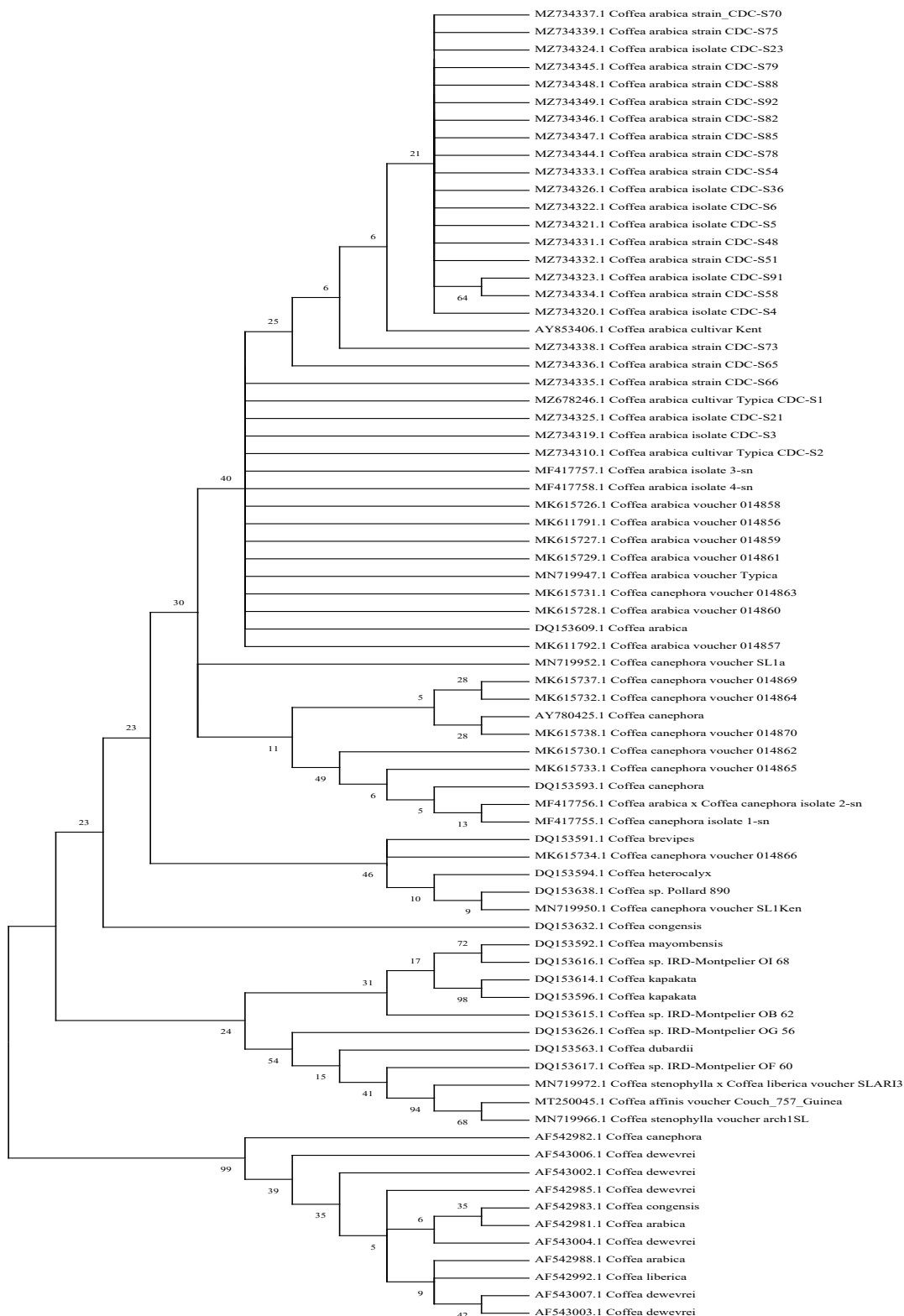


**Figure 4.** Maximum Likelihood tree generated from ITS-2 sequence data. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2000 replicates) are shown next to the branches (Felsenstein, 1985). There were a total of 557 positions in the final dataset. The coffee samples of our study were distributed mainly into three clads, while the distinct clad separation of *C. arabica*, along with our sample species, and the remaining species, elucidates that all of the samples are *C. arabica*.

#### 4. CONCLUSION

This study used molecular phylogenetic analysis to identify and describe the different coffee cultivars grown in Nepal. The analysis also showed that there was only a little amount of evolutionary divergence across the samples, ranging from 0% to 4.3%, with the biggest difference found between CDC-S21 and CDC-S73. These findings contribute to our understanding of the genetic links among the many coffee varieties found in Nepal. The use of a gene-based identification strategy in this work provides a better and more efficient substitute for identifying coffee species using traditional morphological traits. The findings of this study have significance for improving crops and clearing up taxonomical uncertainty. On the basis of these results,

future research might investigate the genetic variety of coffee kinds in Nepal and possibly improve the region's approaches to producing coffee.

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# MORPHOLOGICAL CHARACTERIZATION OF POMEGRANATE (*Punica granatum L.*) GENOTYPES AT DAILEKH

Binod Prasad Luitel<sup>1,\*</sup> and Asmita Khanal<sup>2</sup>

<sup>1</sup> National Horticulture Research Centre, Khumaltar, Lalitpur

<sup>2</sup> Horticulture Research Station, Malepatan, Pokhara, Kaski

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\*Correspondence:

binodsan@yahoo.com;

Tel: +977-9843274202

## ABSTRACT

Pomegranate is an emerging potential fruit crop of Nepal and it can be cultivated successfully from tropical to warm temperate climatic condition of terai to mid-hills. This research was conducted to study the morphological traits and identify the superior pomegranate genotypes at Horticulture Research Station (HRS), Dailekh in 2019. Three pomegranate genotypes (HRDPOM001, HRDPOM004 and HRDPOM004M) were studied for their growth, flowers and pomological characters. Variation in plants, flower and pomological characters was observed in pomegranate genotypes. The highest marketable fruit weight (1.4 kg/plant) produced in HRDPOM001, followed by HRDPOM004 (1.2 kg/plant). The highest aril weight (60.5%) was measured in HRDPOM004. Highest TSS (12.8°Brix) and good taste preferences were recorded in the fruits of HRDPOM004. Pomological attributes for HRDPOM004 was found better than other studied genotypes. Individual fruit weight, fruit length, and fruit diameter showed the significant positive association with total fruit weight, and selection of these traits could improve the fruit yield. Based on the major pomological attributes, HRDPOM004 was found promising and selected to cultivate at the similar agro-climatic regions of Nepal.

## 1. INTRODUCTION

Pomegranate is one of the important ancient domesticated fruits. Asia Minor, Transcaucasia, Iran and Turkmenistan are reported to be the center of origin of pomegranate (Melgarejo and Salazar, 2003). Pomegranate fruit has a high nutritional and medicinal importance, and its cultivation has increased particularly in Iran, India, China, Turkey and the United States. Fruits contain enormous fibers, protein, vitamin C, vitamin K, folate and potassium. It prevents cardiovascular disease, lower the blood pressure and strengthens the digestive system. In Nepal, it is one of the important emerging potential commercial fruit (Atreya *et al.*, 2020).

Pomegranate is well-adapted in sub-tropical, tropical and warm-temperate climate, and it can be grown successfully from terai to an elevation of 1,600 m above sea level (asl). It can be thrived well in drought condition (Atreya, 2014). Pomegranate is considered as deciduous in warm-temperate and temperate climate, but it is an evergreen or partially deciduous fruit in tropical and sub-tropical climate. But high temperature

during fruit ripening (August to October) is necessary to get high quality and quantity of fruits (Karimi and Mirdehghan, 2013). Globally, more than 760 varieties of pomegranate are cultivated (Muhammad *et al.*, 2019). In Nepal, Safeda, Bedana, Spanish Ruby, Local (Darim), Kandhari, Paper Shell, Muscat Red, Ganesh, Mridula, Red Dyana and Bagwa are important varieties and cultivated in different regions. Based on the flowering behavior, it can be categorized into Ambe bahar (February-March), Mrig-bahar (June-July) and Hasta bahar (September-October). In general, one bahar fruiting is regulated to maintain productivity of the plants, but the fruit regulation also depends on market factors and availability of water (Poudel *et al.*, 2017).

In Nepal, pomegranate is cultivated 762 ha with productivity of 6.6 mt/ha, but in Karnali Province, the productive area is estimated to be around 22 ha with the productivity of 7.5 mt/ha (MoALD, 2021). Different varieties are cultivated across the country, but information on fruit traits such as fruit size, peel color,

taste, aril color and seed hardness in different varieties are not available yet. Pomological attributes differ in genotypes. The edible part of pomegranate fruit is arils, which constitute about 50% of the whole fruit. Different pomegranate genotypes have variability for aril-related traits and taste, color, size and seed hardness (Barone *et al.*, 2001). Variability in pomegranate is characterized in terms of domestic, wild, and ornamental types which show the differences in characters such as peel, and aril color, succulence, sugar/acid ratio, fatty acid content, anthocyanin, phenols, and antioxidant activity (Onias *et al.*, 2021). Fruit weight and skin color are the dominant traits for genotypes discrimination of pomegranate (Orhan *et al.*, 2014). Adequate flowering, fruiting, high and regular yield, large fruit size, red peel genotype, red and soft aril, and excellent taste are important selection criteria (Khadivi and Arab, 2021). Besides, no fruit cracking, high and attractive juice, and sour, sweet and sour-sweet taste are considered to choose the desired fruit in pomegranates (Muradoglu *et al.*, 2006).

HRS, Dailekh is assigned to undertake pomegranate research and variety selection as a collaborating research station of Karnali Province (Gotame *et al.*, 2020). Selection of potential genetic resources of pomegranate in the agro-climatic region of Dailekh will help to increase its productivity and income of farmers. The morphological characterization of different pomegranate genotypes is important to select new cultivar of promising characters (Onias *et al.*, 2021; Khadivi and Arab, 2021). Previous studies on morphological and physico-chemical characteristics have been done in several countries of the world (Gazde, 2012; Zaouay *et al.*, 2012; Ferrara *et al.*, 2014; Poudel *et al.*, 2017; Souza *et al.*, 2020). But study on phenotypic characterization of pomegranate genotypes at Dailekh, Karnali Province of Nepal is limited. Therefore, this research was done to characterize plant and pomological attributes, and to select the best pomegranate genotypes for commercial production at the similar agro-climatic regions of Nepal.

## 2. MATERIALS AND METHODS

### 2.1 Experimental site and plant materials

Morphological characters of pomegranate genotypes were evaluated at HRS, Dailekh which is located at 28° 50' 49.8" N longitude and 81° 43' 19.4" E latitude with an elevation of 1, 255m asl in Karnali Province, Nepal. The annual rainfall amount ranged between 140 and 160 mm and its distribution is high around

June-July. The average monthly temperature in fruit bearing period (April-September) at Dailekh ranged from 14.6 to 24.1°C (HRS, 2019). Three genotypes (HRDPOM001, HRDPOM004 and HRDPOM004M) were introduced from National Horticulture Research Centre (NHRC), Khumaltar at HRS, Dailekh for the multi-location evaluation. Six plants of each genotype were planted at the spacing of 5m x 5m in 2015. The trial was designed in randomized complete block and replicated three times, where each replication consisted of two plants. Nutrition, irrigation and pests, and diseases control were done regularly and uniformly for all the genotypes.

### 2.2 Morphological characterization

Plant growth habit, tree growth vigor and canopy density were taken using the descriptor of International Plant Genetic Resources Institute (IPGRI, 2001). Plant growth habit was visually assessed and classified into three types; erect, semi-erect and spreading. Likewise, tree growth habit was also classified into three types; weak, moderate and high. Canopy density was visually assessed and categorized into three types; weak, moderate and high. Flowering and fruit setting were visually observed. Fruits were harvested from each genotype and weighted. Cracked, insect and diseases damaged fruits were considered as non-marketable, and weighted. Uniform and un-cracked fruits were considered as marketable and weighted. Five individual fruits were weighted using digital electronic balance (H-Honda™, India) and averaged it. Fruit length (mm), fruit diameter (mm) and fruit pericarp thickness (mm) were measured using digital caliper (150mm, Model: DC-515). Pericarp weight and aril were calculated on fruit weight basis and expressed as percentage. Number of segments were counted after horizontal cutting of fruits. The total soluble solids (TSS) were determined using a digital refractometer (Model ATAGO, Tokyo, Japan) and expressed in °Brix. For each reading, the refractometer was washed with distilled water each time after use and dried with blotting paper to avoid contamination. Aril color was visually examined and noted using IPGRI (2001) descriptor. Fruit taste was assessed by judge panel of 12 personnel using 1-5 scales (1 = very poor, 2 = poor, 3 = fair, 4 = Good, and 5 = Excellent) and overall preferences for fruit taste were ranked. Seed hardness was assessed using 1-3 scales (1 = Very hard, 2 = Semi-Soft and 3 = Soft) (Khadivi and Arab, 2021). The qualitative traits including fruit shape, fruit peel color, fruit symmetry, fruit feel cracking, aril shape, aril color and fruit quality (based on qualitative

traits) were visually examined and coded using the method described by Mars and Marrakchi (1999).

### 2.3 Data analysis

Plant and fruits data were edited and processed using MS Excel (version 16.0, Microsoft, Redmond, WA, USA) and then, one-way analysis of variance (ANOVA) followed by Tukey's Test, Honestly Significant Difference (HSD) was done using SPSS (SPSS Inc., Chicago, Ill., USA). Phenotypic correlation coefficients among quantitative traits were computed using R version 4.2.2 [R Core Team (2022). R: A language and environment for Statistical Computing, Vienna, Austria. URL, <https://www.R-project.org/>].

## 3. RESULTS AND DISCUSSION

### 3.1 Morphological description

Morphological characters, flowering and fruiting traits of pomegranate genotypes are given in Table 1. Growth habit varied from erect, semi-erect to spreading

type (Figure 1). HRDPOM004M, HRDPOM001 and HRDPOM004 had semi-erect, spreading and erect growth habit, respectively. HRDPOM004 had high tree growth vigor whereas remaining two genotypes had moderate types. Canopy density in HRDPOM001 had high and rests of two genotypes had moderate types. Variability for growth habit, tree growth vigor and canopy density in pomegranate genotypes has also described by previous researchers (Dandachi *et al.*, 2017; Khadivi and Arab, 2021). Flowering was started earlier i.e. second week of March in two genotypes (HRDPOM001 and HRDPOM004) and delayed flowering (second week, April) was observed in HRDPOM004M. Two genotypes (HRDPOM001 and HRDPOM004) had 50% flowered in second week of April. All genotypes started to flower in spring season, but flowering was continued till rainy season (June-July) in HRDPOM004. With respect to first fruiting setting, HRDPOM004M and HRDPOM004 found earlier i.e. third week of April.

**Table 1.** Growth habit, flowering and fruit setting traits of pomegranate genotypes

| Genotypes  | Growth habit | Tree growth vigor | Canopy density | First flower opening | 50% flowering      | Flowering time | First fruit setting |
|------------|--------------|-------------------|----------------|----------------------|--------------------|----------------|---------------------|
| HRDPOM001  | Spreading    | Moderate          | High           | Second week, March   | Second week, April | Spring         | Fourth week, April  |
| HRDPOM004  | Erect        | High              | Moderate       | Second week, March   | Second week, April | Spring-Rainy   | Third week, April   |
| HRDPOM004M | Semi-erect   | Moderate          | Moderate       | Second week, April   | Third week, April  | Spring         | Third week, April   |

### 3.2 Pomological description

The results of the fruit yield and quality traits are presented in Table 2. Genotypes showed significant ( $P < 0.05$ ) differences in total fruit weight, non-marketable and marketable fruit weight, fruit weight, fruit length and diameter, and fruit pericarp thickness. HRDPOM001 produced the highest fruit weight (2.8 kg/plant) and marketable fruit weight (1.2 kg/plant). The highest individual fruit weight was measured in HRDPOM001 (240.4 g), followed by HRDPOM004 (229.4 g). In contrast, in a study of Poudel *et al.* (2017), they reported the highest (355.4 g) fruit weight in Safeda cultivar. Fruit weight is an economic trait which is important for genotype selection (Orhan *et al.*, 2014). Dandachi *et al.* (2017) have explained the variation in fruit weight in pomegranate genotypes. Variation in fruit weight

is mainly governed by genetics, but it also depends on soil and climatic condition (Martinez *et al.*, 2006). Besides, variation in fruit weight ranged from 150.0 to 568.0 g in pomegranate cultivars has been reported (Kazankaya *et al.*, 2003; Ozkan, 2005). In this study, the fruit length and diameter between HRDPOM001 and HRDPOM004 were not significantly different. Fruit size is an essential trait to attract consumers' attention in the fresh food market. Many researchers (Dandachi *et al.*, 2017; Poudel *et al.*, 2017) have reported the variation in fruit length and diameter in pomegranate genotypes. Pericarp thickness was the highest (4.5 mm) in HRDPOM001, but it was not significantly different from HRDPOM004M (4.0 mm). The variation in fruit pericarp thickness in pomegranate genotypes has also reported in previous studies (Dandachi *et al.*, 2017; Khadivi and Arab, 2021).

**Table 2.** Fruit yield and quality traits of pomegranate genotypes

| Genotypes  | Total fruit wt. (kg/plant) | Non-market. fruit wt. (kg/plant) | Marketable fruit wt. (kg/plant) | Fruit wt. (g) | Fruit length (mm) | Fruit diameter (mm) | Fruit pericarp thickness (mm) |
|------------|----------------------------|----------------------------------|---------------------------------|---------------|-------------------|---------------------|-------------------------------|
| HRDPOM001  | 2.8±0.7 a                  | 1.6±0.4 a                        | 1.4±0.4 a                       | 240.4±27.0 a  | 72.6±1.9 a        | 77.0±2.6 a          | 4.5±0.1 a                     |
| HRDPOM004  | 2.0±0.6 a                  | 1.1±0.5 a                        | 1.2±0.1 a                       | 229.4±26.5 b  | 71.1±0.9 a        | 75.4±2.7 a          | 3.4±0.7 b                     |
| HRDPOM004M | 1.2±0.4 b                  | 0.7±0.3 b                        | 0.6±0.1 b                       | 179.7±33.0 c  | 67.2±4.7 b        | 69.1±4.9 b          | 4.0±0.6 a                     |

Mean ± SE (n=5). Means in a column followed by same letter (s) are not significant different at P<0.05 according to Tukey's Test, HSD.

Fruit pericarp weight was significantly ( $P<0.05$ ) different among the genotypes (Table 3). The highest fruit pericarp weight (47.8%) and aril weight (60.5%) was recorded in HRDPOM004. Aril is the fundamental part of fruit which constitute about 50% of the whole fruit and our study showed more than 60% aril weight of the fruit in HRDPOM004 and this might be due to genetic trait. Fruit segments were not significantly different among the genotypes. TSS was measured the highest (12.8°Brix) in HRDPOM004, but it was

statistically similar to HRDPOM004M (11.0°Brix), and the lowest TSS (9.4°Brx) was measured in HRDPOM001. TSS values more than 12% and the juice extracted from the fruits in all genotypes are suitable for commercial uses (Vazquez-Araujo *et al.*, 2014). The superior selected genotype of pomegranate is shown in Figure 1 (D, E and F). Compared to HRDPOM001 and HRDPOM004M, fruits taste of HRDPOM004 was good (4.0) and seed hardness was also soft in HRDPOM004.

**Table 3.** Fruit quality traits of pomegranate genotypes evaluated at HRS, Dailekh, 2019

| Genotypes  | Fruit pericarp wt. (%) | Aril wt. (%) | Fruit segments (no.) | TSS (°Brix) | Fruit taste (1-5) | Seed hardness (1-3) |
|------------|------------------------|--------------|----------------------|-------------|-------------------|---------------------|
| HRDPOM001  | 44.7±1.6 b             | 55.3±1.6 b   | 5.6±0.4 a            | 9.4± 0.4 b  | 2.0               | 2.0                 |
| HRDPOM004  | 47.8±1.4 a             | 60.5±1.9 a   | 5.3±0.3 a            | 12.8±0.3 a  | 4.0               | 3.0                 |
| HRDPOM004M | 44.8±2.5 b             | 55.2±2.4 b   | 5.4±0.6 a            | 11.0±0.3 a  | 3.0               | 2.0                 |

Mean ± SE (n = 5). Means in a column followed by same letter (s) are not significant different at P<0.05 according to Tukey's Test, HSD. Fruit taste (1-5 scales); 1; Very poor, 2; Poor, 3; Fair, 4; Good, and 5; Excellent. Seed hardness (1-3 scales); 1; Very hard, 2; Semi-soft and 3; Soft.

Fruit pomological traits of studied genotypes are given in Table 4. Fruit morphology (fruit shape and fruit peel color) and aril color of pomegranate genotypes are also presented in Figure 1. Fruit shape of HRDPOM004M and HRDPOM001 had round, while the fruits of HRDPOM004 had oval- shaped. Two genotypes (HRDPOM004M and HRDPOM001) contained yellow-red fruit peel color, while HRDPOM004 had red-brown fruit peel color. Fruit skin color is dominant trait for the genotypes discrimination in pomegranate (Orhan *et al.*, 2014). Peel color is one of the important criteria for famers to decide the appropriate harvesting time (Melgarejo-Sanchez *et al.*, 2015). Fruit symmetry was present in HRDPOM004 and remaining two genotypes did not show any fruit symmetry. Fruit peel cracking was present in HRDPOM004M and HRDPOM001 and it was absent in HRDPOM004. Triangular aril shape was recorded in HRDPOM004M, whereas aril of HRDPOM001 and HRDPOM004 contained oval and

prismatic aril shape, respectively. Aril color was red in HRDPOM004M and it was white-red in HRDPOM001 and dark red in HRDPOM004. Dark red aril color is one the important traits to be considered for the variety selection in pomegranate (Ozgen *et al.*, 2008). Aril color is also the predominant trait for cultivar selection (Stover and Mercure, 2007). Diverse aril color from light milky, pink, white red, red and red-black has reported in a study of Khadivi and Arab (2021), but in our study observed white-red, red and dark-red aril color. Fruit qualities (good fruit shape, absence of fruit cracking, and dark-red aril color etc.) was higher in HRDPOM004 than HRDPOM004M and HRDPOM001. Fruit shape, fruit peel color, absence of cracking and aril color are the important traits for commercial importance. No fruit cracking, peel and aril colors are important traits of fruit quality in pomegranate marketing (Mena *et al.*, 2011).

**Table 4.** Fruit pomological traits of pomegranate genotypes

| Genotypes  | Fruit shape | Fruit peel color | Fruit symmetry | Fruit peel cracking | Aril shape | Aril color | Fruit quality |
|------------|-------------|------------------|----------------|---------------------|------------|------------|---------------|
| HRDPOM001  | Round       | Yellow-red       | Absent         | Presence            | Oval       | White-red  | Moderate      |
| HRDPOM004  | Oval        | Red-brown        | Present        | Absence             | Prismatic  | Dark red   | High          |
| HRDPOM004M | Round       | Yellow-red       | Absent         | Presence            | Triangular | Red        | Moderate      |



**Figure 1.** Trees, fruits and aril of pomegranate genotypes. A, B, C represent tree, fruit and aril of HRDPOM001, respectively. D, E, F represent tree, fruit and aril of HRDPOM004, respectively and G, H, I represent tree, fruit and aril of HRDPOM004M, respectively.

### 3.3 Correlation among the measured traits

Phenotypic correlation coefficients of 11 quantitative traits in pomegranate genotypes are presented in Table 4. Total fruit weight/plant was positively and significantly correlated with non-marketable fruit weight/plant ( $r = 0.94$ ), marketable fruit weight ( $r = 0.87$ ), fruit weight ( $r = 0.86$ ), fruit length ( $r = 0.71$ ) and fruit diameter ( $r = 0.80$ ). Non-marketable fruit weight showed positive and significant correlation with fruit weight ( $r = 0.90$ ), fruit length ( $r = 0.76$ ), and fruit diameter ( $r = 0.86$ ). Fruit weight exhibited positive and significant strong correlation with fruit length ( $r = 0.90$ ), fruit diameter ( $r = 0.98$ ), and aril weight ( $r = 0.50$ ). Positive and significant correlation of fruit weight with fruit length and fruit diameter in pomegranate have been reported by previous researchers (Karimi and Mirdehghan, 2013; Khadivi-

Khub *et al.*, 2015; Khadivi and Arab, 2021). Positive and significant correlation between fruit weight and aril weight has also mentioned by Waliullah *et al.* (2021). Fruit length had a significant positive association with fruit diameter ( $r = 0.96$ ) and aril weight ( $r = 0.48$ ) while fruit diameter was positively and significantly correlated with aril weight ( $r = 0.40$ ) and was in line with the previous findings in pomegranate (Khadivi and Arab, 2021). There was a negative and significant correlation between pericarp weight and aril weight ( $r = -0.98$ ). Pericarp weight also showed positive and significant correlation with TSS ( $r = 0.68$ ). In contrast, a negative and significant correlation between aril weight and TSS was observed. Phenotypic correlation of morphological characters gives important information to breeders that they can use in designing a high-performance design

to explore genotypes (Tancred *et al.*, 1995). Besides, this coefficient can help to compare the direct and indirect selections and develop a plan for the traits that

are difficult to select and study (Falconer and Mackay, 1996).

**Table 5.** Phenotypic correlation coefficient of 11 quantitative traits in pomegranate genotypes

| Traits | TFW | NMF    | MWT    | FW     | FL     | FD     | FPT  | PWT   | AWT     | FS     | TSS    |
|--------|-----|--------|--------|--------|--------|--------|------|-------|---------|--------|--------|
| TFW    | 1.0 | 0.94** | 0.87** | 0.86** | 0.71*  | 0.80** | 0.43 | -0.26 | 0.27    | -0.62  | -0.01  |
| NMF    |     | 1.0    | 0.66   | 0.90** | 0.76*  | 0.86** | 0.33 | -0.23 | 0.24    | -0.47  | 0.01   |
| MWT    |     |        | 1.0    | 0.63   | 0.48   | 0.54   | 0.48 | -0.24 | 0.25    | -0.74* | -0.03  |
| FW     |     |        |        | 1.0    | 0.90** | 0.98** | 0.44 | 0.04  | 0.50*   | -0.39  | 0.11   |
| FL     |     |        |        |        | 1.0    | 0.96** | 0.31 | -0.06 | 0.48*   | -0.27  | -0.06  |
| FD     |     |        |        |        |        | 1.0    | 0.41 | 0.04  | 0.40*   | -0.34  | 0.06   |
| FPT    |     |        |        |        |        |        | 1.0  | 0.35  | -0.35   | 0.04   | 0.52   |
| PWT    |     |        |        |        |        |        |      | 1.0   | -0.98** | 0.21   | 0.68*  |
| AWT    |     |        |        |        |        |        |      |       | 1.0     | -0.21  | -0.68* |
| FS     |     |        |        |        |        |        |      |       |         | 1.0    | 0.33   |
| TSS    |     |        |        |        |        |        |      |       |         |        | 1.0    |

\*Significant at  $P < 0.05$ , \*\*Highly significant at  $P < 0.01$ . TFW; Total fruit weight/plant (kg), NMF; Non-marketable fruit weight (kg/plant), MWT; Marketable fruit weight (kg/plant), FW; Fruit weight (g), FL; Fruit length (mm), FD; Fruit diameter (mm), FPT; Fruit pericarp thickness (mm), PWT; Pericarp weight (%), AWT; Aril weight (%), FS; Fruit segment (no./fruit), and TSS; Total soluble solids ( $^{\circ}$ Brix).

## 4. CONCLUSION

This study morphologically characterized the three pomegranate genotypes and found significant variability for plants, flowers and pomological characters. Marketable fruit weight/plant, fruit and aril weight, TSS and fruit taste showed significant variation among the genotypes. Based on the pomological and important traits of commercial importance including high marketable weight, high fruit weight, high aril weight, high TSS, good taste with soft seed, red-brown

fruit peel color and dark red aril and high fruit quality, genotype HRDPOM004 was found to be promising and can be recommended to cultivate directly in the farmer's field of similar agro-climatic regions of Nepal.

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# NEGLECTED AND UNDERUTILIZED CROP SPECIES OF NEPAL: SMART FOODS FOR UNCERTAIN FUTURE

Toyanath Joshi<sup>1\*</sup>, Ram Prasad Mainali<sup>2</sup>, Sabina Bhandari<sup>3</sup>, Prashiksha Acharya<sup>3</sup> and Krishna Hari Ghimire<sup>2</sup>

<sup>1</sup> National Centre for Potato, Vegetables and Spice Crops Development (NCPVS), Kirtipur, Kathmandu

<sup>2</sup> National Agriculture Genetic Resources Center (Gene bank), Khumaltar, Lalitpur, Nepal

<sup>3</sup> Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal

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\* Correspondence:  
joshitoyanath@gmail.com  
Tel.: +977-9845088408

## ABSTRACT

*The majority of crop species that are cultivated for food in Nepal are not prioritized in formal research, education and development making them neglected and underutilized. A narrative review and expert interviews were carried out to investigate the significance of neglected and underutilized species in the realms of food and nutrition, climate change, economic activities, and culture. We listed 65 neglected and underutilized species as future smart food based on their potential value in food and nutrition. Neglected and underutilized species can play a crucial role by improving incomes and enhancing the food and nutrition security of smallholder farmers and rural communities. Furthermore, these crops have the capacity to adapt against extreme climatic conditions and be tolerant to biotic and abiotic stresses. There is an urgent need to broaden the food basket of the country by supporting the promotion and commercialization of neglected and marginalized crops through research and development.*

## 1. INTRODUCTION

Globally, approximately 30,000 edible plant species have been identified. Of them, more than 7,000 plant species have been used for food purposes (Padulosi *et al.* 2002). Over 84% of the global human diet and nutrition come from plants, contributing 90% of the average human diet in Asia (Joshi *et al.* 2019). At present, however, not more than 150 species are commercially cultivated, and of these, just 103 crops provide 90% of the calories in the human diet (Padulosi *et al.* 2013). Just four of these (rice, wheat, maize & potato) account for fully 60% of the human energy supply. Agricultural species that are not among the major staple crops are often grouped as ‘neglected and underutilized crop species’ (NUS) and are sometimes called ‘orphan’ crops. Interestingly, NUS holds the potential to ensure food and nutritional security for the growing global population through their significant contribution to human nutrition and their use as traditional medicine for ages. However, little

attention is paid to or is entirely ignored by agricultural researchers, plant breeders and policymakers to NUS (Shrestha *et al.* 2018). Nevertheless, NUS has tremendous opportunities for fighting poverty, hunger, and malnutrition and building agricultural production systems more resilient to climate change.

Currently, more than 100 national and international organizations are working in Nepal for agricultural research and development, with some involvement in the academic sector as well. The government has given utmost importance to agriculture since the commencement of the third five-year plan (1975-80) for addressing the issues of food and nutritional security. Indeed, three crops, rice, wheat and maize, remain the most priority crops for research and development. A small percentage (approximately 5%) of NUS landraces are utilized in research and breeding programs. (Joshi *et al.* 2020). Currently, landraces of major food crops

provide limited food sources at a national level, even though landraces of NUS provide major sources of food and nutrition in marginal hills and mountains. Agriculture gets priority in different plans and policies; however, every time the emphasis was given to major cereals by both the research and development sector. Apparently, NUS get less priority in research and development programs.

Before the 13<sup>th</sup> three-year plan (2013-2016) of the country, NUS were considered non-commodity crops in national policies and agricultural statistics. Research on NUS was initiated in 1991 and systematic conservation of these crops was started in 2010 (Joshi *et al.* 2019). The Hill Crop Research Program (HCRP), under the umbrella of NARC, has been working in NUS. However, HCRP with minimal human and financial resources is compelled to work on multilocation trials on rice, maize and wheat, resulting in a negligible focus on NUS. This can be seen clearly from the number of varieties released/registered in four decades of research. There are only five varieties of finger millet, one variety each of buckwheat, naked barley and amaranth but no varieties of foxtail millet, proso millet and sorghum, released and or registered as compared with 139 varieties of rice, 32 varieties of wheat and 93 varieties of maize (SQCC, 2019). Similar is the situation of grain legumes. Strategies to encourage NUS production in lower food production areas were given in the country's 14th three-year national plan. Then, the Ministry of Land Management, Agriculture & Cooperative (MoLMAC) announced the 58-point agricultural transformation roadmap in 2018, emphasizing NUS's promotion and conservation. From the fiscal year 2018/19, the promotion of NUS was started in three districts, namely Lamjung, Humla and Bajura, representing three provinces Gandaki, Karnali, and Far-western, respectively. Later this program was extended to 12 districts of Nepal. In 2018, the government of Nepal in collaboration with Pacific of the FAO, qualified six NUS as Future Smart Foods (FSF), namely Tartary buckwheat, Grass pea, Taro, Drumstick, Jackfruit, and Nepal butter tree. These six NUS were selected based on the four-dimensional features such as nutrient-dense, climate-resilient, potentially viable, and locally adaptable.

The NUS are gaining interest in Nepal, reflecting a growing trend within agriculture to identify and develop new crops for national and international markets. Consumer interest is also creating the demand for NUS in the domestic market. The

Government of Nepal also emphasized the promotion and commercialization of NUS in policies, programs and budget. Further, in collaboration with the Food and Agriculture Organization (FAO) of the United Nations and Bioversity International, the National Genebank, NARC of Nepal has focused on improving NUS conservation and use and have been spearheading specific national and international activities to improve the conservation and use of these species over the past few years. However, there is a gap in exploiting NUS potential, their documentation (Joshi and Shrestha 2018) and prioritization. Further, limited literature is available on the nutritional values and climate resilience of NUS. Therefore, the present study aimed to explore the status of NUS in Nepal, their nutritional and medicinal values, and their potential for climate resilience and agrotourism.

## 2. METHODOLOGY

A narrative review was conducted to search for available literature. In this study, we identified the five keywords; (i) underutilized crops, (ii) indigenous crops, (iii) traditional crops, (iv) neglected crops and (v) orphan crops. The keywords were searched online using Google and Google Scholar, Scopus, ScienceDirect and SpringerLink search engines. For the grey literature, information was retrieved from the official websites of related organizations such as the Ministry of Agriculture and Livestock Development (MoALD), National Genebank, Food and Agriculture Organization (FAO), Bioversity International. Relevant policies, laws, and regulations existing in NUS were also taken into consideration. The outcomes of quantitative and qualitative research were combined. This study has been structured into three parts, listing major NUS with their food value in Nepal, their prospects for climate change, and the importance of NUS in maintaining cultural diversity and agrotourism.

## 3. RESULTS AND DISCUSSION

### 3.1 Criteria for NUS

Many researchers defined NUS with different criteria. Adhikari *et al.* (2017) defined NUS as having high nutritional value, but their role in achieving nutrition security is not adequately understood, and they do not feature in food and nutrition policies and programs of the countries. International Plant Genetic Resources Institute (IPGRI) of FAO defined NUS as those crop species neglected by science and development; some, therefore, call them 'orphan crops. Moreover, NUS

are those to which little attention is paid or which are entirely ignored by agricultural researchers, plant breeders and policymakers (Padulosi *et al.* 2013). The IPGRI (2002) reported six features of NUS (Box I).

#### Box I. NUS criteria

- Of local importance in consumption and production systems
- Highly adapted to agro-ecological niches/marginal areas
- Receive scarce attention by national agricultural and biodiversity conservation policies, research and development
- Represented by ecotypes/landraces
- Cultivated and utilized relying on indigenous knowledge
- Scarcely represented in *ex situ* collections

### 3.2 Nutritional value of NUS

NUS are potential Future Smart Foods (FSF) because they are socially acceptable, have strong nutritional values (social sustainability), and are vital to the agrobiodiversity and resilience of farming systems (environmental sustainability) (Joshi *et al.* 2020). NUS provides essential micro-nutrients and thus complements staple foods. Additionally, NUS provides flavoring in local cuisine, strengthens local gastronomic traditions and provides income opportunities for both the rural and urban poor that's why they are also called 'poor man's food'. Many NUS are high in carotenoids and minerals and, therefore, could play a role in improving the micro-nutrient content in the diets of millions of people in food-insecure areas. It was reported that increased consumption of locally available indigenous or traditional fruits, vegetables, grains, roots and tubers could improve human nutrition and increase local

productivity (Padulosi *et al.* 2013; Ebert, 2014).

Many of the people in Nepal are facing the severe challenge of malnutrition. About 41% of children younger than five years of age in Nepal are experiencing chronic malnutrition, and 11% suffer from acute malnutrition (Acharya *et al.* 2018). The situation has alarmed us in achieving the United Nations Sustainable Development Goal (SDG) of zero hunger by 2030. Recently, import-based consumption and dietary shift of Nepalese people have led to the deterioration of such traditional mountain food systems, resulting in a decline in agro-biodiversity and further increasing the risk of food and nutrition security. The national policies on NUS are becoming congenial; however, not in their practical implementation or not observed in the real field; hence NUS are gradually disappearing from food systems. NUS have a considerable potential to be 'future smart foods' if they are revitalized in local farming systems. According to the available literature, and discussion with experts working in the research and development of NUS, we listed major 65 NUS as priority crops for Nepal with their nutritional value and role in food security from the total list of NUS developed by Joshi and Shrestha (2018), Joshi *et al.* (2020) and Joshi *et al.* (2022). It is clearly seen that these NUS provide not only a good calorie supply but also essential nutrients for better health.

**Table 1.** Nutritional values of neglected and underutilized crop species of Nepal

| SN       | Scientific name                             | English name   | Nepali name | Parts used and the local form of consumption             | Nutritional value                                                                                                                                                              |
|----------|---------------------------------------------|----------------|-------------|----------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>1</b> | <b>Cereals</b>                              |                |             |                                                          |                                                                                                                                                                                |
| 1        | <i>Eleusinecoracana</i> (L.) Gaertn.        | Finger millet  | Kodo        | Grains are used for pancake, beverages, fodder, porridge | Rich source of calcium, dietary fiber, phytates, phenolics, thiamine, and riboflavin.                                                                                          |
| 2        | <i>Panicum miliaceum</i> L.                 | Proso millet   | Chino       | Grains are used for pancake, beverages, fodder.          | Rich source of protein, dietary fiber, several B vitamins, and numerous dietary minerals, especially manganese.                                                                |
| 3        | <i>Pennisetum glaucum</i> (L.) R.Br.        | Pearl millet   | Baajraa     | Grains used for bread.                                   | Grains are gluten-free, higher dietary fiber than rice, similar in lipid content to maize, and high essential amino acids (leucine, isoleucine and lysine) than wheat and rye. |
| 4        | <i>Setariaitalica</i> (L.) Beauvois         | Foxtail millet | Kaaguno     | Grains are used for pancake, beverages& fodder           | Rich in protein, fiber, minerals, and phytochemicals. Possesses hypolipidemic, low-glycemic index, and antioxidant characteristics.                                            |
| 5        | <i>Hordeum vulgare</i> L.                   | Barley         | Jau         | Grains are used for beverages & leaves for fodder        | Considered superfoods because it contains a high amount of dietary fibers and phytochemicals.                                                                                  |
| 6        | <i>Hordeum vulgare</i> var. <i>nudum</i> L. | Naked barley   | Uwa         | Grains for bread & leaves for fodder                     | Manganese and carbohydrate-rich fruit support Antioxidant, Anti-microbial, antifungal and Antitumor activity.                                                                  |

| SN                      | Scientific name                                         | English name                | Nepali name  | Parts used and the local form of consumption                                                                                   | Nutritional value                                                                                                                                                                                                 |
|-------------------------|---------------------------------------------------------|-----------------------------|--------------|--------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 7                       | <i>Sorghum vulgare</i> Pers.                            | Sorghum                     | Junelo       | Grains are used for bread& leaves for fodder                                                                                   | The plant is usually used for fodder and has medicinal value also.                                                                                                                                                |
| 8                       | <i>Echinochloa frumentacea</i> Link.                    | Barnyard millet             | Sama         | Leaves for forage and grains for bread                                                                                         | Good source of highly digestible protein and is the least caloric dense compared to other cereals.                                                                                                                |
| <b>2 Pseudo cereals</b> |                                                         |                             |              |                                                                                                                                |                                                                                                                                                                                                                   |
| 1                       | <i>Fagopyrum esculentum</i> Moench                      | Common buckwheat            | Mithe Phapar | Grains for bread, young leaves are used for vegetables& other parts for fodder                                                 | Rich in Vit. B1 and B2 and lysine. The rutin content of buckwheat has a hypotensive effect.                                                                                                                       |
| 2                       | <i>Fagopyrum tataricum</i> (L.) Gaertn.                 | Tartary buckwheat           | Tite Phapar  | Tender leaves used for vegetables & seeds for bread                                                                            | Rich source of amino acid, lysine & rutin (which reduces blood cholesterol count), dietary fiber, lipids & minerals.                                                                                              |
| 3                       | <i>Amaranthus hypochondriacus</i> L.                    | Prince's feather            | Latte dana   | Tender leaves used for vegetables & grains for bread                                                                           | Rich in protein, carbohydrates & minerals (Ca, Fe, Na & K).                                                                                                                                                       |
| 4                       | <i>Amaranthus caudatus</i> L.                           | Foxtail amaranth            | Jhule latte  | Leaves used for vegetable & grains for bread                                                                                   | Rich in protein, fat, ash, starch and dietary fiber. Seeds have a higher content of fiber essential amino acid.                                                                                                   |
| 5                       | <i>Amaranthus cruentus</i> L.                           | Blood/Red amaranth          | Rato latte   | Leaves used for vegetables & grains for bread                                                                                  | Rich source of Ca, K & amino acids.                                                                                                                                                                               |
| <b>3 Pulses</b>         |                                                         |                             |              |                                                                                                                                |                                                                                                                                                                                                                   |
| 1                       | <i>Vigna umbellata</i> (Thunb.)                         | Rice bean                   | Mashyang     | Used as a grain and fodder legume                                                                                              | Contain 25 % higher protein than many cultivated legumes.                                                                                                                                                         |
| 2                       | <i>Pisum sativum</i> L. var. <i>arvense</i> L.          | Small pea, field pea        | Saanokeraau  | Fresh vegetables and other dried food products                                                                                 | Contain starch, protein and other phytochemicals which are anticarcinogenic.                                                                                                                                      |
| 3                       | <i>Mucuna pruriens</i> (L.) DC.                         | Velvet bean, horse eye bean | Kaausesimi   | Used as an important forage, fallow and green manure crop                                                                      | Important forage, fallow and green manure crop (nitrogen fixation). It is also a medicine against snake bites.                                                                                                    |
| 4                       | <i>Lathyrus sativus</i> L.                              | Grass pea                   | Khesari      | Grains for human food and other parts for livestock fodder                                                                     | Seeds are rich in proteins and essential amino acids. Different unsaturated fatty acids the essential PUFA $\alpha$ -linolenic, linoleic and $\gamma$ -linolenic acids are also present.                          |
| 5                       | <i>Vigna mungo</i> (L.) Hepper                          | Black gram                  | Mas          | Grain legume in mid-hills used for daal and fodder for livestock                                                               | It contains high levels of protein, potassium, calcium, iron, niacin, thiamine, and riboflavin.                                                                                                                   |
| 6                       | <i>Glycine max</i> (L.) Merr.                           | Soybean                     | Bhatmas      | Seeds used to prepare baby food, green pods used as green vegetables and dry seeds roasted or fried eaten as snacks            | A green plant serves as a high-quality forage for livestock. The seed contains high-quality protein and oil which is also a good source of vitamin E                                                              |
| 7                       | <i>Phaseolus vulgaris</i> L.                            | Common bean                 | Asaresimi    | Grains used as <i>simi ko daal</i> . <i>Simi ko daal</i> remains a recipe for special occasions                                | Good source of protein, carbohydrates, vitamins & minerals especially for poor populations throughout the world                                                                                                   |
| 8                       | <i>Macrotyloma uniflorum</i> (Lam.) Verdc. curing stone | Horse gram                  | Gahat        | Gains used to prepare the recipe of Gahat ko jhol & to cure kidney stones                                                      | Medicinal uses (tonic, astringent, diuretic) and recommended for rheumatism, neuralgia & other several diseases. Seeds are rich in natural phenols, mostly phenolic acids, flavonoids and the major antioxidants. |
| 9                       | <i>Vicia faba</i> L.                                    | Broad bean, faba bean       | Bakula       | Pods are consumed mostly as green vegetables and dry seeds as roasted beans and small seeds usually split and consumed as soup | Unique legume crops rich in nutrients like proteins, complex carbohydrates, dietary fiber, choline, lecithin, minerals and secondary metabolites such as phenolics                                                |

| SN                        | Scientific name                                                            | English name           | Nepali name      | Parts used and the local form of consumption                                                                                                                                                                                             | Nutritional value                                                                                                                                                                                                                                   |
|---------------------------|----------------------------------------------------------------------------|------------------------|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>4 Root and Tubers</b>  |                                                                            |                        |                  |                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                     |
| 1                         | <i>Ipomoea batatas</i> (L.)                                                | Sweet potato           | Sakharakhand     | Tuber eaten as roasted and boiled                                                                                                                                                                                                        | Rich source of starch, sugar sum, protein, vitamin C, ascorbic acid and minerals (P, Ca & Mg)                                                                                                                                                       |
| 2                         | <i>Manihot esculenta</i> Crantz                                            | Tapioca, cassava       | Simaltarul       | Tuber eaten as roasted in a special festival such as <i>makarsankranti</i>                                                                                                                                                               | Roots and leaves contain a higher level of vitamin C and some minerals. Rich source of starch and fiber                                                                                                                                             |
| 3                         | <i>Dioscoreaalata</i> L.                                                   | Greater yam, white yam | Tarul, ghartarul | Tuber eaten as roasted in a special festival such as <i>makarsankranti</i>                                                                                                                                                               | Higher food energy calories, fiber & minerals (Ca, P & Fe), amino acids (tyrosine arginine, Isoleucine, leucine, lysine)                                                                                                                            |
| 4                         | <i>Colocasia esculenta</i> (L.)                                            | Taro                   | Pindaalu/ Gaba   | Leaves ( <i>karkalo ko paat</i> ), the young stalks ( <i>gaaba</i> ), and the taro tuber or corms ( <i>pidhaalu</i> ) to prepare various dishes. The taro tubers are used as a root vegetable, steamed & preparation of some daal dishes | Good source of thiamin, riboflavin & minerals (Fe, P, Zn, K, Cu & Mn), and an excellent source of vitamin B6, vitamin C, niacin. Taro also contains higher amounts of vitamin B-complex than whole milk.                                            |
| 5                         | <i>Dioscorea nepalensis</i> (Jacquem. ex Prain& Burkill) Sweet ex Bernardi | Deltoid yam            | Vhyakur          | food values and sources of different kinds of medicine<br>Fruits used for food and medicine. Seeds used to wash hair                                                                                                                     | Used as a medicine, hair wash & food. The juice of the root tuber is taken in the evening as a treatment for roundworms. Also used to alleviate constipation.                                                                                       |
| 6                         | <i>Amorphophalluspaeoniifolius</i> (Dennst.) Nicolson                      | Elephant foot yam      | Oal              | Root is eaten as deep-fried, curry, or <i>chokha</i> (a kind of achar)                                                                                                                                                                   | Rich in vitamins, minerals, and energy. Also, has medicinal and therapeutic value                                                                                                                                                                   |
| <b>5 Leafy vegetables</b> |                                                                            |                        |                  |                                                                                                                                                                                                                                          |                                                                                                                                                                                                                                                     |
| 1                         | <i>Chenopodium album</i> L.                                                | Lamb's quarter         | Bethe            | Leaves used as vegetable and medicine                                                                                                                                                                                                    | Contains high amino acids (lysine, methionine & cysteine), protein, carotene & vitamin C                                                                                                                                                            |
| 2                         | <i>Trigonellafoenum-graecum</i> L.                                         | Fenugreek              | Methi            | Seeds used as spices and medicine. Fresh leaves for vegetable                                                                                                                                                                            | Seeds are rich in minerals (P, S, Mg, &Ca.). Seeds and leaves contain biologically active substances (protein, amino acids, biogenic elements, lipids & fatty acids), which are used in traditional medicine, functional food & cosmetics industry. |
| 3                         | <i>Brassica nigra</i> (L.) Koch                                            | Black mustard,         | Rayo             | Fresh leaves for vegetables and seed for oil                                                                                                                                                                                             | Rich in calories, protein & fatty oil, mainly oleic acid                                                                                                                                                                                            |
| 4                         | <i>Dryopteris cocheleata</i> (D. Don) C. Chr.                              | Fern                   | Niuro            | Fresh thallus is used for vegetable                                                                                                                                                                                                      | Contains germacrene D, 1, 3-cyclohexanedion,2-methyl-2 (3-isobutyl), neoisolongifolene,8, 9-dehydro compounds which show medicinal properties                                                                                                       |
| 5                         | <i>Spinacia oleracea</i> L.                                                | Spinach                | Palungo          | Leaves consumed as a vegetable                                                                                                                                                                                                           | Possesses a higher concentration of vitamins, minerals (Ca, P, K & Fe) and protein.                                                                                                                                                                 |
| 6                         | <i>Amaranthus gracilis</i> Desf.                                           | Green amaranthus       | Lunde            | Leaves used as a vegetable                                                                                                                                                                                                               | Contain a higher amount of proteins compared to true cereals. Relatively well-balanced food that has been shown to have medicinal benefits                                                                                                          |
| 7                         | <i>Nasturtium officinale</i> R.Br.                                         | Water cress            | Sim saag         | Leaves used as vegetables and seeds as medicine.                                                                                                                                                                                         | Rich in Vit. K and contains significant amounts of Vit. A, C, riboflavin, B6, Ca & Mn.                                                                                                                                                              |

| SN                | Scientific name                           | English name                    | Nepali name         | Parts used and the local form of consumption                                                                                                                                                                  | Nutritional value                                                                                                                                                                                                                                                                                 |
|-------------------|-------------------------------------------|---------------------------------|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 8                 | <i>Phytolaccaacinos</i> Roxb.             | Indian poke                     | Jaringo saag        | Tender leaves and stalks are used as vegetables.                                                                                                                                                              | Root is antiasthmatic, antibacterial, antidote, antifungal, antitussive, diuretic, expectorant, laxative & vermifuge. The plant has exciting chemistry & it is currently being investigated as a potential anti-AIDS drug                                                                         |
| 9                 | <i>Moringa oleifera</i> Lam.              | Drumstick tree                  | Sitalchini          | Leaves used as food. The roots, leaves, bark & pods are said to have medicinal properties.                                                                                                                    | Contain larger amounts of essential nutrients than the common foods often associated with these nutrients, for instance: two times more protein in milk, four times the calcium in milk, four times the vitamin A in carrot and more. So, they can help to combat malnutrition.                   |
| 10                | <i>Asparagus racemosus</i> Wild.          | Asparagus                       | Kurilo              | Roots, tubers and tender shoots are widely used for food & medicinal purposes.                                                                                                                                | Said to promote fertility and have a range of health benefits, particularly for the female reproductive system. Also, has Antioxidant and Anti-anxiety effects.                                                                                                                                   |
| <b>6 Oilseeds</b> |                                           |                                 |                     |                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                                                   |
| 1                 | <i>Perilla frutescens</i> (L.) Britton    | Perilla                         | Silam               | Seeds used to extract oil and prepare pickles                                                                                                                                                                 | Famous for its medicinal benefits and phytochemical compounds such as phenolic, flavonoids, Phytosterols, Tocopherols, Policosanols & fatty acids.                                                                                                                                                |
| 2                 | <i>Sesamum orientale</i> L.               | Sesamum                         | Til                 | Seeds used to prepare traditional, pickles and used as <i>hawan</i> material during <i>yagya</i>                                                                                                              | Increase plasma g-tocopherol and enhance Vit. E activity, which is believed to prevent human aging-related diseases such as cancer & heart disease                                                                                                                                                |
| 3                 | <i>Aesandra butyraceae</i> (Roxb.) Baehni | Nepali butter tree              | Chyuri              | Seed used to prepare ghee that is used for fuel lamps, and body lotion; the fruit is eaten fresh and used for alcohol distillation, oil cakes are utilized as manure, and the tree itself is used as firewood | Rich in sugars and fatty acids. It also bears medicinal value.                                                                                                                                                                                                                                    |
| 4                 | <i>Linum usitatissimum</i> L.             | Linseed                         | Aalash, Tisi        | Seeds used in bread and cereals as well as oil                                                                                                                                                                | Rich in soluble dietary fiber, oil (saturated fatty acids), protein & Vit. E (gamma-tocopherol)                                                                                                                                                                                                   |
| 5                 | <i>Prinsepia utilis</i> Royle             | Himalayan cherry                | Dhatelo             | Used as an ornamental elsewhere for its striking shiny brown bark                                                                                                                                             | Rich source of unsaturated fatty acids mainly oleic acid & linoleic acid                                                                                                                                                                                                                          |
| 6                 | <i>Ricinus communis</i> L.                | Castor bean or castor oil plant | Ander               | Seed extracted for castor oil to be used as medicine                                                                                                                                                          | Potential tropical feed resource because of its high nutrient profile for farm animals. It has medicinal value and used as a purgative and laxative. Castor toxin, ricin itself is being studied for therapeutic use in cancer chemotherapy, bone marrow transplantation and cell-based research. |
| <b>7 Fruits</b>   |                                           |                                 |                     |                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                                                   |
| 1                 | <i>Annona squamosa</i> L.                 | Custard apple                   | Sariphaa, sitaaphal | Fruit is eaten Fresh                                                                                                                                                                                          | Good dietary supplements have high content of minerals, Vit. C and carbohydrates. Antioxidant potential due to phenolic and flavonoids.                                                                                                                                                           |

| SN | Scientific name                                              | English name          | Nepali name  | Parts used and the local form of consumption                                                                                                     | Nutritional value                                                                                                                                                                                                                                          |
|----|--------------------------------------------------------------|-----------------------|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2  | <i>Tamarindus indica</i> L.                                  | Tamarind, Indian date | Imli         | Fruits used for human consumption                                                                                                                | The fruit pulp is rich in protein fiber, moisture, ash, carbohydrate, fat and minerals (Ca, Mg, Na & K). It also contains alkaloids, cardiac glycosides, flavonoids, phenols, saponins, steroids, tannins & terpenoids.                                    |
| 3  | <i>Myrica esculenta</i> BuchHam. ex D. Don                   | Hairy Bayberry        | Kaphal       | Fruit is eaten fresh                                                                                                                             | Widely used in folk medicine to treat several ailments such as asthma, cough, chronic bronchitis, ulcers, inflammation, anemia, fever, diarrhea, and ear, nose, and throat disorders. Fruits used for preparing syrups, jams, pickles & refreshing drinks. |
| 4  | <i>Aegle marmelos</i> (L.) Correa                            | Golden apple          | Bel          | Fruit and seed used for medicine and religious purpose. The fruit pulp is used as a refreshing juice by mixing the pulp, sugar and ice in water. | Has excellent medicinal, nutritional, environmental and commercial importance. Also, rich in carbohydrates, fiber, minerals & vitamins.                                                                                                                    |
| 5  | <i>Choerospondias axillaris</i> (Roxb.) B.L.Burt & A.W. Hill | Nepalese hog plum     | Lapsi        | Fruit used to make pickle                                                                                                                        | Wood is used as light construction timber and fuelwood; seed stones are used as fuel in brick kilns & the bark has medicinal value. The fruits are rich in vitamin C.                                                                                      |
| 6  | <i>Citrus limon</i> (L.) Burn f.                             | limon                 | Nibuva       | Fruit pulp used as fresh green salad, juice and processed products like chuk, squash, and pickle                                                 | Rich in phytochemicals including essential oils, alkaloids, flavonoids, coumarins, psoralens, carotenoids with a wide range of nutritional components including vitamins, minerals and trace elements.                                                     |
| 7  | <i>Citrus junos</i> Tanka                                    | Yuzu                  | Kali jyamir  | Fruit juice used for making vinegar which has medicinal values                                                                                   | Used industrially in the production of sweets, beverages, cosmetics, perfumes, and aromatherapy products. Important bioactive components present in yuzu fruits include vitamin C, $\beta$ -carotene, flavonoids, limonoids, and dietary fiber.            |
| 8  | <i>Citrus grandis</i> (L.) Osbeck                            | Pummelo               | Bhogate      | Fruit eaten fresh                                                                                                                                | Rich in flavonoids, antioxidants and phenolics content. The peel is also used for beauty purposes.                                                                                                                                                         |
| 9  | <i>Syzygium cumini</i> (L.) Skeels                           | Java plum             | Jamun        | Fruit eaten fresh and as medicine                                                                                                                | Rich in bioactive compounds such as phenols, flavonoids, and tannin which have medicinal value and used to cure cardiometabolic disorders.                                                                                                                 |
| 10 | <i>Juglans regia</i> L.                                      | Walnut                | Okhar        | Fruit has religious importance during worship. Oil extracted from fruit is edible. Fruit is a subsistence food item.                             | Rich in proteins, fat, antioxidants, some vitamins & minerals. A rich source in terms of Omega 3 which is useful for regular health.                                                                                                                       |
| 11 | <i>Artocarpus heterophyllus</i> Lam.                         | Jack fruit            | Rukh katahar | The fleshy pulp is eaten raw. Young-stage fruits used as vegetables. Seeds are eaten boiled, roasted, or cooked into a variety of food dishes    | Rich source of Vit. A, C, thiamin, riboflavin, minerals (Ca, K, Fe, Na, Zn), & niacin. Rich source of potassium and helps to lower blood pressure.                                                                                                         |
| 12 | <i>Phyllanthus emblica</i> L.                                | Indian gooseberry     | Aamala       | Fruit used to make shampoo, hair oil, sauces, candy, dried chips, pickles, jellies and powder.                                                   | All plant parts are used in various Ayurvedic medicine herbal preparations. The fruit is also used for culinary purposes. Also used in inks shampoos and hair oil. Due to its high tannin content, it is used for fixing dyes in fabrics.                  |

| SN                                  | Scientific name                             | English name          | Nepali name               | Parts used and the local form of consumption                                                                                                                                                                                                | Nutritional value                                                                                                                                                                                                                                                        |
|-------------------------------------|---------------------------------------------|-----------------------|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>8 Fruit vegetables</b>           |                                             |                       |                           |                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                          |
| 1                                   | <i>Trichosanthes dioica</i> Roxb.           | Pointed gourd         | Parvar                    | Fruit used as a vegetable                                                                                                                                                                                                                   | Rich in Vit. A, Vit. C, tannins, saponins, alkaloids, a mixture of novel peptides, proteins tetra & pentacyclic triterpenes. Plant leaves used as antipyretic, diuretic, cardiotonic, laxative, antiulcer.                                                               |
| 2                                   | <i>Sechium edule</i> (Jacq.) Sw.            | Chayote               | Skush                     | Fruit used as a vegetable                                                                                                                                                                                                                   | Rich in vitamins, minerals, fiber, water, and several amino acids. It also possesses diuretic, anti-inflammatory, and hypotensive activities.                                                                                                                            |
| 3                                   | <i>Benincasa hispida</i> (Thunb.) Cogn      | Wax gourd             | Kubindo                   | Fruit used as a vegetable when young. ripe fruit are popular in making crystallized candied sweet called "petha"                                                                                                                            | Nutritious vegetables and a good source of natural sugars, amino acids, organic acids, mineral elements and vitamins. Several medicinal properties such as anti-diarrheal, anti-obesity, anti-ulcer, and antioxidant and diuretic have been ascribed.                    |
| 4                                   | <i>Momordica balsamina</i> L.               | Balsam Apple          | Barela                    | Fruit used as a vegetable                                                                                                                                                                                                                   | Important green leafy vegetables as a source of nutrients to supplement another major source. Leaves are used to make soup for lactating mothers and babies to meet up the body's nutrient demand.                                                                       |
| <b>9 Spices</b>                     |                                             |                       |                           |                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                          |
| 1                                   | <i>Zanthoxylum armatum</i> DC.              | Nepal pepper          | Timur                     | Fruit used as a spice in Nepali cooking either fresh or in dried form. A special recipe timur ko chhop is also prepared from seed. Fruit and oil from seed used for therapeutic, pharmaceutical products, flavoring agents and in perfumery | Medicine as a carminative, stomachic, and relieves toothache. Fruits and seeds are also extensively used in fever, dyspepsia, and to kill roundworms. The essential oil of its fruits exhibited excellent antibacterial, antifungal, and antihelminthic activities.      |
| 2                                   | <i>Trachyspermum ammi</i> (L.) Sprague      | Caraway, ajowan, ammi | Jowano                    | Leaves used as vegetables. Seeds used to feed lactating women. Soup from seed has medicinal value                                                                                                                                           | Rich in carbohydrates, glycosides, saponins, phenolic compounds, volatile oil, protein, fat, fiber and minerals (Ca, P, Fe) and nicotinic acid. It also has medicinal value.                                                                                             |
| 3                                   | <i>Bunium persicum</i> (Boiss.) B. Fedtsch. | Black cumin           | Himaalijiraa, kaalo jiraa | Seed used as spice                                                                                                                                                                                                                          | Has antimicrobial and antioxidant properties and has excellent potential for the medicinal and food industries. In traditional medicine, it is used to treat or improve some cases such as digestive and urinary disorders, diabetes, obesity and increased breast milk. |
| <b>10 Hallucinogenic/ Narcotics</b> |                                             |                       |                           |                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                          |
| 1                                   | <i>Cannabis sativa</i> L.                   | Hemp                  | Bhang                     | Seeds have been used in pickle. Hemp fiber from the stem used to prepare a rope. Leaves used to make ganja and insecticides                                                                                                                 | High levels of calcium, fiber, fatty acids and protein. It also contains polyphenols which are well-known antioxidant. Seeds have Omega-3 (linolenic acid).                                                                                                              |
| 2                                   | <i>Papaver somniferum</i>                   | Poppy/Opium           | Aphim/ KhusKhus           | Opium is the dried latex obtained from the opium poppy which contains morphine, an alkaloid, which is also used to produce heroin in some parts of the country illegally.                                                                   | Seeds contain Selenium, which is necessary for human and animal nutrition. It helps several antioxidant enzymes to function normally.                                                                                                                                    |

Source: (Khanal et al., 2014; Joshi and Shrestha, 2018; Joshi et al., 2020)

NUS are the Future-smart food (FSF) because of their high adaptability and also a high potential for food and nutrition (Xuan and Siddique, 2018). Pacific of the FAO (2018) qualified six NUS namely Tartary buckwheat, Grass pea, Taro, Drumstick, Jackfruit, and Nepal butter tree as FSF for Nepal, to reinforce the importance of agricultural diversification. These six NUS fulfill the four-dimensional features of a FSF such as nutrient-dense, climate-resilient, potentially viable, and locally adaptable. Recently, the Government of Nepal has qualified buckwheat and mungbean as a FSF for the hilly and Terai regions, respectively. Prioritizing only a few NUS again leaves so many neglected crops as orphan crops creating problems in the country's food and nutrition security. Therefore, we agreed to prioritize above 65 NUS as a set of certain important crops for an uncertain future.

### 3.3 Prospects of NUS in climate change

NUS are predominantly climate-resilient, but their yield and quality are compromised due to selective breeding. Several orphan crops are identified as nutritious, resilient, and adapted to niche marginal agricultural environments. NUS can also provide a safety net during periods of stress and following disasters and other emergencies (Padulosi *et al.* 2013). NUS yields are typically lower than the main staple crops, often compensating for this by being more resistant to biotic stress and providing reliable harvests under unfavorable climatic conditions or on difficult soils (Padulosi *et al.* 2013). The adaptive capacity under biotic and abiotic stresses is one of the key traits of many NUS. Moreover, they are the most resistant to local pests and diseases. The erosion of agricultural diversity, especially of NUS, thus has serious implications for agriculture – the loss of resilience in the face of climate change, social and economic shocks and less ecosystem functionality. Thus, enhancing the diversity of NUS crops will not only diversify agroecosystems but will also improve their adaptability to extreme climatic conditions and provides resilience to biotic and abiotic stresses (Padulosi *et al.* 2002). Also, their diversity provides environmental services, such as clean water and carbon sequestration. Most of the growing NUS concern also focuses on their ability to contribute to adaptation to climate change (Aliyu *et al.*, 2014). However, other than selected reports of drought and heat stress tolerance, there is limited research confirming their suitability for future climates. For the NUS priority, research should focus on evaluating the impacts of climate change on their production for selected bioclimatic regions, with a focus on fitting them into semi-arid and arid regions.

### 3.4 NUS for agro-tourism and cultural diversity

NUS could be a potential crop for enhancing agro-tourism or culinary tourism in the country. The use of plants has long been an intimate part of local cultures and traditions. Many NUS play a role in keeping alive cultural diversity associated with food habits, health practices, rituals, and social exchanges. NUS have its most significant cultural importance at the local level, which puts greater emphasis on NUS as an important way to promote cultural diversity in an increasingly globalized world. The food culture that emphasizes NUS could fit into sustainable development programs such as agro-tourism, which highlights the agricultural and cultural inheritance of traditional knowledge, the agricultural landscape and agro-diversity. Localization of NUS into tourist areas might be the reason for tourist attraction, and promotion NUS by branding their geographic identification (GI). There should be a provision in a national policy that every homestay in the tourist area must feed the food to tourists, which constitutes at least 60% of the local food. Localization of local food not only promotes tourism but also promotes the diversity of neglected and underutilized food crops.

## 4. CONCLUSION

Nepal has a great diversity of NUS. These species have enormous nutritional, medicinal, environmental and economic values, and have the potential to contribute to poverty reduction, mainly in food-insecure areas. Considering their huge values for food and nutrition security, environment rural transformation, NUS promotion and utilization have been supported by national policies and programs. However, the promotion and commercialization of NUS are not gaining momentum. The reason behind this is the unavailability of seed materials, changing consumer behavior and less priority in research and development. For the utilization and promotion of NUS, it is essential to have a specialized research organization. Further, educational institutions should also include the NUS in their curriculum. NUS, in particular, should be studied for adaptability to extreme climate and nutritional and economic values. Considering the situation, we recommend that, for the next coming decade, national and international research and development organizations in the field of agriculture should provide grants for research and development of NUS. Mainstreaming these crops into national programs and policies, as well as integrating them into local food systems, is imperative. Besides, it is crucial to utilize

advanced breeding technologies for unlocking the true potentials of NUS as this is crucial for ensuring national food and nutritional security, promoting biodiversity, strengthening local communities, and ultimately achieving the United Nations Sustainable Development Goals (SDGs).

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## NEPALESE FINE AND AROMATIC RICE LANDRACES: A REVIEW

Kamana Rayamajhi<sup>1,2,\*</sup> and Bishwas Malla Thakuri<sup>1,3</sup>

<sup>1</sup> Institute of Agriculture and Animal Science (IAAS), Tribhuvan University, Nepal.

<sup>2</sup> Nepal Agricultural Research Council (NARC), Nepal Seed and Fertilizer Project, Khumlatar, Lalitpur.

<sup>3</sup> Inland Norway University of Applied Sciences, Postboks 400, 2418 Elverum, Norway.

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#### \*Correspondence:

kamana.rmj@gmail.com;  
Tel.: +977 9840069424

### ABSTRACT

*With the recent issue of Geographical Indication (GI) tag claim by India on Basmati rice, Nepalese rice sector got an urgent call to conserve and promote fine and aromatic rice landraces. Fine, medium, coarse landraces were further classified into aromatic and non-aromatic. Some of the aromatic landraces popularized by their local dialect names as Basmati anadi, Basmati anpjhutte, Choti basmati, etc. were cultivated in diverse environment by local farmers but utilization in national rice improvement program is insufficient. Just handful of landraces were used as parental lines to develop improved varieties viz. Jarneli in Khumal-2, Pokhreli masino in Khumal 4, Khumal -5 and Palung 2, pureline of Jethobudho as Pokhreli jethobudho, pureline of Lalkabasmati as Lalkabasmati. With the prevailing trend of fast-track registration of the exotic improved lines, our grassroots level efforts to study the genetic makeup and unique attributes of our landraces are overshadowed. However, Jarneli, Kalo marshi, Kalo nuniya, Mansara, etc. were found to be popular for traits nutritious, medicinal, abiotic stress tolerance and resilience to low fertility soil. Furthermore, the modern tools of molecular characterization are yet to be utilized prominently to identify such valuable genes and understand their phylogenetic diversity rewarding for future rice breeding program.*

### 1. INTRODUCTION

As the principle food grain of Nepal, rice (*Oryza sativa*) contributes 20% to the AGDP and 53% to the total cereal food production with the total area (14,58,195 ha), production (55,50,878 Mt) and productivity (3.804 Mt/ha) (MoALD, 2021). The aromatic rice comprises of group of varieties that emit aroma in cooking and consists of excellent eating (softness) qualities, fetching premium price in both national and international markets. The Basmati rice of Nepal is one of the most scented aromatic fine rices cultivated in lower belts of churiya range hills and some plains nearby Koshi river (Joshi *et al.*, 2017). Basmati dhan is also known as ‘Aghani’ in mithila language and used as various socioeconomic purposes like making ‘Acchata’ and burning ‘Diya’ in a clay pot as a religious belief, ‘Khuechha’ and ‘lawā’ during marriage ceremony and ‘Guchha’, ‘Jutti’ and ‘Samachakhewa’ as decorative items made from panicles of Basmati rice (Mandal, 2021).

Nepal used to export about 200 metric tonnes of fine quality aromatic rice per annum earning 41 million

rupees price in 1977 (Giri & Shahi, 1978). A total of 48 fine and aromatic rice varieties including both improved and local landraces are found to be grown in Nepal. Out of total area under rice cultivation, 16.9% (258855 ha) of area is covered by fine and aromatic rice of which the share of mountains, hills and terai are 5.92%, 44.20% and 49.88%, respectively (MOAD, 2015). The dietary pattern is changing over the years with change in living standard and economic status of the people. Nowadays, medium, and higher income groups prefer fine and aromatic rice over traditional coarse and sticky ones. The consumption of fine rice was 71.1 g per day and coarse rice was 245 grams per day (CBS, 2010/11). There is increasing demand of fine and aromatic rice in Nepal, but the demand is largely fulfilled by importing from open borders. The rice breeding program in Nepal is still dependent on foreign germplasm despite having 2500 rice landraces and 8,389 rice accessions recorded (Joshi, 2017).

Considering this scenario, the government of Nepal launched the first mission program on “Fine and

Aromatic Rice” in 20 districts in 2015, aimed at increasing area, production, and productivity of fine and aromatic rice for reducing imports in Nepal (CDD, 2015). With targets to cultivate fine and aromatic rice in 7500 ha and attain yield at 3 t/ha, the mission had major activities like 50% subsidies in foundation seed, 75% in commercial or improved seed, 100% in green manure (*Sesbania aculeata*) crop seed and provision of Zinc Sulphate for the reclamation of Zinc deficiency in rice field (MOAD, 2015).

In context of unfulfilled demand of fine aromatic rice on one side and the loss of important landraces on the other side, there seems to have a potential gap somewhere. If we do not work out immediately, there is high chance of not knowing even the names of most of our landraces by our future generation. So, the review highlights the status of fine and aromatic rice varieties in Nepal including major activities done for identification and analysis of fine quality and aromatic rice genotypes in Nepal. The article collects evidence of presence of landraces with high aromatic quality preferred and named locally as various types of ‘Basmati Dhan’.

### 1.1 Fine and aromatic landraces with preferred quality traits.

For milled rice the length of fine, medium, and coarse rice is > 6mm, 5.0-5.99mm and < 5mm, respectively (FAO, 2010). The diversity within and among fine and aromatic rice line is simply fascinating due to difference in size of the grains and varying degree of aroma (Table 1). And more specifically, special traits like earliness, drought tolerance, nutritious and medicinal value (Table 2). So, the differences in landraces are utilized according to consumer’s preferences.

**Table 1.** Classification of landraces based upon their fine and aromatic trait.

| S.N. | Groups        | Description                 | Landraces                                                                                                                                                                                                                                             |
|------|---------------|-----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1.   | Fine aromatic | Small size grain with aroma | Jorayal Basmati, Achame masino, Bayarni, Biramphool, Gauria, Gurdi kalo, Gurdi seto, Khalte kholo, Mansara, Masino, Sisuwa panheli, Jethobudho, Panhele, Anpjhutte, Rato Basmati, Motisar, Kalo masino, Chobo masino, Manakote, battisara, Thapachini |

| S.N. | Groups             | Description                     | Landraces                                                                                                                                                                                                                                                                                                                          |
|------|--------------------|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2.   | Fine nonaromatic   | Small size grain without aroma  | Andheri, Chhote, Gola, Gudura masino, Gurdi, Jerneli, Khusremni, Lamjunge, Manamuri, Panheli masino, Samundraphini, Tunde, Marshi, Jhinuwa, Mutmur, Anjani                                                                                                                                                                         |
| 3.   | Medium aromatic    | Medium size grain with aroma    | Laji, Mahajogani, Aanga, Chiniya puri, indrabeli, Jhinuwa, Jhinuwa ghaiya, Jhinuwa Masino                                                                                                                                                                                                                                          |
| 4.   | Medium nonaromatic | Medium size grain without aroma | Anjana, Kohile Jadhaniya                                                                                                                                                                                                                                                                                                           |
| 5.   | Coarse aromatic    | Large size grain with aroma     | Anjana, Gudgudo, Gudura, Sali dhan, Anadi Basnadar, Dudhe marshi, Pakhe jhinuwa                                                                                                                                                                                                                                                    |
| 6.   | Coarse nonaromatic | Large size grain without aroma  | Aanga, Aanpjhutte, Anadi rato, Bange jhinuwa, Budho thakale, Darmali, Dudhe juwari, Golaya tauli, Gorkhali, Anadi, Jadan, Germani, Pakhe jerneli, Jhaurijhutte juwari, Juwari, Jhinuwa, Kathe marsi, Kathe juwari, Naltumme, Naulo madhise, Sali dhan, Seto thakale, Silange pakhe, Thimaha, Tauli, Thulo masishe, mutmur, Jarneli |

Source: Joshi, 2017; Rijal, 1998

The farmers are very selective towards growing Landraces with the unique traits that add onto extra value to our cultivated varieties like Jarneli, Kalo Marshi, Kalo nuniya, Mansara, Mutmur, Pakhe jhniwa, Sali Dhan, Samudraphij, Sotwa, Sukirato, etc. The Landraces with their important unique traits are listed in Table 2.

**Table 2.** Landraces with their unique traits.

|     | Lines         | Trait                                                         |
|-----|---------------|---------------------------------------------------------------|
| 1.  | Jarneli       | Drought tolerance                                             |
| 2.  | Kalo marshi   | Tasty, easy milling, Andilo (giving energy for longer period) |
| 3.  | Kalo nuniya   | Nutritious and sticky                                         |
| 4.  | Mansara       | Adapted to Low fertility rainfed marginal environment.        |
| 5.  | Mutmur        | Grows in poor, rainfed soils                                  |
| 6.  | Pakhe jhinuwa | Early maturity and High Yield under low input.                |
| 7.  | Sali dhan     | Bold grain, scented upland                                    |
| 8.  | Samudraphij   | Suitable in swampy Land                                       |
| 9.  | Sotwa         | Medicinal Property                                            |
| 10. | Sukirato      | Early maturity, red grain, good in low fertility              |

Source: Joshi, 2017

Most of the landraces in Table 2 are cultivated and preserved by local farmers for their distinct properties but there are no efforts for conservation through registration at national level.

## 1.2 History of fine and aromatic rice

### Before 1980

1950: Initially, the rice varietal improvement in Nepal started as the selection of the local seeds (CDD, 2015; Rijal, 1998; Mallick, 1981) after which the exotic germplasm was introduced and tested in Agronomy farm, singhadurbar and parwanipur, Bara. However, none of them were found performing better than the popular local variety, thapachiniya with yield 2541 kg/ha.

1972: The establishment of National Rice Research Program at Parwanipur, Bara, initiated the systematic research followed by release of some semi dwarf IRRI lines IR 20 and IR 22. Moreover, the other high yielding varieties were released like Mansuli, Durga, Janaki, Jaya, and Sabitri with average grain yield of 4-5 t/ha (CDD, 2015).

1970s to 1980s: The Mansuli which was of intermediate height and fine grain was widely accepted by farmers

and popularized in late in the terai and inner terai regions of Nepal (CDD, 2015).

### 1981 to 2000

The increasing popularity of Mansuli in terai and inner terai region indicated that the farmers preferred traits like intermediate height, fine to medium grain size and acceptable cooking qualities. So, the focus was drawn to those traits to develop the varieties khumal 2, khumal 4 for midhills and palung 2 for palung and similar climatic areas of high hills. Despite of high popularity of Mansuli, the blast susceptibility decreased its demand and increased the search for blast resistant variety like Sabitri started in terai and inner terai regions. In the meantime, the varieties with intermediate to tall heights like Radha 7 and Radhakrishna 9 were developed by National Breeding Program for terai region (CDD, 2015; NNRP, 1997). The exotic germplasm Rampur mansuli was introduced (a blast resistant variety of Mansuli type) was released as a substitute of Mansuli variety in 1999 (CDD, 2015; Khatiwada & Upreti, 2008).

### 2001 Onwards

As alternative to Mansuli, Mithila and Ram dhan were released for irrigated and Loktantra was released for rainfed lowland and Radha 13 for low fertility and rainfed/partially irrigated lowlands. All these varieties were specifically selected for the mansuli type grain color. The popular aromatic rice Pokhreli jethobudo was released in 2006 for pokhara valley and its surroundings upto 600-900 masl, followed by exotic aromatic germplasm, Sunaulo sugandha with 3.8 mt per ha production was released in 2008 for terai and inner terai. In 2010, the release of Lalka basmati for main and inner terai region was done through Local selection method (CDD, 2015).

These varieties Pokhreli jethobudo and Sunaulo sugandha were developed by the joint venture of Local Initiatives for Biodiversity, Research and Development (LIBIRD) and Nepal Agricultural Research Council (NARC). The increasing involvement of private sector in recent period has initiated the hybrid rice research and use of markers assisted breeding (CDD, 2015; Joshi *et al.*, 2009). Moreover, the popular high demanded varieties Jumli marshi, khumal 4 and mansuli were utilized in molecular breeding for development of blast resistant varieties (CDD, 2015).

## 2. UTILIZATION IN VARIETAL IMPROVEMENT PROGRAM

Despite having approximately 2500 rice Landraces growing under diversified environment, very few germplasms have chance to reach up to varietal development stage. Mostly, Pokhrel masino, Jarneli, Jumli marshi and Jethobudho are popularized in Nepalese market as well as utilized in the development of improved varieties. The details of parental lines used for developing varieties are listed in Table 3.

**Table 3.** List of fine and aromatic varieties developed from local landraces.

| S.N. | Varieties         | Parent Lines                                            | Released Year |
|------|-------------------|---------------------------------------------------------|---------------|
| 1.   | Khumal-2          | Jarneli/ Ku-16-361-BLK-2-8                              | 1987          |
| 2.   | Khumal-4          | IR 28/ Pokhrel masino                                   | 1987          |
| 3.   | Palung -2         | BG- 94-2/ Pokhrel masino                                | 1987          |
| 4.   | Khumal -5         | Pokhrel masino/ KA-IB-361-BLK-2-6                       | 1990          |
| 5.   | Manjushree 2      | Jumli Marsi/IR 9129-159-3\kn-lb-361-1-8-6-3             |               |
| 6.   | Khumal-8          | Jumli Marsi/ IR 36                                      | 2007          |
| 7.   | Khumal-10         | IR 36/ Khumal 5                                         | 2011          |
| 8.   | Pokhrel jethobudo | Pureline selection from Jethobudho of Pokhara Valley.   | 2006          |
| 9.   | Lalka basmati     | Pureline selection from Lalka basmati of eastern terai. | 2010          |

Source: Upreti, 2017

Most of the popular aromatic varieties, with high yield potential favoured by commercial farmers in Terai region viz. Mansuli, Sunaulo sugandha, Sugandhit dhan 1 were developed from exotic germplasm of Malaysia and India respectively and varieties with submergence tolerant gene like Swarna Sub-1 and Samba Mansuli Sub-1 were introduced through International Rice Research Institute (IRRI) as shown in Table 4.

**Table 4.** List of fine aromatic varieties developed from exotic germplasm.

| S.N. | Variety             | Parentage                                                                          | Origin         |
|------|---------------------|------------------------------------------------------------------------------------|----------------|
| 1.   | Mansuli             | Mayang ebos 80*2/ Taichung 65                                                      | Malaysia, 1973 |
| 2.   | Sunaulo sugandha    | Selection of Pusa basmati1 treated by y rays*Unknown Parent                        | India, 2008    |
| 3.   | Sugandhit dhan 1    | Pusa basmati/ IET 12603                                                            | India, 2016    |
| 4.   | Swarna sub-1        | Swarna/ IR49830-7-1-2-3//2*Swarna (Swarna/ IR49830-7-1-2-3)                        | IRRI, 2011     |
| 5.   | Samba mansuli Sub-1 | Samba mahsuri/ IR49830-7-1-2-3//2* Samba Mahsuri (Samba mansuli / IR49830-7-1-2-3) | IRRI, 2011     |

Source: SQCC, 2021

## 3. STATUS (DIVERSITY AND DISTRIBUTION PATTERN) OF FINE AND AROMATIC LANDRACES

The need for preservation of landraces arises for their specific preferred traits, various socio-economic and cultural values, and better local adaptation. For fine and aromatic traits, landraces like Lalka basmati, Kariyakamod, Kanakjira in Bara, Kalonuniya in Jhapa, Jethobudho, Jhinuwa and Panhele in Kaski, Gudgudo in Gulmi and Gudo (kalo and seto) in Dailekh were conserved in cultivation.

The glory of mid western mountains is that, our landraces viz. Basmati, Jumli marshi\Kali marshi can be grown at the highest altitude while the mountains at central regions have evidences of growing Pokhrel masino, Anadi, Marsi and Anpjhutte. The landraces grown in the western hills (Jethobudho, Jarneli, Jhinuwa, Anadi, Gauriya, Gurdi, Anpjhutte, Mansara), mid western hills (Danda mansuli, Basmati, Marshi, Pokhrel masino, Paran pyuli, Pyuthani seto) far western hills (Thapachini, Jorayal basmati, Shyamjira, Marsi, Jaulo, Ratodhan, Dudhe) and Central hills (Pokhrel masino, Basmati, Manbhog, Mansara, Bhogati) have their own locally preference whose genetic similarities and distinctiveness are yet to be studied at genomic level.

**Table 5.** Distribution pattern of locally preferred landraces.

| S N. | Rice cultivars     | Distribution                                                                                                                                                                                                                                       |
|------|--------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1.   | Achhame masino     | 200-800masl (Chitwan, Jhapa, Makwanpur, Morang)                                                                                                                                                                                                    |
| 2.   | Anadi dhan         | < 700 masl (Arghakhanchi, Rupandehi, Kailali, Siraha)                                                                                                                                                                                              |
| 3.   | Basmati            | 200-100masl (Bara, Bajura, Dadeldhura, Darchula, Dhanusa, Doti, Humla, Jhapa, Kapilvastu, Kanchanpur, Kathmandu, Lalitpur, Mahottari, Morang, Parsa, Pyuthan, Ramechhap, Rautahat, Rupendehi, Sarlahi, Siraha, Sidhupalchowk, Taplejung, Udayapur) |
| 4.   | Basmati anadi      | <300masl (Bara)                                                                                                                                                                                                                                    |
| 5.   | Basmati anpjhutte  | <800masl (Dolakha)                                                                                                                                                                                                                                 |
| 6.   | Chhoti basmati     | <800masl (Jhapa, Morang, Sunsari)                                                                                                                                                                                                                  |
| 7.   | Danda basmati      | 1530 (Dadeldhura)                                                                                                                                                                                                                                  |
| 8.   | Gola basmati       | <500masl (Sunsari)                                                                                                                                                                                                                                 |
| 9.   | Jetho budo         | 600-1250 (Kaski, Myagdi, Syanga, Tanahu, Parbat, Sunsari)                                                                                                                                                                                          |
| 10.  | Jhinuwa            | <600masl (Baglung, Doti, Gorkha, Kaski, Lamjung, Kailali, Kanchanpur, Kathmandu, Myagdi, Nuwakot, Parbat, Sankhuwasbha, Sidhupalchowk, Sunsari, Syanga, tanahu)                                                                                    |
| 11.  | Jirasari           | <600masl (Jhapa, Morang, Panchthar, Ramechhap, Sunsari)                                                                                                                                                                                            |
| 12.  | Joroyal basmati    | <800masl (Doti)                                                                                                                                                                                                                                    |
| 13.  | Jorpal basmati     | <1200masl (Jhapa, Morang, Sunsari)                                                                                                                                                                                                                 |
| 14.  | Kalo basmati       | <1200masl (Dhankuta, Kathmandu, Jhapa, Morang, Sunsari)                                                                                                                                                                                            |
| 15.  | Kalo nuniya        | 60-300masl (Jhapa, Morang, Sunsari)                                                                                                                                                                                                                |
| 16.  | Kalo nuniya thulo  | 60-300masl (Jhapa, Morang, Sunsari)                                                                                                                                                                                                                |
| 17.  | Kalo tunde basmati | <300masl (Jhapa, Morang, Sunsari)                                                                                                                                                                                                                  |
| 18.  | Kanak jira         | <600masl (Bara, Bardiya, Chitwan, Jhapa, Morang, Sunsari, Kailali, Kanchanpur, Kapilvastu, salyan, sunsari, syanja)                                                                                                                                |
| 19.  | Kanak jira basmati | <300masl (Jhapa, Morang, Sunsari)                                                                                                                                                                                                                  |
| 20.  | Kasturi            | <500masl (Bara, Kailali, Parsa)                                                                                                                                                                                                                    |
| 21.  | Krishnabhog        | <1400masl (Achham, Dhankuta, Kanchanpur, Ramechhap)                                                                                                                                                                                                |
| 22.  | Lalka basmati      | 60-300 masl (Bara, Dhanusa, Kailali, Rautahat)                                                                                                                                                                                                     |
| 23.  | Masino basmati     | 900masl (Dhading, Khotang)                                                                                                                                                                                                                         |
| 24.  | Pahade basmati     | <1000masl (Ilam)                                                                                                                                                                                                                                   |
| 25.  | Rato basmati       | 60-300 masl (Jhapa, Morang, Sunsari)                                                                                                                                                                                                               |
| 26.  | Rato basmati sano  | 60-300 masl (Bara, Parsa, Mahottari, Siraha Jhapa, Morang, Sunsari)                                                                                                                                                                                |
| 27.  | Ratotunde basmati  | <300 masl (Jhapa, Morang, Sunsari)                                                                                                                                                                                                                 |
| 28.  | Seto basmati       | 60-300 masl (Bara, Parsa, Jhapa, Morang, Sunsari)                                                                                                                                                                                                  |
| 29.  | Shyamjira          | 60-300 masl (Banke, Doti, Kailai, Kanchanpur, Jhapa, Morang, Sunsari)                                                                                                                                                                              |
| 30.  | Thapachini         | 200-1400masl (Achham, Bajhang, bajura, Kailali, Deldeldhura, lamjung, tehrathum)                                                                                                                                                                   |
| 31.  | Tulsiprasad        | 200-1400masl (Nawalparasi, Bara, Dhanusa)                                                                                                                                                                                                          |
| 32.  | Tulsiphool         | 60-300 masl (Dhanusa, Morang, Siraha, Sapatari, Sindhuli, Sunsari, Udayapur, Mahottari)                                                                                                                                                            |
| 33.  | Ujarka basmati     | 60-300 masl (Bara, Parsa, Rautahat)                                                                                                                                                                                                                |

Source: Upadhyay &amp; Joshi, 2003

Table 5. shows that most of the Basmati rice viz. Ujarka, Ratotunde, Rato basmati, kalo tunde basmati, Basmati anadi, Kanak jira, etc. are grown at less than 300 masl and some of them like Basmati anpjhutte, Choti, Gola, Joroyal basmati, Kasturi etc are better grown at 600-800masl.

While Landarces like Jetho budo, Masino, Kalo basmati, Jorpal basmati, Pahade and Masino basmati can also be grown above 800masl up to 1250masl. This provides a good opportunity for famers of hills and mountains for

growing fine aromatic rice at higher altitudes as well.

#### 4. VALUE OF OUR FINE AND AROMATIC RICE LANDRACES AND MAJOR ISSUES

With the major storage protein called glutelin i.e., oryzenin (Kulp & Ponte, 2000), rice has highest protein and energy digestibility among the cereals due to its low dietary fibre and tannin content (FAO, 2021). Additionally, it is good source of thiamine, riboflavin, niacin, and highest level of selenium among cereals (10-13 mg/100 gm). The long white grain rice is

relatively good source of carbohydrates, calcium, iron, thiamine, pantothenic acid, folate, and vitamin E than other cereals. Though, long grain varieties have higher chances of breakage during milling than short grain, it is assumed that higher the length-breadth ratio, higher will be quality (Bhattacharya, 1969).

Dhungana, 2017 evaluated the grain quality of ten aromatic landraces and found the highest hulling percentage (83.24 %) in jhinuwa and that of lowest in indrabeli (75.03%) both of which have capacity of strong aromatic emission. The highest Length to Breadth ratio was obtained in Joroyal Basmati (3.8) whereas the lowest was found in gudgudo (1.6). Based upon the L/B ratio, the studied varieties were classified as Bold (Anjana, Gudgudo, Gudura), Medium (Aanga, Chiniya puri, indrabeli, Jhinuwa, Jhinuwa ghaiya, Jhinuwa masino) and Slender (Joroyal basmati). In a comparative study of four types of Basmati rice varieties, Red, white, Black and Pokhareli, the highest protein content was found in red basmati rice 7.74%. The highest hulling percentage (72.02%) and head rice recovery (67.46%) was found in pokhareli basmati. Red Basmati was found to have better cooking quality due to highest kernel elongation and volume expansion ratios 1.62 and 2.85 respectively, minimum water uptake ratio (2.18) and gruel loss (1.05%) and highest starch iodine value of 0.21 (Ojha *et al.*, 2018).

In molecular characterization of aroma in Jethobudo, seven enhanced jethobudo populations were evaluated for aroma with three known aromatic varieties: (Azucena japonica cultivar from IRRI, Pusa Basmati-1: a popular indian aromatic rice, Rato basmati: local aromatic rice of Nepal) and one nonaromatic modern variety IR 36 (Kandel & Shrestha, 2018). The seven SRR primers associated with aromatic quality were used to characterize the jethobudo populations using biomolecular technique (McCouch *et al.*, 1997).

Panta *et al.*, 2012 found out the relative abundance of rice landraces and varieties in farmers field as 31-40% Basmati, 21-30% Sona mansuli and Anadi, 10-20% Jethobudho and less than 10% Rato anadi, seto anadi gurdi, jhinuwa, Jarneli, Mansara, Masula, Biramful etc. Furthermore, over 30 % of the farmers were growing Basmati rice and the varieties like Basmati, Sona mansuli and anadi were the most popular ones. So, the authors concluded that the value of the aromatic trait of Basmati or other local landraces can be about one-fourth of the value of rice produced. That implies the aromatic traits of rice have values of about NPR 11

billion (\$148.6m) and tasty traits over NPR 2 billion (\$27m) per annum (Panta *et al.*, 2012).

The aroma was perceived as a relative term by the local farmers who believed that the degree of the aroma decreases if consumed on a regular basis. The application of chemical fertilizers would also decrease the pure aromatic taste, the degree of aroma decreases with storage time and aroma depends on the milling process i.e., rice milled with local Dhiki is more aromatic as compared to milled in a rice huller (Rijal *et al.*, 1998). Moreover, the farmers realized the quality of rice was superior when grown on irrigated low land as compared to swampy lands. Thus, it was well accepted fact that the aroma is not only characteristic to the varietal genetic property, rather a collective result of well executed production practice.

According to a study conducted by Local Initiatives for Biodiversity, Research and Development (LIBIRD) and International Development Research Centre (IDRC) on the 'Inventory of Indigenous Rainfed and Aromatic Landraces in Seti River Valley, Pokhara, Nepal' the local landraces grown widely by farmers for their quality traits have been reported to have low yield potential, susceptible to insect-pests and prone to lodging. These were Jetho Budo, Gurdi, Anadi, Panhele, Gauria, Biramphool, Ramani, Mansara, Aanpjhutte, Jerneli, Khalte kholo. Though, the introduction of improved varieties could have replaced these landraces by farmers for their low Yield potential, the varieties Jetho budo, Anadi and Panhele were hardly replaced (Joshi *et al.*, 1996).

## 5. CONCLUSION

To fulfil the demand of increasing population and substitute the cereals import with export in this changing global environment, the systematic development and deployment of high yielding and climate resilient varieties is the only option. This has drastically increased the trend of registering exotic improved varieties leading to increased rate of replacement of traditional rice varieties. However, this scenario i.e., pressure to feed the increasing population is leading to loss of authenticity in rice improvement program in Nepal as evident in any other sectors.

Despite of unceasing efforts of our dedicated researchers and local farmers in the conservation and promotion of fine quality rice landraces, these issues could be well addressed only through efficient utilization of preferred quality traits in rice breeding programs. The various

types of Basmati dhan, Sona mansuli, Anadi, Pokhrel, ethobudo, Mansara, Jhinuwa, Masula, Jarneli etc. are in the phase of gradual extinction despite of having quality traits viz., drought tolerance, growth in adverse environment, good aroma, medicinal value. Such varieties must be characterized at agro morphological and evaluated at molecular level for their valuable genes to be identified and utilized substantially in breeding

programs. The easy way of fast-tracking registration of improved varieties via private sectors should not overshadow the value of our local landraces. Rather, the present rice research must be willing to deliver conscious efforts on evaluating our native germplasm and incorporate their valuable genes through modern breeding techniques in rice varietal improvement program.

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# PHYTOCHEMICAL SCREENING AND ALLELOPATHIC POTENTIAL OF IMPORTANT MEDICINAL PLANTS USED BY DHIMAL COMMUNITY IN URLABARI MUNICIPALITY

Sonika Poudel <sup>1,\*</sup> and Subodh Khanal <sup>2</sup>

<sup>1</sup> Institute of Agriculture and Animal Science, Tribhuvan University, Kathmandu, Nepal

<sup>2</sup> Gauradaha Agriculture Campus, Institute of Agriculture and Animal Science, Tribhuvan University, Gauradaha, Jhapa

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\*Correspondence:

poudelsonika07@gmail.com;

Tel: +977-9817316046

## ABSTRACT

A study was conducted to document the major ethnobotanicals used against different ailments from Dhimal community of Urlabari Municipality. Deductive research approach employed through household survey using semi-structured questionnaire with 115 respondents for research work. Out of 23 documented plants, *Cuscuta reflexa* Roxb., *Mimosa pudica* L., *Azadirachta indica* A. Juss, *Achyranthes aspera* L. and *Acorus calamus* L. were screened for further phytochemical analysis based on fidelity level. Five treatments maintained as 3 different concentrations (5, 10, and 15% of stock solution), and control were arranged in a completely randomized design with three replications for evaluating allelopathic potential where selected botanical extracts on germination, radical, and plumule growth of wheat seedlings were examined under invitro condition. *Mimosa pudica* L. had the highest alkaloid (15.39%), *Cuscuta reflexa* Roxb. had the highest terpenoid (9.17%) and *Acorus calamus* L. had the highest saponin (5.49%) when calculated via gravimetric method. Germination, radical and plumule growth found under control treatment were  $3.24^{ab} \pm 0.09$ ,  $2.94^a \pm 0.09$ cm and  $2.70^a \pm 0.17$ cm respectively. The stock solution of *Acorus calamus* L. with 15% concentration extract resulted in the maximum reduction of radicle length ( $1.14^e \pm 0.22$ cm) and plumule length ( $1.04^h \pm 0.17$ cm), along with heavily controlled germination ( $1.05^f \pm 0.17$ ) of wheat seedlings. Thus, *Acorus calamus* L. proved the highest allelopathic potential on wheat seedling growth indicating further investigation on other crops.

## 1. INTRODUCTION

Nepal is a multi-ethnic, multi-lingual and multi-religious nation with each ethnic communities having significant customary knowledge on utilization of plant and plant parts and there is a long tradition of transferring this indigenous knowledge from generation to generation (Acharya & Acharya, 2009). The ethnic people residing in different geographical belts of Nepal depend on wild plants to meet their basic requirements and all the ethnic communities have their own pool of secret ethnomedicinal and ethnopharmacological knowledge about the plants available in their surroundings. Strengthening the wise use and conservation of indigenous knowledge of useful plants may benefit and improve the wellbeing of poor people (Kunwar & Bussmann, 2008). Dhimal is one among 59 ethnic groups identified by the Government of Nepal (Bhattarai & Niraula, 2020). Dhimal's settlement is mainly concentrated in Morang and Jhapa districts of Koshi Province of Nepal. The main settlement area of Dhimal is stretched east to west along the foothills

around the forest belts of Chure mountain range. It is said that the Dhimal people belong to Kiratis and are believed to have settled down along the northern or north-eastern Himalayan region long before 1000 B.C. or even before (Dhimal, 1973).

Ethnobotany is a set of empirical local practices embedded in the indigenous knowledge of a social group often transmitted from generation to generation (Kunwar & Bussmann, 2008) which influence the health problems and intent to overcome such problems (Njoroge *et al.*, 2004). The concept of ethnobotany considers and includes all studies, which focus on the mutual relationship between plants and traditional people (Harshberger, 1896). People still depend upon the traditional practitioners who have been using the indigenous ethnobotanical knowledge (Koirala & Khaniya, 2009). The possibility to better understand the relationships among the people, their culture and the environment has central importance because it

allows the characterization of social systems through their particular environmental perception, and provides useful tools (Toledo *et al.*, 2009). The ethnobotanical plants have the potential to treat a wide range of illnesses and there is the need to validate their uses for therapeutic application with further research on the isolation and characterization of the plant active compounds for the discovery of potential drugs (Asiimwe *et al.*, 2021). The indigenous knowledge is an important part of the primary health care system and are the last storehouse of traditional knowledge which is under the constant threat of losing valuable information (Khajuria *et al.*, 2021) especially the far-flung areas. These areas, one of the last storehouses of traditional knowledge are under the constant threat of losing this valuable information as it moves from one generation to another through word of mouth. Modernization, migration, education, and changing socio-economic status of people also affect the perpetuity of traditional knowledge. Therefore, time-to-time updation of information regarding the ethnomedicinal plants must be carried out so that any addition to the traditional knowledge is recorded and further phytochemical and pharmacological studies may be conducted for developing new drugs. Aim of the study: The study aimed at documenting the traditional knowledge and practices about the medicinal plants used by the inhabitants of Pauri district of Uttarakhand. Besides, the study strives to identify plants for future phytochemical and pharmacological studies. Material and methods: The information was collected through semi-structured questionnaire from 98 informants distributed in 15 villages of Pauri. The data was analyzed for use-reports (UR). The loss of traditional medicinal knowledge in a culture that is undergoing a rapid change is as irreversible as the loss of plant species (Joshi & Joshi, 2000). Ethnobotanical knowledge should be consolidated with modern biotechnologies to achieve desired end products with scientific validation. The ethnobotanicals can be an alternative to problem of anti-biotic resistance (Sohail *et al.*, 2012).

Phytochemical screening is the scientific process of analyzing, examining, extracting, experimenting, and identifying different classes of phytoconstituents in various parts of the base for the discovery of drugs, the active components could be further taken for investigation. The outcome of the investigation could be fruitful in developing potent drugs against various diseases (Sharma *et al.*, 2020). Chemicals released from plants and imposing allelopathic influences are termed as allelochemicals and they can exist in several parts of plants including roots, rhizomes, leaves, stems,

pollen, seeds and flowers. These allelochemicals could be the main reason for the restricted growth of other plant species near their colony. The allelopathic plants pose threat on seed germination, seedlings radicle and plumule lengths of wheat and barley (Tessema & Tura, 2018). Ethnomedicinal documentation, combined with phytochemical and bioactivity screening, is a convincing method for identifying new drugs from medicinal plants that play an essential role in human health (Sher & Aldosari, 2012). Also, determining the allelopathic effect of those plants helps to provide accessible, safe, reliable and inexpensive sources of control of various weeds (Gautam *et al.*, 2021). Allelopathic potentials of plants which persuades identifying and purification of allelopathic substances, may result in controlling specific weeds through their use as natural herbicides (Hassan *et al.*, 2012).

Ethnobotanicals have been studied elsewhere for their chemical composition and biological activities. However, the published data are scant regarding the ethnobotany and pharmacological properties of the ethnobotanical plants. Most of the ethnomedicinal studies conducted in recent years in Nepal have only documented whether the community people have knowledge about the use of plants or not but have not mentioned about the phytochemical screening along with the allelopathic potential of these plants. Therefore, along with documentation and phytochemical screening of the ethnobotanicals, test for allelopathic potential would preserve and promote its use as an ingredient in agriculture and other industries. Efforts should be made to document the medicinal use of the plants before much of this is eliminated, or before the inhabitants of the region abandon their traditional medical practices (Joshi & Joshi, 2000).

## 2. MATERIALS AND METHODS

### 2.1 Conceptual framework

The focus of the research was to document the ethnomedicinal plants used by Dhimal community of Urlabari municipality, their phytochemical screening and discovering their allelopathic potential. Overall conceptual framework of the research is presented as:

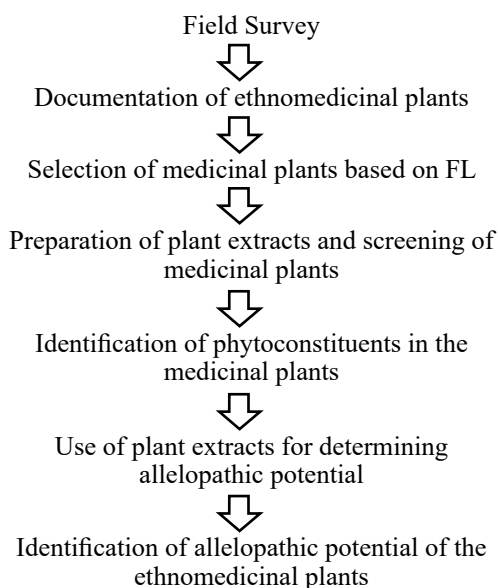


**Figure 1.** Conceptual framework for the research

## 2.2 Study area

Urlabari municipality of Morang district was selected for the survey. The main basis of selection of this site was because of the accessibility and dominance of Dhimals (7.55%). The ethnomedicinal plants were collected from the survey site which is located at 26°39' N and 87°37' E. The laboratory activities were carried out at the Institute of Agriculture and Animal Science (IAAS), Gauradaha Agriculture Campus, Gauradaha, Jhapa located at 26°33' N and 87°43' E. Geographically, the survey and experimental location both lies in the fertile Terai plains of the Koshi Province of eastern Nepal.

## 2.3 Fidelity level (FL)

The fidelity level (FL) was expressed as the percentage of informants claiming the use of a certain plants for the same major purpose and calculated as per Alexiades (1996):

$$FL \% = \frac{I_p}{I_u} \times 100$$

Where,  $I_p$  = the number of informants who independently suggested the use of a plant species for a particular disease and  $I_u$  = total number of informants who mentioned the same plant for any disease.

## 2.4 Plant extract preparation

The medicinal plant parts were collected, cleaned with running water and dried under shade at room temperature (30 ± 5°C) for 10 days (Handa *et al.*, 2008).

### 2.4.1 Preparation of crude powder

After about 10 days of shade drying, well dried plants parts were grinded and then product was subjected to mass sieving to obtain fine powder. The obtained powder was then kept in a plastic jar with airtight lid and stored for the required period (Khanal, 2021).

### 2.4.2 Preparation of stock solution

100 grams crude powder of each collected parts was soaked in 1000 ml of distilled water separately and left for overnight in an airtight plastic bottle for maceration. Mixture was then filtered and used further (Khanal, 2021). The prepared extract of all the ethnomedicinal plants were used for phytochemical screening.

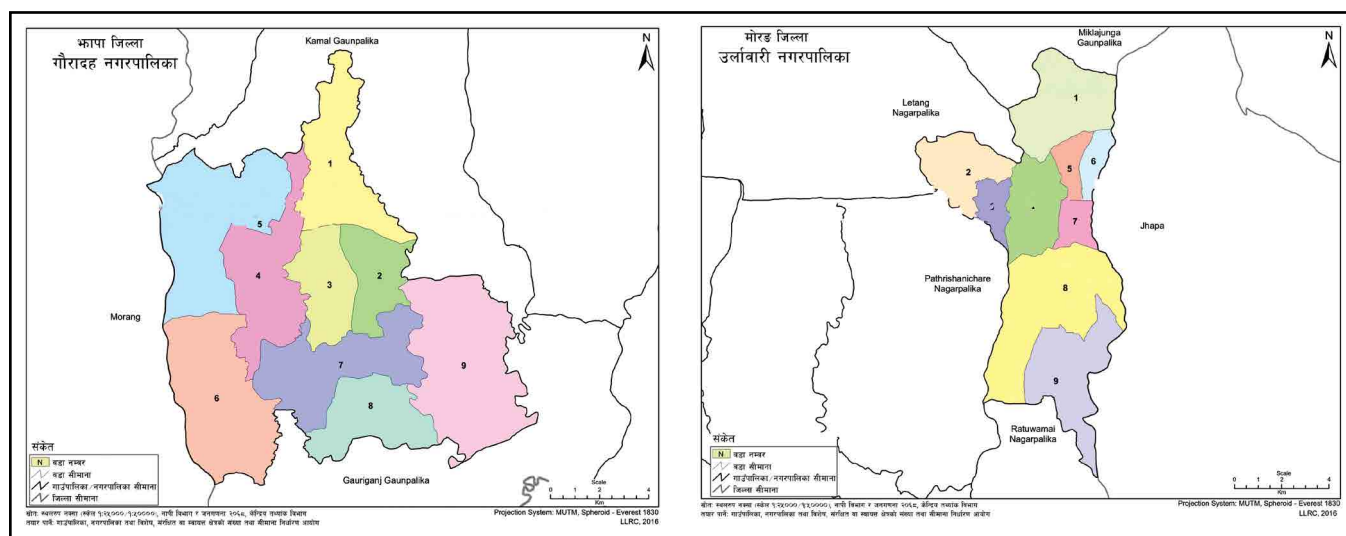


**Figure 2.** Research sites

Source: LLRC, 2016

## 2.5 Quantitative Phytochemical Analysis

Quantitative test of phytochemical was done by the gravimetric method described as per Harborne (1973):

### 2.5.1 Test for alkaloid

Five grams of sample dust was dissolved in 100 ml of 10% acetic acid. It was well soaked and left for 4 hours. The solution was then filtered and the filtrate was evaporated to  $\frac{1}{4}$ <sup>th</sup> of its original volume. Concentrated Ammonium hydroxide (NH<sub>4</sub>OH) was added drop wise to precipitate the alkaloid content. Solution was then filtered again and washed with 1% NH<sub>4</sub>OH. Filter paper containing precipitate was dried in the oven at 60°C for 30 minutes and was weighed after being allowed to cool for a few minutes. The total alkaloid content was then calculated as per Harborne (1973):

$$\text{Alkaloid \%} = \frac{W_2 - W_1}{W_1} \times 100$$

Where,  $W_1$  = weight of empty filter paper,  $W_2$  = weight of paper + alkaloid precipitate

### 2.5.2 Test for terpenoid

Dried plant extract 10 grams ( $W_i$ ) was taken and soaked in 90 ml of ethanol. The extract after filtration was mixed with 10 ml of petroleum ether and again filtered using a separating funnel. The extract was then waited for its complete drying and measurement was taken ( $W_f$ ). The total terpenoids contents was measured as per Indumathi *et al.* (2014):

$$\text{Terpenoid \%} = \frac{W_i - W_f}{W_i} \times 100$$

Where,  $W_i$  = dried plant extracts,  $W_f$  = extracts after drying

### 2.5.3 Test for saponin

The plant extract i.e., 25 ml was placed in a round bottom flask. 100 ml of 50% alcohol was added, boiled

for 30 minutes and filtered while hot through a filter paper. 2 gm of charcoal was added to the filtrate and it was boiled and filtered while hot. The filtrate was then cooled and an equal volume of acetone was added to completely precipitate the saponins. The precipitated saponins was collected and calculated as per Muhammad & Abubakar (2016):

$$\text{Saponin \%} = \frac{W_2 - W_1}{W_1} \times 100$$

Where,  $W_1$  = weight of filter paper,  $W_2$  = weight of residue + weight of filter paper

### 2.5.4 Test for allelopathy

The phytotoxic activity of different plant extracts on the selected plant was investigated by using different concentrations of the extracts. Out of 23 plants, 5 plants were selected on the basis of fidelity level and the plant extracts were then subjected to test allelopathy. Wheat seeds were used as experimental plants and it was assumed that Vijaya variety of wheat would perform better even in the laboratory. A total of twenty wheat seeds of Vijaya variety (soaked overnight) were placed in each petri-plate with moistened filter paper inside. Each seed was then placed at equal distances and moisture was provided according to the concentration of extracts. Data of germinated seeds, radicle and plumule length of wheat seedlings were recorded and the temperature and relative humidity of laboratory during the whole experiment were also noted. Altogether, 14 treatments and one control with distilled water and 3 replications were arranged in the laboratory of Gauradaha Agriculture Campus in completely randomized design (CRD). Each treatment was maintained as 5, 10 and 15% of prepared stock solution (Gyawali *et al.*, 2021).

**Table 1.** Treatment details for assessing allelopathic potential of important medicinal plants

| Plants                            | Concentration (%) | Treatment |
|-----------------------------------|-------------------|-----------|
| Control                           | 0                 | T0        |
| <i>Azadirachta indica</i> A. Juss | 5                 | T1        |
| <i>Azadirachta indica</i> A. Juss | 10                | T2        |
| <i>Azadirachta indica</i> A. Juss | 15                | T3        |
| <i>Cuscuta reflexa</i> Roxb.      | 5                 | T4        |
| <i>Cuscuta reflexa</i> Roxb.      | 10                | T5        |
| <i>Cuscuta reflexa</i> Roxb.      | 15                | T6        |
| <i>Mimosa pudica</i> L.           | 5                 | T7        |
| <i>Mimosa pudica</i> L.           | 10                | T8        |
| <i>Mimosa pudica</i> L.           | 15                | T9        |
| <i>Achyranthes aspera</i> L.      | 5                 | T10       |
| <i>Achyranthes aspera</i> L.      | 10                | T11       |
| <i>Achyranthes aspera</i> L.      | 15                | T12       |
| <i>Acorus calamus</i> L.          | 5                 | T13       |
| <i>Acorus calamus</i> L.          | 10                | T14       |
| <i>Acorus calamus</i> L.          | 15                | T15       |

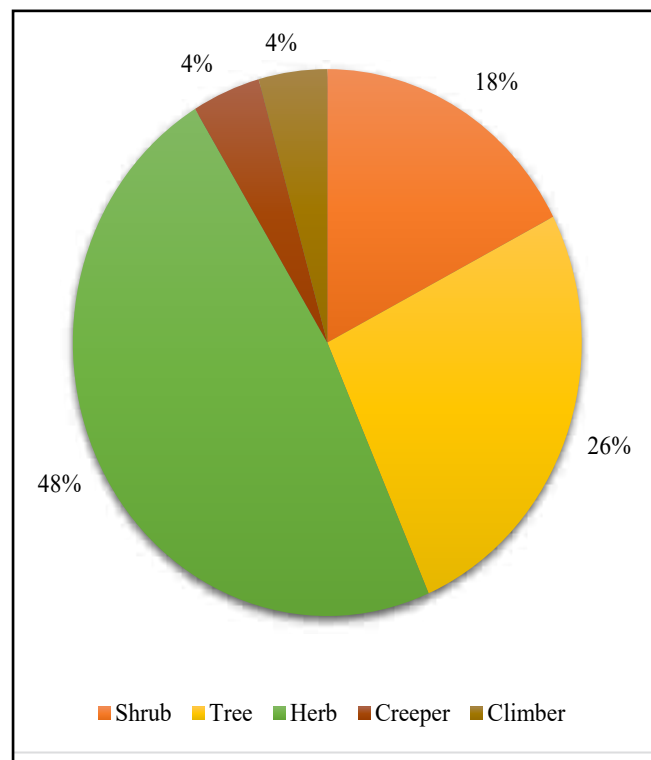
## 2.6 Data entry and analysis

The survey was taken with the help of m-Water Surveyor while other experimental data were entered in MS Excel, followed by tabulation and arrangement. Data were subjected to square root transformation before further analysis. Statistical analysis was carried out using R-stat version (4.2.1) and mean separation by DMRT (Duncan Multiple Range Test) at 5% level of significance. Package “Agricole” was used for mean separation and “Rstatix” for calculating standard error.

## 3. RESULTS AND DISCUSSION

### 3.1 Plant characteristics

A total of 23 medicinal plants belonging to 20 families were recorded during the survey. Majority of the plants (52%) were obtained in cultivated form. Leaves were used mostly rather than other parts because of easy collection and presence of secondary metabolites available in leaves. The life forms of the plants were presented below in figure 4.

**Figure 3.** Laboratory set-up for the test of allelopathic potential**Figure 4.** Life form of medicinal plants

### 3.2 Knowledge and concern regarding the use of medicinal plants

Majority of the respondents (80.87%) agreed that they have been sharing the knowledge regarding ethnobotanicals to their family members. Talking about the need of documentation in scale, more than half of the respondents (57.39%) suggested the strong need of documentation of ethnomedicinal plants while the least (1.74%) gave a neutral opinion regarding documentation. Majority of the respondents (54%) didn't show any interest at all and were not ready to take concern in the use and promotion of ethnomedicinal plants. Majority (87%) supported pharmaceutical drugs more accessible and used for instant healing than ethnobotanicals, yet all (100%) agreed on the economic viability of ethnobotanicals. So, ethnomedicinal plants could be assessed and explored for potential drugs

(Sharma *et al.*, 2020) from which economically weak households also can be benefitted for cure of ailments.

### 3.3 Fidelity level (FL)

While selecting the most preferred plant species for each ailment category, the highest fidelity level values were considered in each category of ailment. *Cuscuta reflexa* Roxb., *Mimosa pudica* L. and *Azadirachta indica* L. were found to have maximum FL (100%) followed by *Achyranthes aspera* L. (81.33%) and *Acorus calamus* L. (65.44%). The plant species with the highest FL is considered the most preferred and important species for a particular purpose (Hoffman & Gallaher, 2007). Plants with the highest FL values were an indication of their good healing potential in their respective ailment categories.

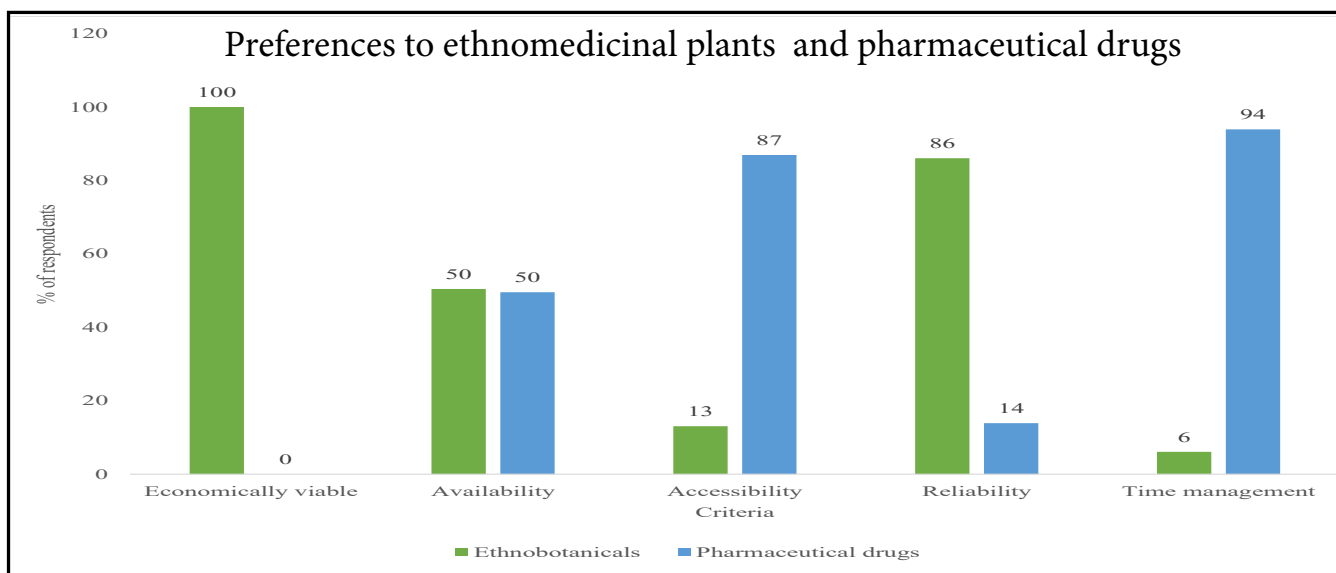


**Figure 5.** Preference to ethnomedicinal plants and pharmaceutical drugs based on different criteria

**Table 2.** Fidelity level of different medicinal plants

| Ailment categories                 | Medicinal plants                  | Np | N   | FL (%) |
|------------------------------------|-----------------------------------|----|-----|--------|
| Against jaundice                   | <i>Cuscuta reflexa</i> Roxb.      | 67 | 67  | 100.00 |
| Against pneumonia                  | <i>Mimosa pudica</i> L.           | 29 | 29  | 100.00 |
| Against skin disease               | <i>Azadirachta indica</i> A. Juss | 29 | 29  | 100.00 |
| Against fever                      | <i>Achyranthes aspera</i> L.      | 61 | 75  | 81.33  |
| Against cough, cold & sore-throat  | <i>Acorus calamus</i> L.          | 89 | 136 | 65.44  |
| In burn, cuts & wound              | <i>Aloe barbadensis</i> Mill.     | 9  | 14  | 64.29  |
| Against high blood pressure        | <i>Artemisia vulgaris</i> L.      | 16 | 25  | 64.00  |
| Against gastro-intestinal disorder | <i>Oroxylum indicum</i> L.        | 26 | 42  | 61.90  |
| Against urinary problem            | <i>Centella asiatica</i> L.       | 8  | 14  | 57.14  |
| Against body pain                  | <i>Melia azedarach</i> L.         | 2  | 4   | 50.00  |

Np=Citation for the particular ailment, N=Particular plant used, FL=Fidelity level

### 3.4 Quantitative evaluation of medicinal plants

Quantitative analysis of phytochemicals was done as explained by Harbone (1973) by the gravimetric method. Phytochemical screening of alkaloid, terpenoid and saponin was done in the selected medicinal plants (*A. indica* A. Juss, *C. reflexa* Roxb, *M. pudica* L., *A. aspera* L. and *A. calamus* L.) and the result was tabulated. It was observed that *Mimosa pudica* L. had the highest alkaloid (15.39%), *Cuscuta reflexa* Roxb. had the highest terpenoid (9.17%) and *Acorus calamus* L. had the highest saponin (5.49%) when calculated. The contents of alkaloid and saponin obtained from the leaves of *A. indica* A. Juss were in agreement with Khanal (2021). Variation in result among the tested medicinal plants may be due to the plant parts used, geographical factor, solvent used, procedure followed and other external factors. Limited research has been done for the quantitative analysis of phytoconstituents for only a few medicinal plants while some plants have not been mentioned till date for phytochemical analysis.

**Table 3.** Phytoconstituent composition in important medicinal plants

| Medicinal plants                  | Alkaloid (%) | Terpenoid (%) | Saponin (%) |
|-----------------------------------|--------------|---------------|-------------|
| <i>Azadirachta indica</i> A. Juss | 11.05        | 5.00          | 2.32        |
| <i>Cuscuta reflexa</i> Roxb.      | 5.67         | 9.17          | 1.92        |
| <i>Achyranthes aspera</i> L.      | 7.97         | 3.00          | 1.58        |
| <i>Mimosa pudica</i> L.           | 15.39        | 0.83          | 2.05        |
| <i>Acorus calamus</i> L.          | 12.66        | 3.17          | 5.49        |

### 3.5 Allelopathic potential of medicinal plants

Germination of wheat seedlings was found to be maximum in control ( $3.24^{ab} \pm 0.09$ ) while heavily controlled germination was observed in 15% stock solution of *A. calamus* L. ( $1.05^f \pm 0.17$ ) as shown in appendix 2. Germination of seedlings were found to be constant after 6 days of placement of seeds. It was observed that with increase in concentration of plant extracts, resulted in reduction of germination i.e., inhibitory activity was dependent on the extract concentrations and the higher extract concentration resulted in the stronger inhibitory activity (Salam & Kato-Noguchi, 2010; Salam, 2018).

Radicle and plumule growth found under control treatment were  $2.94^a \pm 0.09$ cm and  $2.70^a \pm 0.17$ cm respectively which was maximum. The 15% of stock

solution of *Acorus calamus* L. extract resulted in a highly significant ( $p < 0.01$ ) and maximum reduction in radicle length ( $1.14^c \pm 0.22$ cm) and plumule length ( $1.04^h \pm 0.17$ cm). It was observed that the effectiveness of plant extracts was greater on root growth to that of the shoot growth of the plants used for allelopathy test which was supported by (Asgharipour & Armin, 2010). Similar finding was observed in mungbean which concluded that the allelopathic effect was important in controlling the seedling growth and only the control treatment bored germination with proper radicle and plumule development (Gyawali *et al.*, 2021).

## 4. CONCLUSION

The majority of plants under investigation were contributing majorly herbs followed by tree, shrub and creeper and climbers belonging to 20 families. The most frequently medicinally utilized plant parts were leaves and bark followed by root and rhizome. *Mimosa pudica* L. had the highest alkaloid and lower terpenoid, *Cuscuta reflexa* Roxb. had the highest terpenoids and the lowest alkaloids, *Acorus calamus* L. had the highest saponin and *Achyranthes aspera* L. had the lowest saponin. This indicates that different medicinal plants are known to produce secondary metabolites which influence the growth of other plants. *Acorus calamus* L. was discovered with the highest allelopathic potential on wheat seedling growth which might be fruitful for weed management in other crops as well which needs further investigation. Various studies have given clear ideas regarding the phytoconstituents along with their inhibitory effects against either ailments or weed management. Thus, along with the documentation of important medicinal plants which have been used from early days, proper studies and researches regarding their different properties can be carried out. Research activities related to ethnobotanics can be fruitful in exploring and discovering new drugs. These ethnomedicinal plants have some allelopathic potential and can be an alternative for weed management in agriculture. Considering their availability, effectiveness and reliability, use of these ethnomedicinal plants should be encouraged rather than being limited within the indigenous community.

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## ANNEX

Appendix 1. List of documented plants along with their other information

| S.N | Local name     | Common name | Scientific name                           | Family         | Type of plant | Life form of plant | Parts used   | Method of Use and Administration                           | Ethno-medicinal uses                                           |
|-----|----------------|-------------|-------------------------------------------|----------------|---------------|--------------------|--------------|------------------------------------------------------------|----------------------------------------------------------------|
| 1   | Aank           | Ank         | <i>Calotropis gigantea</i> L.             | Apocynaceae    | Wild          | Shrub              | Root         | Root juice is extracted.                                   | In burn, cuts & wound                                          |
| 2   | Amala          | Amla        | <i>Phyllanthus emblica</i> L.             | Euphorbiaceae  | Cultivated    | Tree               | Fruit        | Fruits are consumed directly.                              | Against gastro-intestinal disorder & jaundice                  |
| 3   | Ambak          | Guava       | <i>Psidium guajava</i> L.                 | Myrtaceae      | Cultivated    | Tree               | Bark         | Bark extract is obtained by crushing the bark.             | Against gastro-intestinal disorder                             |
| 4   | Bakaino        | Bakaino     | <i>Melia azedarach</i> L.                 | Meliaceae      | Wild          | Tree               | Leaves       | Leaf juice is extracted.                                   | Against urinary problem & body pain                            |
| 5   | Barro          | Barro       | <i>Terminalia bellirica</i> Gaertn. Roxb. | Combretaceae   | Cultivated    | Herb               | Fruit        | Fruits are consumed directly.                              | Against cough, cold & sore-throat                              |
| 6   | Bayer          | Indian plum | <i>Ziziphus mauritiana</i> Lam.           | Rhamnaceae     | Wild          | Tree               | Root         | Juice is extracted by crushing the roots.                  | Against fever                                                  |
| 7   | Besar          | Turmeric    | <i>Curcuma longa</i> L.                   | Zingiberaceae  | Cultivated    | Shrub              | Rhizome      | Powder is extracted from dried rhizome.                    | Against cough, cold & sore-throat & gastro-intestinal disorder |
| 8   | Bhringraj jhar | False daisy | <i>Eclipta prostrata</i> L.               | Asteraceae     | Wild          | Herb               | Whole plant  | Plant is crushed and juice is extracted.                   | In cuts & wound                                                |
| 9   | Bojho          | Sweet flag  | <i>Acorus calamus</i> L.                  | Acoraceae      | Cultivated    | Herb               | Rhizome      | Small piece of dried rhizome is chewed or tied on the neck | Against cough, cold & sore-throat                              |
| 10  | Gheukumari     | Aloe vera   | <i>Aloe barbadensis</i> Mill.             | Liliaceae      | Cultivated    | Herb               | Leaves       | Jel is extracted, used or consumed.                        | Against fever & in burn, cuts & wound                          |
| 11  | Godhtapre      | Godhtapre   | <i>Centella asiatica</i> L.               | Apiaceae       | Wild          | Herb               | Leaves       | Leaf juice is extracted.                                   | Against gastro-intestinal disorder & urinary problem           |
| 12  | Gurjo          | Gurjo       | <i>Tinospora cordifolia</i> L.            | Menispermaceae | Cultivated    | Climber            | Stem, leaves | Decoction of fresh leaves and stem.                        | Against fever                                                  |

| S.N | Local name     | Common name    | Scientific name                    | Family         | Type of plant | Life form of plant | Parts used     | Method of Use and Administration                                                   | Ethno-medicinal uses                          |
|-----|----------------|----------------|------------------------------------|----------------|---------------|--------------------|----------------|------------------------------------------------------------------------------------|-----------------------------------------------|
| 13  | Lajjawati jhar | Lajjawati jhar | <i>Mimosa pudica</i> L.            | Fabaceae       | Wild          | Crepper            | Root           | Roots are crushed and taken orally.                                                | Against pneumonia                             |
| 14  | Neem           | Neem           | <i>Azadirachta indica</i> A. Juss  | Meliaceae      | Wild          | Tree               | Leaves         | Leaf extract is obtained and used for bathing                                      | Against skin disease                          |
| 15  | Pahelo lahara  | Cuscuta        | <i>Cuscuta reflexa</i> Roxb.       | Convolvulaceae | Wild          | Herb               | Stem           | Juice is made, filtered and consumed or use for bathing                            | Against jaundice                              |
| 16  | Parijat        | Parijat        | <i>Nyctanthes arbor-tristis</i> L. | Oleaceae       | Cultivated    | Shrub              | Leaves, flower | Leaves and flowers are crushed and consumed.                                       | Against fever, cough, cold & sore-throat      |
| 17  | Patharchattia  | Bryophyllum    | <i>Kalanchoe pinnata</i> L.        | Crassulaceae   | Herb          |                    | Leaves         | Fresh leaves are chewed. Leaf juice is also extracted.                             | Against gastro-intestinal disorder            |
| 18  | Pudina         | Pudina         | <i>Mentha spicata</i> L.           | Lamiaceae      | Cultivated    | Herb               | Leaves         | Juice is extracted from leaves. Fresh leaves consumed directly.                    | Against gastro-intestinal disorder & fever    |
| 19  | Sadabahr       | Periwinkle     | <i>Catharanthus roseus</i> L.      | Apocynaceae    | Cultivated    | Herb               | Leaves, flower | Leaves and flowers are consumed directly.                                          | Against high blood pressure                   |
| 20  | Titepati       | Common mugwort | <i>Artemisia vulgaris</i> L.       | Asteraceae     | Wild          | Herb               | Leaves         | Decoction of fresh leaves. Leaf juice is also extracted.                           | Against high blood pressure & skin disease    |
| 21  | Totelo         | Tatelo         | <i>Oroxylum indicum</i> L.         | Bignoniaceae   | Wild          | Tree               | Leaves, bark   | Decoction of fresh leaves. Bark extract is also obtained.                          | Against gastro-intestinal disorder & jaundice |
| 22  | Tulsi          | Tulsi          | <i>Ocimum tenuiflorum</i> L.       | Lamiaceae      | Cultivated    | Shrub              | Whole plant    | Juice is obtained from the leaves or consumed directly. Decoction of fresh leaves. | Against fever, cough, cold & sore-throat      |
| 23  | Ulto jara      | Apamarg        | <i>Achyranthes aspera</i> L.       | Amaranthaceae  | Wild          | Herb               | Root           | Juice is extracted by crushing the roots.                                          | Against fever cough, cold & sore-throat       |

## Appendix 2. Effect of plants extract on germination of wheat seedlings

| Treatment  | Germinated seeds           |                            |                             |                             |                             |                             |                             |
|------------|----------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|            | 2 DAP                      | 3 DAP                      | 4 DAP                       | 5 DAP                       | 6 DAP                       | 7 DAP                       | 8 DAP                       |
| T0         | 3.24 <sup>ab</sup> ±0.09   | 3.24 <sup>ab</sup> ±0.09   | 3.24 <sup>ab</sup> ±0.09    | 3.24 <sup>ab</sup> ±0.09    | 3.24 <sup>ab</sup> ±0.09    | 3.24 <sup>ab</sup> ±0.09    | 3.24 <sup>ab</sup> ±0.09    |
| T1         | 2.76 <sup>abc</sup> ±0.32  | 2.99 <sup>abc</sup> ±0.33  | 3.06 <sup>abc</sup> ±0.26   | 3.06 <sup>abc</sup> ±0.26   | 3.06 <sup>abc</sup> ±0.26   | 3.06 <sup>abc</sup> ±0.26   | 3.06 <sup>abc</sup> ±0.26   |
| T2         | 2.00 <sup>bcd</sup> ±0.65  | 2.12 <sup>bcd</sup> ±0.71  | 2.23 <sup>abcde</sup> ±0.77 | 2.23 <sup>abcde</sup> ±0.77 | 2.23 <sup>abcde</sup> ±0.77 | 2.23 <sup>abcde</sup> ±0.77 | 2.23 <sup>abcde</sup> ±0.77 |
| T3         | 1.77 <sup>cde</sup> ±0.59  | 1.93 <sup>cde</sup> ±0.62  | 2.06 <sup>bcd</sup> ±0.68   | 2.06 <sup>bcd</sup> ±0.68   | 2.06 <sup>bcd</sup> ±0.68   | 2.06 <sup>bcd</sup> ±0.68   | 2.06 <sup>bcd</sup> ±0.68   |
| T4         | 2.09 <sup>abcd</sup> ±0.26 | 2.40 <sup>abcd</sup> ±0.44 | 2.55 <sup>abcd</sup> ±0.40  | 2.60 <sup>abcde</sup> ±0.44 | 2.60 <sup>abcde</sup> ±0.44 | 2.60 <sup>abcde</sup> ±0.44 | 2.60 <sup>abcde</sup> ±0.44 |
| T5         | 2.34 <sup>abcd</sup> ±0.12 | 2.73 <sup>abcd</sup> ±0.11 | 2.73 <sup>abcd</sup> ±0.11  | 2.73 <sup>abcde</sup> ±0.11 | 2.73 <sup>abcde</sup> ±0.11 | 2.79 <sup>abcde</sup> ±0.12 | 2.79 <sup>abcde</sup> ±0.12 |
| T6         | 0.71 <sup>e</sup> ±0.00    | 1.56 <sup>de</sup> ±0.19   | 1.56 <sup>de</sup> ±0.19    | 1.64 <sup>ef</sup> ±0.26    | 1.64 <sup>ef</sup> ±0.26    | 1.64 <sup>ef</sup> ±0.26    | 1.64 <sup>ef</sup> ±0.26    |
| T7         | 3.38 <sup>a</sup> ±0.15    | 3.44 <sup>a</sup> ±0.10    | 3.48 <sup>a</sup> ±0.13     | 3.48 <sup>a</sup> ±0.13     | 3.48 <sup>a</sup> ±0.13     | 3.48 <sup>a</sup> ±0.13     | 3.48 <sup>a</sup> ±0.13     |
| T8         | 2.40 <sup>abcd</sup> ±0.18 | 2.67 <sup>abcd</sup> ±0.17 | 2.74 <sup>abcd</sup> ±0.11  | 2.86 <sup>abcde</sup> ±0.06 | 2.86 <sup>abcde</sup> ±0.06 | 2.86 <sup>abcde</sup> ±0.06 | 2.86 <sup>abcde</sup> ±0.06 |
| T9         | 2.41 <sup>abcd</sup> ±0.59 | 2.96 <sup>abc</sup> ±0.15  | 3.02 <sup>abc</sup> ±0.20   | 3.02 <sup>abcd</sup> ±0.20  | 3.02 <sup>abcd</sup> ±0.20  | 3.02 <sup>abcd</sup> ±0.20  | 3.02 <sup>abcd</sup> ±0.20  |
| T10        | 2.28 <sup>abcd</sup> ±0.81 | 2.56 <sup>abcd</sup> ±0.69 | 2.68 <sup>abcd</sup> ±0.58  | 2.68 <sup>abcde</sup> ±0.58 | 2.68 <sup>abcde</sup> ±0.58 | 2.68 <sup>abcde</sup> ±0.58 | 2.68 <sup>abcde</sup> ±0.58 |
| T11        | 2.74 <sup>abc</sup> ±0.00  | 2.80 <sup>abcd</sup> ±0.06 | 2.80 <sup>abcd</sup> ±0.06  | 2.80 <sup>abcde</sup> ±0.06 | 2.80 <sup>abcde</sup> ±0.06 | 2.80 <sup>abcde</sup> ±0.06 | 2.80 <sup>abcde</sup> ±0.06 |
| T12        | 2.64 <sup>abc</sup> ±0.30  | 2.91 <sup>abc</sup> ±0.17  | 2.91 <sup>abc</sup> ±0.17   | 2.91 <sup>abcde</sup> ±0.17 | 2.91 <sup>abcde</sup> ±0.17 | 2.91 <sup>abcde</sup> ±0.17 | 2.91 <sup>abcde</sup> ±0.17 |
| T13        | 1.72 <sup>cde</sup> ±0.51  | 1.79 <sup>cde</sup> ±0.56  | 1.86 <sup>cde</sup> ±0.60   | 1.86 <sup>cdef</sup> ±0.60  | 1.86 <sup>cdef</sup> ±0.60  | 1.86 <sup>cdef</sup> ±0.60  | 1.86 <sup>cdef</sup> ±0.60  |
| T14        | 1.10 <sup>de</sup> ±0.39   | 1.10 <sup>e</sup> ±0.39    | 1.66 <sup>de</sup> ±0.22    | 1.74 <sup>def</sup> ±0.27   | 1.74 <sup>def</sup> ±0.27   | 1.74 <sup>def</sup> ±0.27   | 1.74 <sup>def</sup> ±0.27   |
| T15        | 0.71 <sup>e</sup> ±0.00    | 1.05 <sup>e</sup> ±0.17    | 1.05 <sup>e</sup> ±0.17     | 1.05 <sup>f</sup> ±0.17     | 1.05 <sup>f</sup> ±0.17     | 1.05 <sup>f</sup> ±0.17     | 1.05 <sup>f</sup> ±0.17     |
| Grand mean | 2.14                       | 2.39                       | 2.48                        | 2.50                        | 2.50                        | 2.50                        | 2.50                        |
| CV         | 32.08                      | 27.48                      | 25.92                       | 26.21                       | 26.21                       | 26.19                       | 26.19                       |
| MS error   | 0.47                       | 0.43                       | 0.41                        | 0.43                        | 0.43                        | 0.43                        | 0.43                        |
| LSD        | 1.14                       | 1.09                       | 1.07                        | 1.09                        | 1.09                        | 1.09                        | 1.09                        |
| F-value    | 4.01***                    | 3.69***                    | 3.33**                      | 3.11**                      | 3.11**                      | 3.12**                      | 3.12**                      |

Mean followed by the same letter in a column are not significantly different by DMRT at 5% confidence level. DAP=Days after placement; CV=Coefficient of variation; MS error= Mean standard error; LSD=Least significant differences; \*\* significant 1% and \*\*\*significant 0.1% level of significance

**Appendix 3.** Effect of plants extract on radicle and plumule length of wheat seedlings

| Treatment  | Plumule length           |                            | Radicle length            |                          |
|------------|--------------------------|----------------------------|---------------------------|--------------------------|
|            | 4 DAP                    | 7 DAP                      | 4 DAP                     | 7 DAP                    |
| T0         | 2.32 <sup>a</sup> ±0.11  | 2.70 <sup>a</sup> ±0.17    | 2.55 <sup>a</sup> ±0.15   | 2.94 <sup>a</sup> ±0.09  |
| T1         | 1.46 <sup>d</sup> ±0.10  | 1.61 <sup>defg</sup> ±0.11 | 1.65 <sup>cdc</sup> ±0.09 | 1.84 <sup>cd</sup> ±0.01 |
| T2         | 1.04 <sup>ef</sup> ±0.17 | 1.12 <sup>gh</sup> ±0.21   | 1.20 <sup>ef</sup> ±0.25  | 1.24 <sup>c</sup> ±0.27  |
| T3         | 1.03 <sup>ef</sup> ±0.16 | 1.09 <sup>h</sup> ±0.19    | 1.18 <sup>ef</sup> ±0.24  | 1.24 <sup>c</sup> ±0.27  |
| T4         | 1.30 <sup>dc</sup> ±0.05 | 1.40 <sup>efgh</sup> ±0.07 | 1.52 <sup>dc</sup> ±0.03  | 1.65 <sup>dc</sup> ±0.03 |
| T5         | 1.26 <sup>dc</sup> ±0.06 | 1.35 <sup>efgh</sup> ±0.08 | 1.42 <sup>def</sup> ±0.05 | 1.60 <sup>dc</sup> ±0.04 |
| T6         | 0.93 <sup>ef</sup> ±0.04 | 1.14 <sup>gh</sup> ±0.08   | 1.15 <sup>ef</sup> ±0.02  | 1.35 <sup>dc</sup> ±0.02 |
| T7         | 2.04 <sup>ab</sup> ±0.03 | 2.16 <sup>bc</sup> ±0.03   | 2.18 <sup>ab</sup> ±0.07  | 2.35 <sup>bc</sup> ±0.02 |
| T8         | 1.91 <sup>bc</sup> ±0.04 | 2.09 <sup>bcd</sup> ±0.06  | 2.12 <sup>abc</sup> ±0.04 | 2.28 <sup>bc</sup> ±0.01 |
| T9         | 1.53 <sup>cd</sup> ±0.01 | 1.76 <sup>cde</sup> ±0.10  | 1.73 <sup>bcd</sup> ±0.07 | 1.88 <sup>cd</sup> ±0.08 |
| T10        | 2.24 <sup>ab</sup> ±0.03 | 2.35 <sup>ab</sup> ±0.03   | 2.37 <sup>a</sup> ±0.03   | 2.46 <sup>b</sup> ±0.04  |
| T11        | 1.87 <sup>bc</sup> ±0.13 | 2.06 <sup>bcd</sup> ±0.13  | 2.17 <sup>ab</sup> ±0.11  | 2.30 <sup>bc</sup> ±0.11 |
| T12        | 1.62 <sup>cd</sup> ±0.35 | 1.70 <sup>cdef</sup> ±0.35 | 1.81 <sup>bcd</sup> ±0.33 | 1.84 <sup>cd</sup> ±0.34 |
| T13        | 1.01 <sup>ef</sup> ±0.15 | 1.20 <sup>fgh</sup> ±0.25  | 1.20 <sup>ef</sup> ±0.25  | 1.36 <sup>dc</sup> ±0.33 |
| T14        | 1.01 <sup>ef</sup> ±0.13 | 1.31 <sup>efgh</sup> ±0.07 | 1.19 <sup>ef</sup> ±0.16  | 1.47 <sup>dc</sup> ±0.03 |
| T15        | 0.81 <sup>f</sup> ±0.06  | 1.04 <sup>h</sup> ±0.17    | 0.92 <sup>f</sup> ±0.11   | 1.14 <sup>c</sup> ±0.22  |
| Grand mean | 1.46                     | 1.63                       | 1.65                      | 1.81                     |
| CV         | 15.36                    | 16.55                      | 16.31                     | 15.93                    |
| MS error   | 0.05                     | 0.07                       | 0.07                      | 0.08                     |
| LSD        | 0.37                     | 0.45                       | 0.45                      | 0.48                     |
| F-value    | 14.37***                 | 10.72***                   | 10.62***                  | 10***                    |

Mean followed by the same letter in a column are not significantly different by DMRT at 5% confidence level.

DAP=Days after placement;

CV=Coefficient of variation;

MS error=Mean standard error;

LSD=Least significant differences;

\*\* significant 1% and \*\*\*significant 0.1% level of significance.



## SATELLITE REMOTE SENSING OF RICE CROPS IN NEPAL : A REVIEW

Keshav Thapa Magar <sup>1</sup>

<sup>1</sup> Nepal Open University, Lalitpur, Nepal

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\* Correspondence:  
ksabmagar7@gmail.com  
Tel: +977-

### ABSTRACT

*Remote sensing, based on satellite data, is useful in studying rice crops for their several parameters such as cropped area, crop-coverage, growth stages, production, yield estimation and other statistics useful in scientific investigations. Despite some satellite-remote-sensing based researches have been reported in the country, such appliance in agricultural studies is very sparse in Nepal. In the current situation, Nepal could benefit from remote sensing technology for betterment of its agricultural planning and development specifically in the rice sub-sector for its national economic and food security importance. This review article is an attempt to highlight the importance of rice remote sensing in Nepal and list research articles in the field as bookmarks to benefit future rice researchers and interested readership.*

### 1. INTRODUCTION

Remote sensing is the scientific technology to acquire physical characteristics of distant objects by the measurement of the reflected and emitted spectral information (USGS, 2022). The radiation collected as the spectral information from sensors mostly based in satellite, aircraft or flying bodies, such as drones, enables the ability to observe larger areas of the surface. Such prepared information over longer duration, even more than a decade, adds advantage to study the changes in the area. These are useful in studying the changes induced by humans in different landscapes and ecosystems (Kerr & Ostrovsky, 2003). Unlike other sensors, the benefits of satellite remote sensing are its readiness and free availability from the providers which has been used in multiple areas including agriculture and crop studies, and importantly in rice crops (Corvino *et al.*, 2018).

Remote sensing in agriculture is used in crops monitoring as an assistive tool for decision making even at the farm level (Gao, 2021; Hatfield *et al.*, 2020). Remote sensing aids in crop yield estimation process, management of the crops in drought and unpredicted weather conditions and proper policy planning for betterment of crops and agriculture in direct relations with food security (Kumar *et al.*, 2022). The benefits

of remote sensing are in its beneficial involvements in different crop stages for proper optimization of the yield whose research trends are increasing in current years (Khanal *et al.*, 2020).

Application of remote sensing in rice has been increasing since mid 2000s with the MODIS sensors data (due to its easy access and with its higher temporal resolution or its ability to acquire the image every one or two days) and the main research objectives of rice remote sensing have been in retrieval and study of the rice characteristics such as Leaf Area Index (LAI), nitrogen and chlorophyll contents, biomass which have the guiding roles in the rice agronomic managements (Xu *et al.*, 2022). The research publications are mostly seen from the developed nations that includes the United States of America (USA), China and Japan, but genuinely, the remote sensing has been boon in other nations as well for monitoring of rice crops mainly for the crop mapping (DDNS, 2016; Tiwari *et al.*, 2021; Torres, 2022).

Multiple objectives of rice remote sensing can be surely pointed depending on what satellite sensors and spectral information are being used such as observing rice plant diseases, methane emissions from rice fields,

and rice growing ecosystem as pointed by Kuenzer & Knauer (2013), but the primary goals of the rice remote sensing are rice mapping and the yield estimation. Rice mapping simply are the process to delineate the rice growing areas where multiple methods are applied, including but not limited to, using vegetation indices (mathematical operations of the different spectral bands), radar backscatter (reflectance of microwave portion from the rice growing area) and phenology based approaches from different rice growing stages (Dong & Xiao, 2016). For the rice yield estimation, different models are developed, where satellite generated parameters are empirically related with the yield with current innovative algorithms of machine learning and deep learning (Torre *et al.*, 2021).

## 2. A BRIEF HISTORY

The history of the agriculture remote sensing dates as early as 1970s or even before, but the use of satellite remote sensing can be formally dated with the launch of Landsat-1, which was used for the estimation of wheat production under the project Large Area Crop Inventory Experiment (LACIE) (Macdonald, 1984). With similar aims in Europe in 1988, the Monitoring Agriculture using Remote Sensing (MARS) was launched for estimation of crop yield. By the 1990s, radar based satellite sensors were already launched, whose additional advantages were over its use in the clouds, as microwave energy could penetrate over the clouds, and were used in the studies of rice phenology, rice acreage estimation and rice biophysical parameters (Macdonald, 1984).

History of satellite remote sensing in Nepal dates back with the use of the satellite imageries in the map preparation such as demographic maps in 1991 (Oli, 2002). The potential of remote sensing was long realized for the sensitive ecology of the Himalayan region of Nepal (Millette *et al.* 1995). National Remote Sensing Centre (NRSC) of Nepal was established in 1981, by the cooperation between the then government of Nepal and USAID, which merged with the Forest Survey Division in 1989 and has worked since then for monitoring of forest resources (Juwa, 2009). The noteworthy initiation of application of remote sensing in agriculture can be pointed to the Agriculture Inventory Project of Nepal in the 1980s. However, the project itself had slight or no importance towards the agricultural aspects, and the full exploration of remote sensing in crop studies is still ambiguous (Johnson *et al.*, 1984).

## 3. CURRENT ACTIVITIES IN NEPAL

Current rice remote sensing activities, including other crops, have been mainly performed by International Centre for Integrated Mountain Development (ICIMOD) such as the observation of drought conditions in the agricultural lands (SERVIR, 2016) and the training for crop monitoring using satellite remote sensing aimed at agriculture officials (SERVIR, 2014). The praiseworthy activity is in the ICIMOD's joint approach with Ministry of Agricultural and Livestock Development (MoALD) of Nepal in developing the crop agricultural statistics using the satellite data with machine learning methods, while ICIMOD's important goal has constantly been in aiming to uplift technological capacity of MoALD in Nepal (Qamer *et al.*, 2023).

## 4. LISTABLE WORKS

Discussing the approaches applied in the rice remote sensing will still be early in the context of Nepal. Due to limited works being conducted and the remote sensing works yet to be seen, the best will be still to list the available published works.

The current works can be suggestively divided into three main groups:

- works done for the rice mapping and yield estimation precisely done for Nepal (Table 1),
- works done with the similar goals but not aimed particularly for Nepal, but includes Nepal in the research process (Table 2), and
- works involving rice and some aspects of satellite remote sensing in Nepal but include broader themes not being limited to rice mapping and yield estimation (Table 3).

No particular search method was applied in search of the research articles, which were rather collected during personal studies of the author. Title of the article with distinct object identifier (doi) along with the publication year has been provided as the bookmarked list in table 1, 2 and 3 as grouped in three main sections. Short phrases as the brief, generalising the research works, have been provided, but the interested readers still need to seek each article for comprehensive understanding of the methods applied.

Table 2. presents the research mainly done for the South Asian countries and includes Nepal as one of their study areas.

**Table 1.** Rice Mapping and Yield Prediction precisely done for Nepal

| <b>Title. doi</b>                                                                                                                                                                                                                         | <b>Year</b> | <b>Brief</b>                                                                 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|------------------------------------------------------------------------------|
| Temporal changes in rice-growing area and their impact on livelihood over a decade: A case study of Nepal. <a href="https://doi.org/10.1016/j.agee.2011.06.010">https://doi.org/10.1016/j.agee.2011.06.010</a>                            | 2011        | rice mapping from MODIS sensor from 2000 to 2010                             |
| Operationalizing crop monitoring system for informed decision making related to food security in Nepal. <a href="http://dx.doi.org/10.5194/isprsarchives-XL-8-1325-2014">http://dx.doi.org/10.5194/isprsarchives-XL-8-1325-2014</a>       | 2014        | rice crop area estimation of terai districts of 2013 and 2014                |
| Sentinel-2 Multi-Temporal Data for Rice Crop Classification in Nepal. <a href="https://doi.org/10.1109/IGARSS39084.2020.9323771">https://doi.org/10.1109/IGARSS39084.2020.9323771</a>                                                     | 2020        | deep learning methods for the preparation of the rice maps                   |
| Tracking the dynamics of paddy rice cultivation practice through MODIS time series and PhenoRice algorithm. <a href="http://dx.doi.org/10.1016/j.agrformet.2021.108538">http://dx.doi.org/10.1016/j.agrformet.2021.108538</a>             | 2021        | preparation of the rice map of Nepal from 2003 to 2018                       |
| Rice-Yield Prediction with Multi-Temporal Sentinel-2 Data and 3D CNN: A Case Study in Nepal. <a href="https://doi.org/10.3390/rs13071391">https://doi.org/10.3390/rs13071391</a>                                                          | 2021        | deep learning method for the rice yield predictions of terai districts       |
| Mapping Spatial Distribution Of Main Season Rice Fields In Eastern Nepal Using Multi-Temporal Landsat 8 Images. <a href="http://doi.org/10.26480/bda.01.2021.01.05">http://doi.org/10.26480/bda.01.2021.01.05</a>                         | 2021        | rice mapping of Sunsari and Morang district using Landsat data               |
| Rice Modeling Using Long Time Series of High Temporal Resolution Vegetation Indices in Nepal. <a href="https://doi.org/10.1109/Agro-Geoinformati.cs55649.2022.9858981">https://doi.org/10.1109/Agro-Geoinformati.cs55649.2022.9858981</a> | 2022        | relating vegetation indices with the rice yield through modelling approaches |

**Table 2.** Rice Mapping and Yield Prediction done for South Asian countries

| <b>Title. doi</b>                                                                                                                                                                                                                                                         | <b>Year</b> | <b>Brief</b>                                        |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|-----------------------------------------------------|
| Mapping paddy rice agriculture in South and Southeast Asia using multi-temporal MODIS images. <a href="https://doi.org/10.1016/j.rse.2005.10.004">https://doi.org/10.1016/j.rse.2005.10.004</a>                                                                           | 2005        | rice mapping of South Asian countries               |
| Integrating remote sensing, census and weather data for an assessment of rice yield, water consumption and water productivity in the Indo-Gangetic river basin. <a href="http://dx.doi.org/10.1016/j.agwat.2009.09.021">http://dx.doi.org/10.1016/j.agwat.2009.09.021</a> | 2009        | relates satellite based parameters with the yield   |
| Mapping rice areas of South Asia using MODIS multitemporal data. <a href="https://doi.org/10.1117/1.3619838">https://doi.org/10.1117/1.3619838</a>                                                                                                                        | 2011        | rice mapping of South Asian countries               |
| Mapping of rice-cropping pattern and cultural type using remote-sensing and ancillary data: a case study for South and Southeast Asian countries. <a href="https://doi.org/10.1080/01431161.2015.1110259">https://doi.org/10.1080/01431161.2015.1110259</a>               | 2015        | rice mapping of South Asian countries               |
| Rice Grain Yield Estimation Over Some Asian Countries Using Isro's Scatsat-1 Ku-Band Scatterometer Data. <a href="https://doi.org/10.5194/isprs-archives-XLII-3-W6-257-2019">https://doi.org/10.5194/isprs-archives-XLII-3-W6-257-2019</a>                                | 2019        | rice yield estimation using microwave based sensors |

Table 3. presents the research done with the broader themes in which specific location of the study has been chosen within Nepal. The research can be seen mostly

aligned with the climate change and the trends of the crop production in the years. Rice has also been studied along with remote sensing based crop cycle observation.

**Table 3.** Remote Sensing with broader themes

| Title. doi                                                                                                                                                                                                               | Year | Brief                                                                                             |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|---------------------------------------------------------------------------------------------------|
| Rice and Bricks: Environmental Issues and Mapping of the Unusual Crop Rotation Pattern in the Kathmandu Valley, Nepal. <a href="https://doi.org/10.1007/s00267-006-0167-0">https://doi.org/10.1007/s00267-006-0167-0</a> | 2007 | uses satellite imagery to observe the brick kilns, observes their impacts in the crop production  |
| Climate Change Impact and Adaptation Practices in Agriculture: A Case Study of Rautahat District, Nepal. <a href="https://doi.org/10.3390/cli4040063">https://doi.org/10.3390/cli4040063</a>                             | 2016 | uses satellite derived moisture data with other data to observe the trends of the crop production |
| Crop Cycles and Crop Land Classification in Nepal Using MODIS NDVI. <a href="https://doi.org/10.1007/s41976-018-0002-4">https://doi.org/10.1007/s41976-018-0002-4</a>                                                    | 2018 | observation of the crop cycle based on MODIS sensor                                               |
| Impact of climate change on agricultural productivity and food security in the Himalayas: A case study in Nepal. <a href="https://doi.org/10.1016/j.agsy.2019.01.008">https://doi.org/10.1016/j.agsy.2019.01.008</a>     | 2019 | observation of the future crop production trends                                                  |
| RICA: A rice crop calendar for Asia based on MODIS multi year data. <a href="https://doi.org/10.1016/j.jag.2021.102471">https://doi.org/10.1016/j.jag.2021.102471</a>                                                    | 2021 | preparation of rice crop calendar using remote sensing data                                       |
| Land Cover Change and Its Impact in Crop Yield: A Case Study from Western Nepal. <a href="https://doi.org/10.1155/2022/5129423">https://doi.org/10.1155/2022/5129423</a>                                                 | 2022 | observation of the impacts of the land cover change in the crop production                        |

For the further summary of the research, few of the things can be highlighted as seen from the list of the research.

- MODIS sensor based satellite data is found to be most used in most of the research.
- Satellite generated data such as soil moisture data can also be seen in the research with the main objectives of studying the crop future trends.
- Very few affiliations of the authors in the research article are from within Nepal emphasizing the sparse research status of the country although the involvements of the Nepalese authors in the works are admirable.
- More research works are still to be seen involving satellite based rice remote sensing in Nepal.

## 5. CONCLUSION

To summarize, the direct advantage of satellite remote sensing is in its generation of the crop related statistics. In the current contexts of rice imports in increasing trends within Nepal, satellite remote sensing will be a guiding tool to observe the nation's rice statistics closely.

Suggestions from the research findings are pointable much towards the improvement and more participation of Nepalese institutions in the remote sensing based applications. In the current situation of the country, rice remote sensing based on other tools such as drones or aerial based remote sensing cannot fully be cost friendly compared to satellite remote sensing. More emphasis should be put on the use of satellite remote sensing and the currently freely available platforms such as earth engine or planetary computer are still applicable for the institutional needs within the country. And in the continuously developing geospatial domain with more earth observation satellites being launched, Nepal can use those data in the studies of its crops like rice. For this, interests from all the institutions within Nepal, and interested individuals should be jointly involved.

The other blessings of being concentrated in the rice remote sensing will be towards the shared methods applicable toward the other crops of Nepal. This will be surely observable if more and more technicalities within the rice remote sensing are functional and these processes start containing the other crops as well. In coming decades, we are sure to see more sophisticated applications of remote sensing applications in crops, which we already see in the current years as well, where countries like Nepal deserve those being applied as well.

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# SCREENHOUSE EVALUATION OF THE FUNGICIDES AND BIO-CONTROL AGENTS FOR THE MANAGEMENT OF FUSARIUM WILT (Foc Race 1) OF BANANA IN NEPAL

Bimala Pant<sup>1,\*</sup>, Prem Bahadur Magar<sup>1</sup>, Chetana Manandhar<sup>1</sup>, Shrinkhala Manandhar<sup>1</sup>, Ram Bahadur Khadka<sup>1</sup>, Pratiksha Sharma<sup>1</sup>, Ganga G. C.<sup>2,3</sup> and Suraj Baidya<sup>1</sup>

<sup>1</sup> National Plant Pathology Research Center, Nepal Agricultural Research Council, Nepal

<sup>2</sup> Trichandra Multiple Campus, Kathmandu, Nepal

<sup>3</sup> Central Department of Microbiology, Kirtipur Kathmandu, Nepal

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\*Correspondence:

binu\_pant2000@yahoo.com  
Tel: +977-9843181250

## ABSTRACT

Banana is affected by a wide number of diseases, of which, *Fusarium wilt* caused by *Fusarium oxysporum f.sp. cubense* has played a major role in devastating Malbhog (Silk) banana plantations in Nepal. The objective of this study was to evaluate the commonly available fungicides (Carbendazim 50%, Fenamidone 10%+ Mancozeb 50%, Chlorothalonil and biocontrol agents (*Trichoderma* spp., *Bacillus thuringiensis* and *Pseudomonas* species) for their efficacy against Foc. The effectiveness of fungicides and biocontrol agents to Foc was examined in screenhouse. The experiment was laid out in a completely randomized design with three replications and eight treatments. Among the chemicals, the most effective fungicide to reduce *Fusarium wilt* severity (root disease) was Bavistin with an efficacy of 55.33% followed by Sectin 46.33% and Chlorothalonil 35%. Similarly, maximum efficacy over control was recorded in *Trichoderma harzianum* with 41% and lowest was 32% in *Pseudomonas* sp. In the case of leaf disease, the lowest severity was 21.33% recorded in Bavistin and highest was 40.33% in Chlorothalonil. Similarly, among biocontrol agents, the lowest severity was found in *Bacillus thuringiensis* whereas highest was found in *Trichoderma viride*. A Suppression of disease by biocontrol agents might be due to their fungicidal activity, which means they produce a variety of antifungal compounds. These compounds induce plant defense mechanisms. Further field evaluations of the fungicides and biocontrol agents are required to determine if the effect observed in the screenhouse can be integrated into field management of *Fusarium wilt*.

## 1. INTRODUCTION

Banana (*Musa* spp.) is an important crop in many tropical and subtropical regions of the world, providing a source of food and income for millions of people. In terms of the potential growing area, production and domestic consumption, banana is one of the major fruit crops of Nepal. It is cultivated on 10,557 ha of land, with a total production of 204, 009,69 tons and productivity of 19.32 ton/ha (MoALD, 2020).

Malbhog is a variety of banana known for its sweet taste and juicy texture, making it a popular choice for both fresh eating and cooking. In Nepal, Malbhog bananas are typically grown in the terai region and are important crop for local farmers. Banana is also an important source of food and nutrition for many families in the country and suffer production losses due to *Fusarium oxysporum f.sp. cubense*. The disease is

highly destructive to the Cavendish variety, which is the most widely grown banana variety in the world. The soil-borne fungus, *Fusarium oxysporum f. sps. cubense* causes the fusarium wilt of banana (Panamá disease) (E.F. Smith) Snyder and Hansen (*Foc*) (Stover, 1962). The fungus attacks the plants vascular system, causing wilting and eventually leading to plant death. This fungus also survives in plant debris and finds its way into the banana plant in most cases through damage to the roots. *Fusarium wilt* has been known to be a destructive disease all over the world where bananas are grown (Castle, 2009). Although, this disease probably originated in Southeast Asia (Ploetz & Pegg, 2000), it was first discovered in Australia in 1876. Recently most of the Malbhog banana growers in Nepal are switching to wilt resistant variety (Grand Nain) because of fear of

wilt disease (Paudel, 2020).

Panama wilt has been in existence since 19<sup>th</sup> Century, but its effect was noticed in the 20<sup>th</sup> Century where it wiped out plantations of crop in Panama and other parts of the world. The most popular variety in Panama: 'Gros Michel' comprised all the export production then. It's susceptibility to *Fusarium* wilt threatened complete wipe out of banana production (Castle, 2009). This disease is present in major banana growing regions of Nepal such as Chitwan, Nawalparasi, Bardia, Kailali and Kanchanpur district. Affected variety in Nepal is Malbhog (Silk, AAB) where upto 80% of the banana field may show symptoms of the disease (Personal communication with farmers).

Currently, there are three races of *F. oxysporum* f. sp. *cubense*, based on their banana hosts, Race 1 (Foc 1), Race 2 (FoC 2) and Race 4 (FoC 4). Race 4 has two strains that are TR4 (Tropical Race 4) and STR4 (Subtropical Race 4). TR4 (Tropical Race 4) is the most virulent and widespread race that affects Cavendish bananas and all the cultivars that are sensitive to other Races of *Foc* (Cheng *et al.*, 2019a, 2019b). It is important to note that each race has different virulence factors and can cause different levels of damage to banana. Mainly, the severity of this disease is influenced by the level of stress, for example during the extended period of floods, imbalance of nutritional contents and limited or low temperatures and increased levels of salinity. The disease development is favored by the soil of warm, moist, and increased high levels of temperature with optimized soil temperatures for root infection being 30°C or above (Castle, 2009).

*Fusarium* wilt is most serious problem to banana production in the Chitwan, Nawalparasi and Bara districts of Nepal (Personal communication with farmers) and has been causing destruction of banana fields. This has led to the abandonment of hectares of land. An irreversible and untreatable infection is caused by Tropical Race 4 (TR4) of *Fusarium oxysporum* f.sp. *cubense*, therefore its economic impact is profound. The global industry of banana is under serious threat due to the ease with which *Fusarium* wilt spread (Siamak & Zheng, 2018). Rhizome and infected suckers are the main source of disease spread. The spread may also occur through movement of infested soils adhering to vehicles, footwear, and animal feet over long distances. It can be spread within short distances through roots networks and in surface presence of run-off water (Daly & Walduck, 2006). From the discovery of Panama

wilt disease, different control measures such as soil fumigation and Fungicides (Herbert & Marx, 1990); Crop rotation (Hwang, 1991; Su, Hwang, & Ko, 1986); Flood fallowing (Wardlaw, 1961) have been evolved and used, yet, the disease could not be controlled effectively except by planting of resistant varieties (Moore, Bentley, Pegg and Jones, 1995). Because of consumers preference, planting of resistant varieties also cannot be implemented (Viljoen, 2002). Under such circumstances, use of antagonistic microbes which protect and promote plant growth could be an alternative approach for the management of banana wilt. Several strategies have been tried on banana for the management of this disease, however, only a few strategies such as replacement of susceptible cultivar with resistant cultivar (Gand Nain) being the alternative control means in Nepal (Personal communication with farmers).

*Trichoderma* species are soil borne fungi and are known for their ability to produce various enzymes and secondary metabolites that have antifungal activity and can induce plant defense mechanisms. *Pseudomonas* and *Bacillus* species also produce a variety of antifungal compounds.

The aim of this study was to determine the efficacy of Fungicides and biocontrol agents in the management of *Fusarium oxysporum* f.sp. *cubense* race 1 of banana in Screenhouse condition.

## 2. MATERIALS AND METHODS

An experiment was conducted during 2019/20 and 2020/21 at National Plant Pathology Research Center of Nepal Agricultural Research Council, Nepal to find out the effective fungicides and biocontrol agents against *Fusarium oxysporum* f. sp. *Cubense* race 1 *in-vivo*. The experiment was conducted in Complete Randomized design with three replications and eight treatments including control. Per treatments there were three plants.

### 2.1. Isolate used and inoculum preparation.

An isolate of *Foc* race-1 used in this study was isolated from diseased banana plants (Malbhog variety) collected from Nawalparasi district of Nepal. For the evaluation of fungicides and biocontrol agents, pathogenic isolate of *Foc* was grown for 7 days on PDA at 25-27°C in an incubator. Conidial spore suspension was prepared by transferring mycelia into 250ml conical flask containing water. The flasks were placed on a shaker operating at 170 rpm at 25°C for 5 days, after which the conidia

suspension was passed through muslin cloth to separate the mycelium from the spores. Concentration of spores in the remaining liquid was determined by using a hemocytometer, and diluted with sterile distilled water to a final concentration of  $10^6$  spores/ml.

## 2.2. Selection of fungicides and biocontrol agents

Three fungicides, named Carbendazim 50% (Bavistin), Fenamidone 10%+ Mancozeb 50%, (Sectin) and Chlorothalonil were selected for in vivo evaluation against *Foc*. These fungicides were selected based on their performance in the in vitro evaluation. Four biocontrol agents were also selected for evaluation against *Foc*. The name of different treatment used in this study were: T1-Bavistin, T2- Sectin, T3- *Trichoderma viride*., T4- *Trichoderma harzianum*, T5- Chlorothalonil, T6- *Bacillus thuringiensis*, T7- *Pseudomonas* sp. and T8- Water.

## 2.3. In- vivo evaluation of fungicides and biocontrol agents

Tissue cultured plantlets of banana of Malbhog variety were obtained from biotech banana nursery at Lalitpur. Banana plants with well-developed roots were selected for pot trial evaluation of fungicides and biocontrol agents against Fusarium wilt. The sand and soil combination were steam-sterilized before *Foc* inoculation. The potting mixture (sand, soil, and manure) was then deposited into pots. The Soil drenching method of application for disease inoculum and treatments (fungicides and biocontrol agents) was performed. Drenching for inoculum (100ml/ plant) was done after three days of planting. Four days after disease inoculation, drenching treatments with same dose was performed. Control plants were treated with 100ml of distilled water as a treatment. Disease development was estimated by observing wilt severity of external and internal appearance of plants. There was regular inspection of plants showing typical symptoms of wilting and leaf yellowing. The pseudostems of symptomatic individuals were further examined for the presence of the reddish-brown vascular discoloration characteristics of *Foc* infection. In the case of greenhouse experiments, when 50% of the control plants displayed leaf symptoms, then plants were considered ready for rating (Viljoen *et al.*, 2017). Symptoms were recorded every 4 days until all the untreated control plantlets showed clear disease symptoms. Disease rating scale for external symptoms (leaf) was 1-5, where 1=No disease, 2= Little chlorosis and wilting with no petiole bending, 3= Moderate

chlorosis and splitting of leaf base and wilting with some petiole bending, 4= Severe chlorosis and wilting, petiole bending and stunting of newly emerged leaf and 5= Dead.

(LDS) Leaf disease severity (%) = [Total sum of numerical rating/ (Total number of plants observed x Maximun category in the score chart)]  $\times 100$

Similarly, disease rating scale for internal symptoms or Rhizome discoloration was 1-6, where 1= No internal symptoms, 2= Few internal spots, 3=  $< 1/3$  rhizome discolored, 4=  $1/3$  to  $2/3$  rhizome discolored, 5=  $> 1/3$  discolored and 6= entire inner rhizome discolored (International Musa Testing Programme rating scale).

(RDS) Root disease severity (%) = [Total sum of numerical rating/ (Total number of plants observed x Maximun category in the score chart)]  $\times 100$

The efficacy of treatments was determined based on the severity of Fusarium wilt. Efficacy of the treatment was calculated using the formula as follow:

Efficacy = [(Disease severity of control - Disease severity of treatment)/ Disease severity of control]  $\times 100$

## 2.4. Inoculum preparation for trichoderma, bacillus and pseudomonas spp

Two *Trichoderma* isolate such as *Trichoderma Viride* and *Trichoderma harzianum* were obtained from Nepalese type culture collection of National Plant Pathology Research Center, Khumaltar, NARC. Inoculum was prepared by inoculating 5 days old culture of *Trichoderma* isolate in potato dextrose broth (Potato starch infusion 4gm/lit, and Dextrose 20gm/ lit) with subsequent incubation for 5 days at  $28^\circ\text{C}$  in an incubated shaker. Then the spore suspension was obtained by filtering the broth through muslin cloth. The spore density was determined by hemocytometer and filtrate was then diluted to  $10^7$  spores/ml.

*Bacillus* and *Pseudomonas* sp. used in this study were obtained from Central Department of Microbiology, Kritipur Kathmandu, Nepal. Formulation of *Bacillus* and *Pseudomonas* sp. was performed by submerged fermentation (SmF) /liquid fermentation (LF) technique as described by (Ralte, Nachimuthu, & Guruswami, 2016) with slight modification by inoculating the isolates in a 250ml conical flask containing sterilized Luria-Bertani (LB) broth (Tryptone 10g/lit, NaCl 10g/ lit, and yeast extract 5g/lit). The conical flask was

placed in a shaker water bath at 35°C for 90hrs until the colony forming unit (cfu/ml) was greater than  $10^9$ . The inoculum was then adjusted to  $10^7$  spores/ml.

## 2.5. Data Analysis

The statistical package IRRI STAR was used for the analysis of variance to test the significance of treatment effect on wilt severity of *Fusarium oxysporum* f.sp. *cubense*. Tukeys Honest Significant Difference test was used to compare the values of significant treatment means at 0.05% level of significance.

## 3. RESULTS AND DISCUSSION

An isolate of the pathogen was collected from banana

fields in Nawalparasi in 2019. Pathogenicity test of the isolate was confirmed on cultivar Malbhog. Different chemicals and biocontrol agents were tested against *Fusarium oxysporum* f.sp. *cubense* in pot trial condition. After 8 weeks of pathogen inoculation, the symptoms of Fusarium wilt disease were observed and recorded. The first indication of the disease was yellowing and drooping of lower leaves. Some plants exhibited dark brown vascular discoloration, splitting of pseudostem and death. Therefore, the pathogenicity of tested isolate on banana variety Malbhog was clearly distinct. The same fungus was reisolated from the discolored pseudostem and rhizome of the diseased plant.



**Figure 1.** Scoring of disease development according to the rhizome discoloration

Identification of *Fusarium oxysporum* f.sp. *cubense* was based on microscopic observation of different spores such as Microconidia, Macroconidia and Chlamydospores (Fourie, Steenkamp, Gordon, & Viljoen, 2009). The mycelial color of the pathogen was white cottony with purple tinge. Microconidia were borne on conidiophores and are small, thin walled. Macro- conidia were larger, thick walled and produced in chains and chlamydospores were thick-walled, spherical structures.

In our study, chemical fungicide Bavistin showed least leaf disease severity of 21.33%, which is consistent with the study of (Nel, Steinberg, Labuschagne, & Viljoen, 2007), that showed chemical fungicide belonging to the group of benzimidazole including carbendazim had limited success against Fusarium wilt. Similarly, Sectin and Chlorothalonil gave 29%

and 35.67% wilt severity respectively. The results of the performed test indicated that Bavistin was the most effective fungicide with efficacy of 70.33% followed by Sectin 59.33% and Chlorothalonil 44%. However, the use of fungicide alone is not always sufficient to control Fusarium wilt, as the disease is often associated with soil borne pathogens that can persist in the soil for many years. Therefore, other control options such as the use of biocontrol agents are often recommended to manage Fusarium wilt in banana plants. Application of biocontrol agents as drenching after 4 days of disease inoculation significantly decreased mean leaf disease severity compared to *Foc* alone inoculated control plants. Among these isolates, *Trichoderma harzianum* and *Bacillus thuringiensis* gave the lowest wilt severity of 35.67%. A study carried out by ((Thangavelu & Mustafa, 2012) showed that use of *Bacillus* strain,

KY-21 results in 35% wilt severity and 18.3% severe vascular discoloration. and *Trichoderma viride* showed wilt severity of 39.33% and *Pseudomonas* sp. showed highest wilt severity of 40%. In this study, biocontrol agents *Bacillus* and *Trichoderma harzianum* showed efficacy of 50%. According to (Bubici, Kaushal, Prigigallo, Gómez-Lama Cabanás, & Mercado-Blanco, 2019) 40-42% efficacy had been obtained by the application of *Bacillus* spp. in the field condition. An efficacy of 45.33% was found in *Pseudomonas* sp. applied treatment and 44% was found in *T. viride*. In the study of (Sivamani & Gnanamanickam, 1988), *P. fluorescens* showed less severe wilting and internal discoloration due to *Foc* in green house experiment in the seedlings of *Musa balbisiana*. Similarly in the case of root disease scoring, chemical fungicide Bavistin

gave 29% severity followed by Sectin 34.33% and chlorothalonil 43.67%. Application of biocontrol agents such as *Pseudomonas* showed the highest wilt severity of 43.67% whereas *Trichoderma harzianum* gave 38% wilt severity. *Bacillus thuringiensis* and *Trichoderma viride* showed 42% wilt severity as compared to control. *Foc* alone- inoculated plants had a mean wilt severity of 64%. Among chemical fungicides, 55% efficacy was found in Bavistin applied treatment whereas lowest efficacy 33% was found in Chlorothalonil. Similarly, in the case of biocontrol agents, *Trichoderma harzianum* was found to be most efficient with efficacy of 41% followed by *Trichoderma viride* and *Bacillus thuringiensis* 35% and *Pseudomonas* sp. 32% as compared to control (Table 1).

**Table 1.** Effectiveness of Fungicides and biocontrol agents against *Fusarium oxysporum* f. sp. *cubense* (mean of first and second year)

| Treatments                    | Internal scoring |          | External scoring |          |
|-------------------------------|------------------|----------|------------------|----------|
|                               | RDS              | Efficacy | LDS              | Efficacy |
| Bavistin                      | 29.00e           | 55.00a   | 21.33e           | 70.33a   |
| Sectin                        | 34.33d           | 46.33b   | 29.00d           | 59.33b   |
| <i>T. harzianum</i>           | 38.00cd          | 41.00bc  | 35.67c           | 50.00c   |
| <i>T. viride</i>              | 42.00bc          | 35.00cd  | 40.00bc          | 44.00c   |
| Chlorothalonil                | 43.67b           | 33.00d   | 40.33b           | 44.00c   |
| <i>Pseudomonas</i> sp.        | 43.67b           | 32.00d   | 39.33bc          | 45.33c   |
| <i>Bacillus thuringiensis</i> | 42.00bc          | 35.00cd  | 35.67c           | 50.00c   |
| Control                       | 64.00a           | 0.00e    | 71.33a           | 0.00d    |
| CV %                          | 3.56             | 7.26     | 4.15             | 4.74     |
| Tukey's HSD                   | 4.24             | 7.09     | 4.58             | 6.07     |
| P value                       | 0.0000           | 0.0000   | 0.0000           | 0.0000   |

Disease severity is the mean of three replications of both years. Values in the same column followed by similar letters are not significantly different. HSD: Tukey's Honest significant difference

During 2020, the leaf disease severity was found lowest in Bavistin (22.33%) and highest in Chlorothalonil (46.67%) as compared to control. Similarly, the highest efficacy was found in Bavistin (74.33%) followed by Sectin (61.67%) and Chlorothalonil (46.33%) among

fungicides. For biocontrol agents, *Trichoderma viride* showed the highest disease severity (49%) and lowest was found in *Bacillus thuringiensis* (35.65%). The maximum efficacy was found in Bt (59%) and least was found in *T. viride* (44%) as shown in Figure 2.

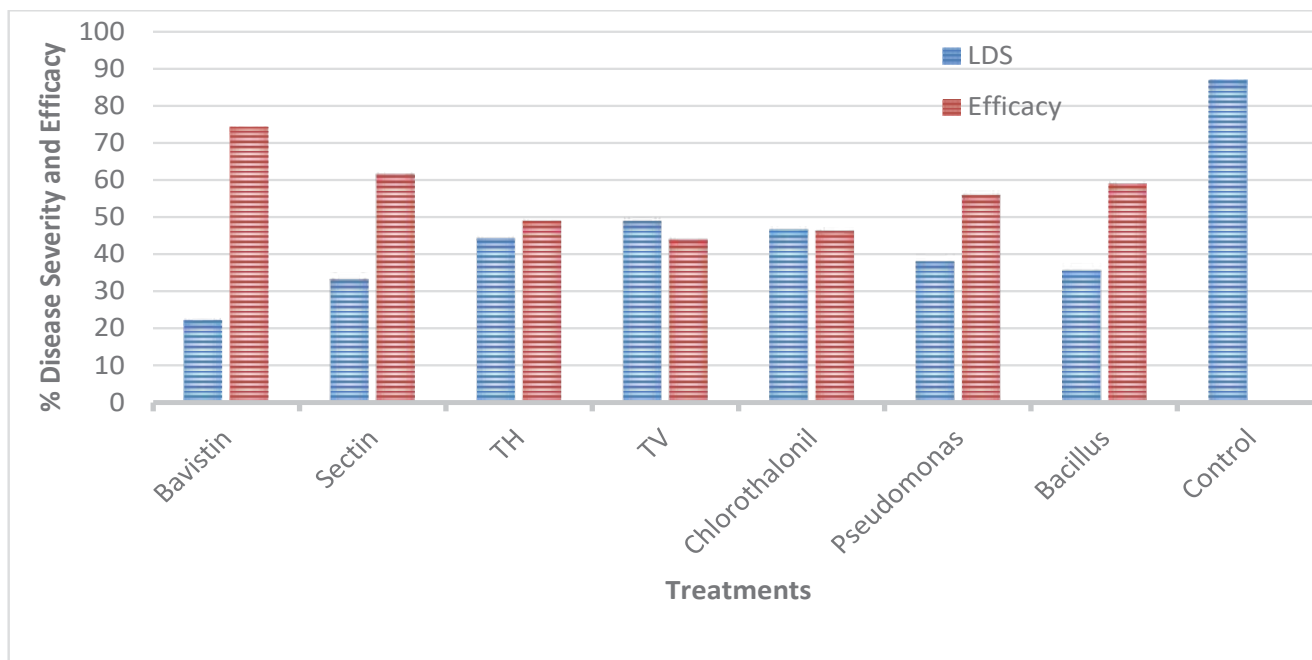


**Figure 2.** Leaf disease severity of *Fusarium* wilt and efficacy of different treatments during 2020 under screenhouse condition.

In the case of root disease severity, among fungicides, the highest severity was found in Chlorothalonil (48%) and lowest in Bavistin (31.33%). The highest efficacy was found in Bavistin (48.67%) and lowest was found in Chlorothalonil (21.33%). Similarly in the case of

biocontrol agents, *T. viride* showed the maximum disease severity of (52%) and least was in Bt (42.67%). The maximum efficacy was shown by Bt (33.33%) and least was found in *T.viride* (14.67%) as shown in Figure 3.

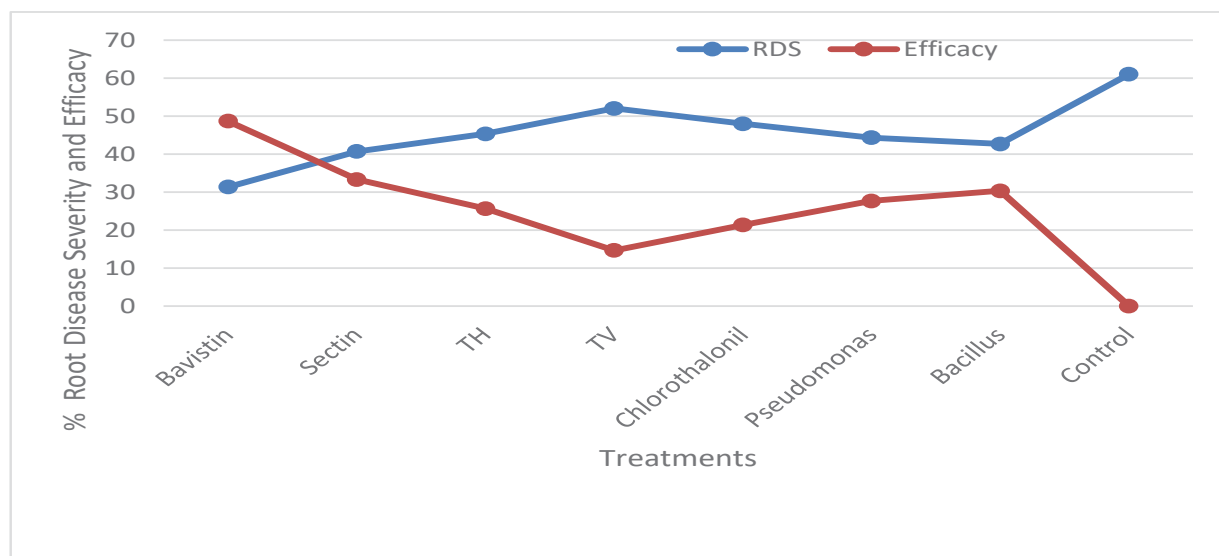


**Figure 3.** Root disease severity and efficacy of different treatments during 2020 under screenhouse condition

During 2021, the leaf disease severity was found least in Bavistin and highest in Chlorothalonil as compared to control whereas highest efficacy was found in Bavistin and lowest in Chlorothalonil. Similarly in the case of biocontrol agents, the maximum disease severity

was found in *Pseudomonas* and least in *T. harzianum* whereas highest efficacy was recorded in *T. harzianum* and lowest was in *Pseudomonas* sp. as shown in Figure 4.

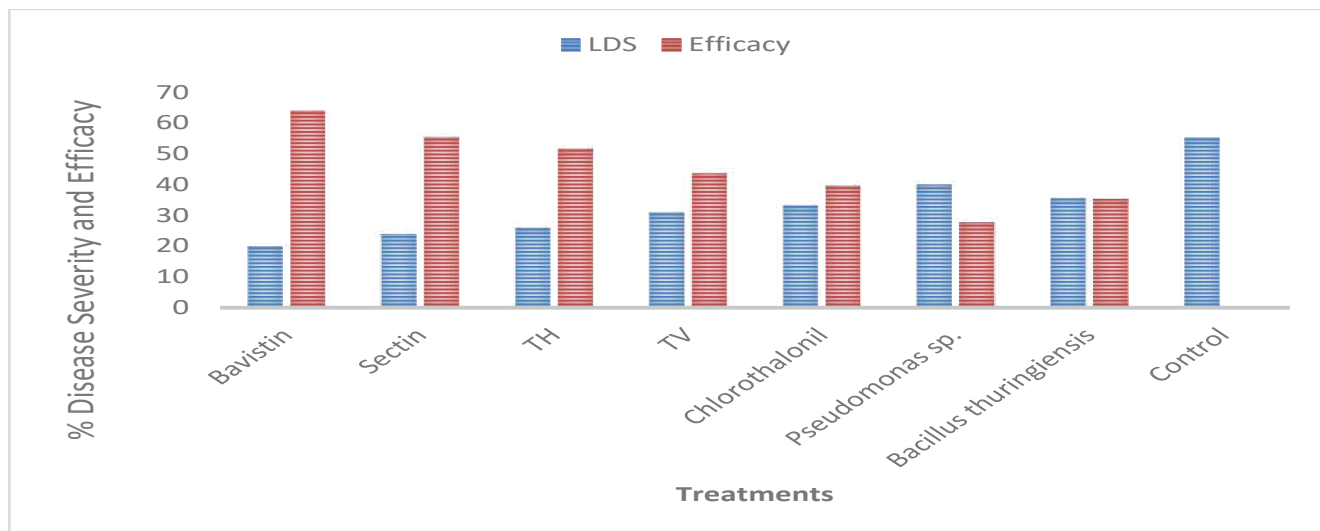


**Figure 4.** Leaf disease severity and efficacy of different treatments during 2021 under Screenhouse condition.

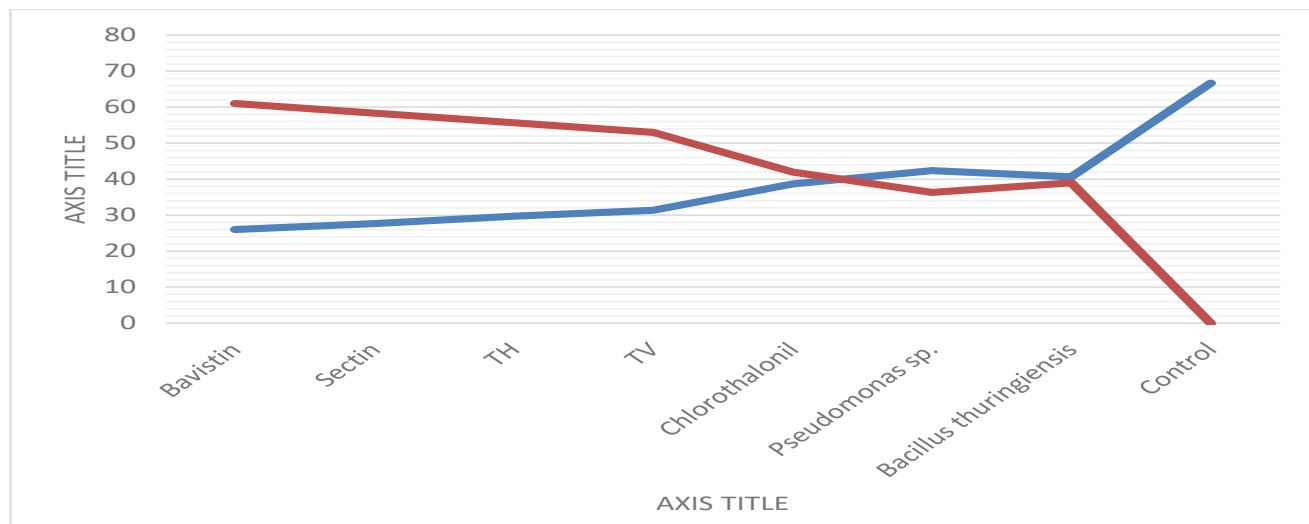


**Figure 5.** Root disease severity and efficacy of different treatments during 2021 under Screenhouse condition

Disease severity is mean of three replications. RDS= Root disease severity

In the case of root disease severity, the maximum severity was recorded in Chlorothalonil and least was in Bavistin whereas highest efficacy was recorded in Bavistin and lowest was in Chlorothalonil. Similarly for biocontrol agents, the highest severity was found in *Pseudomonas* sp. and lowest was in *T. harzianum* whereas highest efficacy was recorded in *T. harzianum* and lowest was in *Pseudomonas* sp. as shown in Figure 5.

#### 4. CONCLUSION

Fusarium wilt of banana is a serious disease, caused by soil borne fungus *Fusarium oxysporum* f.sp. *cubense*. Although different Fungicides and biocontrol agents have been tried against Fusarium wilt disease, this lethal disease could still not be controlled completely. The biocontrol agents tested against Fusarium wilt of banana have not yet reached the banana growers,

because the biocontrol experiment was not conducted against Panama wilt in Nepal. This is mainly because of lack of confidence in the efficacy of biocontrol agents in controlling the disease. Therefore, effective biological control methods for the management of *Fusarium* wilt disease are a) Mixture of biocontrol agents along with or without fungicides or botanicals have to be tried to improve extent of disease control under different environmental and soil conditions b) Suitable and easy method of mass production and delivery system must be selected, c) Mass produced biocontrol-agents should be applied at right quantity (the initial inoculum level of bioagents should be more than the inoculum level of the pathogen) at the right place (at the soil around the rhizosphere) at the right time. For the management of this disease, fungicides such as Bavistin and Chlorothalonil are commonly used. This study evaluated the efficacy of different fungicides and biocontrol agents against *Foc* in banana plants under screenhouse conditions.

The results of the performed test showed that all the used fungicides were effective in reducing severity,

but Bavistin was more effective than Sectin and Chlorothalonil (table 1.)

An effective and sustainable approach to control *Fusarium* wilt disease in banana plants is the use of biocontrol agents such as *Trichoderma*, *Pseudomonas* and *Bacillus* species. However, their effectiveness depends on several factors such as environmental conditions, banana variety and strain of the pathogen. In Nepal, Bt as a biocontrol agent against *Fusarium* wilt of banana is still in early stage of development. Further research is needed to optimize their use in banana farming.

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# STRENGTHENING FOOD SAFETY GOVERNANCE IN NEPAL THROUGH COLLABORATIVE CAPACITY DEVELOPMENT AND PRIVATE SECTOR ENGAGEMENT

Prateek Joshi <sup>1,\*</sup>, Sanjeev Kumar Karn<sup>1</sup> and Pramod Koirala <sup>1</sup>

<sup>1</sup> Ministry of Agriculture and Livestock Development, Singhadurbar, Kathmandu, Nepal

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\*Correspondence:  
prateeklokanthali@gmail.com  
Tel: +977-9841220104

## ABSTRACT

*This article discusses the policy and regulatory challenges faced by Nepal in strengthening food safety governance and the potential solutions to address them. Capacity building through training programs and technical assistance, regional collaboration, and private sector engagement are the key strategies identified for improving food safety standards in the country. The article highlights the need for a comprehensive legal framework and the establishment of a centralized food safety authority in Nepal. The challenges faced by the private sector in complying with food safety standards are also discussed, particularly the difficulties faced by small and medium-sized enterprises. The potential benefits of regional collaboration in the food sector, including the harmonization of food standards, the development of regional frameworks for food safety based on the Codex, and the creation of a regional food safety rapid alert system, are also examined. The article concludes that addressing policy and regulatory challenges through capacity building, regional collaboration, and private sector engagement can lead to improved food safety governance in Nepal and help the country to overcome its food safety challenges.*

## 1. INTRODUCTION

Food safety measures in developing countries are insufficient, scattered and lack efficacy in safeguarding consumer health or enabling countries to compete for export markets. The cost of enhancing food safety measures is usually high, and many developing countries encounter difficulties in enforcing food safety legislation. Despite the introduction of codex and ISO standards, several years ago, these countries continue to experience food safety problems, including food fraud, in their domestic market. Although progress has been made in developing or adopting international standards, the full implementation of these standards, and a significant reduction in food safety-related incidents, have yet to be realized (WHO, 2015).

The effective implementation of food safety regulations and standards is crucial in developing countries to ensure consumer health, trade and economic growth, and food security. Low- and middle-income countries (LMICs) are more vulnerable to foodborne illnesses, leading to significant medical expenses and loss of productivity. Unsafe food can also impede international trade, hindering economic development. Adopting international standards

can help LMICs meet trade requirements and create a culture of transparency and accountability, building consumer trust in the food system while preventing foodborne illnesses and safeguarding public health. According to the World Health Organization (WHO), unsafe food is responsible for an estimated 600 million cases of foodborne illnesses and 420,000 deaths annually, most of which occur in low- and middle-income countries (WHO, 2020).

Nepal has made progress in improving its food safety standards through the establishment of a National Codex Committee and adoption of the HACCP system. However, implementation and enforcement remain a challenge, especially in rural areas where access to resources and training is limited. The level of compliance with food safety regulations in Nepal is generally low, indicating a need for capacity building among regulators and food handlers to ensure the safety and quality of food products (WHO, 2014).

Food safety is a growing concern in Nepal, as it has a significant impact on public health and international trade.

Nepal's membership in the World Trade Organization (WTO) requires the country to adopt its food safety regulations aligning with international standards, such as the Sanitary and Phytosanitary (SPS) agreement. As a result, Nepal has been working to update its existing food safety regulations to meet these standards. However, there are still regulations that need to be harmonized with Codex principles, and the implementation of Hazard Analysis and Critical Control Point (HACCP) principles in the food industry is not yet mandatory (Bajagai, 2012).

To ensure food safety standards in Nepal, it is crucial to focus on enhancing awareness, building capacity, and improving the infrastructure needed for effective implementation and enforcement of food safety regulations. This includes providing training and resources to regulators and food handlers, improving food testing facilities, promoting public education and awareness on food safety, and encouraging the adoption of international standards such as the Hazard Analysis and Critical Control Points (HACCP) system. By prioritizing these efforts, Nepal can create a safe and reliable food supply system that will not only protect public health but also promote economic growth and trade opportunities.

## 2. REGULATORY CHALLENGES IN ENSURING FOOD SAFETY STANDARDS IN NEPAL

Around 61 years ago, Nepal introduced the concepts of food safety, technology, and nutrition through its first periodic plan from 1956 to 1961, which highlighted the importance of exporting ghee to Tibet. This period marked the emergence of a problem in the export process, as there was a lack of proper processing skills for ghee, which resulted in a loss of around 10 million NRs (~ 100 thousand USD at that time) (Dahal *et al.*, 2017). The food safety system of Nepal is primarily guided by the Codex principles but still the country is not able to adopt all international standards and guidelines due to lack of adequate resources.

While the regulation of food safety in Nepal started in 1966 with the enforcement of the Food Act by the government, it wasn't until the country became a member of the World Trade Organization (WTO) in 2004 that food safety-related issues became a matter of increased concern and a priority for the government (Bajagai, 2012). The primary government institutions tasked with ensuring food safety and quality management in Nepal are the Department of Food Technology and Quality Control (DFTQC) and its subordinate offices, which fall under the purview of the Ministry of Agriculture and Livestock Development.

Other government institutions are also supplementing the regulation of the food safety and quality in the food and feed chain. The Department of Agriculture (DoA) and the Department of Livestock Services (DLS) share some of these responsibilities alongside the Department of Food Technology and Quality Control (DFTQC), which operates under the Ministry of Agriculture and Livestock Development. Additionally, the Nepal Bureau of Standards and Metrology (NBSM) governs some voluntary standards related to food safety. To further strengthen the regulation of food safety in Nepal, the government approved and implemented the Food Safety Policy 2019 on June 23, 2019. Moreover, a new food safety and quality regulation is in the pipeline, which is expected to enhance the existing food safety framework in the country.

Food safety incidents have gained widespread attention and pose significant challenges for national food safety regulation. Recent occurrences of food safety incidents worldwide have brought into question the capacity of food safety regulatory agencies. As a result, there is a growing demand for these authorities to take a more prescriptive and proactive approach to tackle food safety issues and ensure appropriate level of consumer protection (Cope *et al.*, 2010; Hoffman and Harder, 2010; Chen *et al.*, 2015). Food safety is a critical issue in Nepal, as the country faces several regulatory challenges in the design, implementation and enforcement of safety standards across the value chain which is shown in Table 1. Insufficient investment in this sector and lack of stakeholder engagement and cooperation are major obstacles. Partnerships/collaborations among federal, state, and local governments with industry, universities, private organizations, and consumers are also lacking. The prevailing laws/regulations are too old and do not address some issues clearly, such as nutrition labeling, newer additives, and export-import issues, and focus mainly on end-product testing rather than a preventive approach. There is also a lack of clear coordination mechanisms among different government institutions, including DFTQC, DoA, DLS, NBSM, and the Ministry of Health and Population. Additionally, there are gaps and overlaps in the roles and responsibilities of government institutions. Furthermore, the country has been unable to respond and adapt to new technology and changing consumer needs, leading to challenges in the regulation of food safety. In order to overcome the challenges facing the food safety sector in Nepal, it is essential for stakeholders to work collaboratively and invest in the implementation of modern technologies, regulations, and coordinated efforts.

**Table 1.** Regulatory challenges in enforcing food safety standards in Nepal

| S.N | Regulatory Challenge                                             | Description                                                                                                                                             |
|-----|------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1.  | Insufficient investment                                          | Lack of resources and funding for food safety programs and infrastructure, making it difficult to enforce regulations and conduct inspections.          |
| 2.  | Lack of stakeholder engagement                                   | Limited partnerships and collaborations among federal, state, and local governments with industry, universities, private organizations, and consumers.  |
| 3.  | Outdated laws and regulations                                    | Prevailing laws/regulations are too old and do not address some issues clearly, such as nutrition labeling, additives, and export-import issues.        |
| 4.  | Reactive rather than preventive approach                         | Focus mainly on end-product testing rather than a preventive approach.                                                                                  |
| 5.  | Lack of coordination                                             | Lack of coordination among different government institutions, including DFTQC, DoA, DLS, NBSM, and the Ministry of Health and Population.               |
| 6.  | Gaps and overlaps in roles and responsibilities                  | Gaps and overlaps in the roles and responsibilities of government institutions.                                                                         |
| 7.  | Inability to adapt to new technology and changing consumer needs | The country has been unable to respond and adapt to new technology and changing consumer needs, leading to challenges in the regulation of food safety. |

### 3. CAPACITY CONSTRAINTS AND POTENTIAL SOLUTIONS IN ENFORCING FOOD SAFETY STANDARDS

The capacity constraints in the enforcement of food safety standards in poor countries, such as inadequate investment, lack of stakeholder engagement, insufficient partnerships and collaborations, unclear implementation plans can lead to increased risks of foodborne illnesses, economic losses, and damage to reputation and public trust in the food industry (Hoffmann *et al.*, 2019). The food safety governance in Nepal faces several challenges such as a limited structure of the governing body, insufficient risk-based inspection, and inadequate laboratories and manpower to carry out surveillance and monitoring. Additionally, the country suffers from a shortage of skilled personnel to operate advanced equipment, and a limited number of food safety standards that are not harmonized with Codex. Furthermore, there is a lack of a mechanism and resources to detect, address and prevent foodborne illnesses, and the research budget is also inadequate. These challenges have resulted in an ineffective implementation of food safety standards and regulations, leading to a high incidence of foodborne illnesses and other related problems in the country.

To address the capacity constraints in ensuring food safety standards in Nepal, several possible solutions can be considered which is summarized in the Table 2 given below. Firstly, a database for food safety control system can be established for better organization and management of food safety information. Secondly, a structured and well-planned food safety surveillance system can be implemented, with adequate monitoring and surveillance. The development of risk assessment methodologies can help in identifying and prioritizing potential food safety risks. Technical collaboration and networking of regional laboratories can improve the capacity of food safety testing and analysis. Thirdly, it is essential to ensure good practices in food production, processing, manufacturing, retail sale, transportation, preparation, and handling. This can be achieved through appropriate technical assistance and education for stakeholders. Additionally, a food chain approach and integrated food safety management system can be implemented to ensure comprehensive food safety management. Finally, developing a National FBD (Food borne disease) surveillance system can help in the early detection and response to food safety incidents.

**Table 2.** Capacity constraints and possible solutions in ensuring food safety standards in Nepal

| S.N | Capacity Constraint                                                                                                  | Possible Solutions                                                                                                                                                    |
|-----|----------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1   | Lack of organization and management of food safety information                                                       | Establish a an integrated database for food safety system                                                                                                             |
| 2   | Inadequate monitoring and surveillance                                                                               | Implement a structured and well-planned food safety surveillance system                                                                                               |
| 3   | Insufficient capacity for food safety testing and analysis                                                           | Develop risk assessment methodologies, and collaborate and network regional laboratories                                                                              |
| 4   | Poor practices in food production, processing, manufacturing, retail sale, transportation, preparation, and handling | Ensure good practices through appropriate technical assistance and education for stakeholders, enforcement of GMP and HACCP guidelines in food manufacturing premises |
| 5   | Inadequate food safety management system                                                                             | Implement a food chain approach and integrated food safety management system                                                                                          |
| 6   | Lack of early detection and response to food safety incidents                                                        | Develop a National FBD surveillance system                                                                                                                            |

#### 4. LEARNING FROM OTHER COUNTRIES: BEST PRACTICES AND EXPERIENCES IN ENSURING FOOD SAFETY STANDARDS

Nepal can learn from the experiences of neighboring countries like India and China, which have faced similar challenges in ensuring food safety. For instance, India has established a strong food safety regulatory framework with the creation of the Food Safety and Standards Authority of India (FSSAI), which has a well-defined organizational structure, comprehensive legal framework, and efficient enforcement mechanisms.

Similarly, China has made significant progress in food safety regulation by implementing a risk-based approach and strengthening laboratory capacity (Chen *et al.*, 2021). Nepal can adopt similar strategies, including the establishment of a centralized food safety authority, risk-based inspection, and strengthening laboratory infrastructure. Collaborating and sharing best practices with neighboring countries could also help Nepal to develop a more effective food safety regulatory framework.

In order to improve the food safety standards in Nepal, the country can learn from the best practices and experiences of other countries. One key learning is the implementation of Good Agricultural Practices (GAP), Good Hygienic Practices (GHP), and Good Veterinary Practices (GVP) at the production level, as well as Good Manufacturing Practices (GMP), GHP, ISO, and HACCP at the processing level. Other best practices include ensuring Good Storage Practices (GSP) during storage, product recall, and traceability during distribution, strengthening and accrediting laboratories, and establishing Food Safety Indicators. Additionally, the government can offer subsidies for system certification and authorize

private laboratories to facilitate timely and effective food testing and inspections. The establishment of a Food Epidemiology Unit can also help identify and prevent foodborne illnesses. Nepal can also adopt a risk-based inspection system and promote technical collaboration and networking among stakeholders, as well as a holistic approach to food safety management through the food chain approach.

Furthermore, Nepal can also learn from other countries' experiences with consumer education and awareness campaigns. For instance, Japan's Consumer Affairs Agency has launched a comprehensive food safety education program to promote safe food consumption among consumers, including schoolchildren, parents, and the elderly (Kimura, 2011). Another example is South Korea's "Smart Consumer" campaign, which aims to educate consumers about food safety issues and empower them to make informed choices (Söng-yong *et al.*, 2022). These programs have been effective in increasing public awareness and reducing food safety incidents.

Moreover, Nepal can also learn from innovative technologies and practices that are being developed and implemented in other countries. For example, the use of block chain technology in food traceability and supply chain management has shown promise in ensuring transparency and accountability in the food industry (Tian *et al.*, 2016). Additionally, the application of artificial intelligence and machine learning in food safety inspections can enhance the efficiency and accuracy of the inspection process.

## 5. COMPLIANCE WITH FOOD SAFETY STANDARDS IN NEPAL: CHALLENGES FACED BY THE PRIVATE SECTOR

In poor countries, the compliance with food safety standards is a major concern for the private sector, who often face difficulties in ensuring compliance due to limited resources, inadequate infrastructure, and a lack of proper training and knowledge (Henson & Jaffee, 2007). The private sector in Nepal faces several challenges in complying with food safety standards, hindering their ability to provide safe and high-quality products to consumers. While tools such as Good Manufacturing Practices (GMP) and Good Hygienic Practices (GHP) are widely used in export items like tea and coffee, they are less commonly utilized in domestic market products. Moreover, the government's communication on food safety largely occurs through associations of particular commodities, which creates a disjointed and inconsistent

approach to food safety regulation. Additionally, private labs are not authorized by the government, which can make it difficult for businesses to conduct necessary testing and analysis of their products. The challenges faced by the private sector along with its possible solutions is summarized in Table 3.

Another challenge is that compliance with food safety standards is easier for large-scale food industries, while small and medium-scale industries often struggle to comply with these regulations. This results in violations of the law, particularly among street vendors, hotels, and restaurants. Small and medium-scale industries are often unable to afford system certification, which can be costly and time-consuming. Thus, there is a need for training and strengthening of these industries to improve their compliance with food safety standards and ensure the safety of the food supply chain.

**Table 3.** Challenges faced by the private sector in compliance with food safety standards in Nepal

| Challenges                                                  | Possible Solutions                                                                                                                                                                                                                                                                                                                                                 |
|-------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Limited resources and inadequate infrastructure             | Provide financial support, grants, and subsidies to assist small and medium-sized enterprises (SMEs) in upgrading their infrastructure and acquiring necessary equipment for food safety compliance. Improve access to loans or credit facilities for businesses to invest in food safety measures.                                                                |
| Lack of proper training and knowledge                       | Conduct comprehensive training programs and workshops for food industry personnel to enhance their understanding of food safety regulations, best practices, and techniques. Collaborate with educational institutions and industry associations to provide specialized courses and certifications in food safety management.                                      |
| Disjointed and inconsistent approach to regulation          | Strengthen coordination and collaboration among government agencies, industry associations, and relevant stakeholders to develop unified and consistent food safety regulations.                                                                                                                                                                                   |
| Limited availability of authorized testing facilities       | Establish authorized private laboratories with the necessary accreditation to conduct food safety testing. Strengthen and expand the capacity of government laboratories to meet the demand for testing services. Encourage partnerships between public and private sectors to improve testing facilities and promote quality assurance.                           |
| Compliance challenges for small and medium-scale industries | Provide financial incentives or subsidies specifically targeted at small and medium-sized enterprises to assist them in meeting food safety compliance requirements. Offer technical assistance and mentorship programs to guide smaller businesses in implementing food safety practices and acquiring necessary certifications.                                  |
| Lack of awareness and understanding among SMEs              | Launch awareness campaigns and educational initiatives to promote the importance of food safety among SMEs. Provide resources, such as guidelines, toolkits, and online platforms, to facilitate access to information and best practices. Collaborate with industry associations and trade organizations to disseminate information effectively.                  |
| Inadequate monitoring and surveillance                      | Strengthen regulatory agencies' capacity for regular monitoring and surveillance of food products. Increase inspections, random sampling, and product testing to ensure compliance with food safety standards. Implement stricter penalties and enforcement measures for non-compliance. Encourage public reporting mechanisms to identify and address violations. |
| Limited technical assistance and education                  | Establish partnerships between government agencies, NGOs, and international organizations to provide technical assistance, capacity building programs, and mentorship to the private food sector. Facilitate knowledge exchange platforms and industry forums to foster learning and sharing of best practices.                                                    |

In addition to the challenges mentioned above, the private sector in Nepal also faces difficulties in accessing technical assistance and education regarding compliance with food safety standards. There is a lack of awareness and understanding among small and medium-sized enterprises (SMEs) regarding the importance of food safety, which often leads to non-compliance. Furthermore, the absence of authorized private laboratories and the limited capacity of government laboratories pose a significant challenge for the routine service delivery to the private sector in Nepal. This lack of laboratory infrastructure and testing facilities makes it difficult for small and medium-sized enterprises to ensure the safety and quality of their products. Inadequate monitoring and surveillance of food products in the market *also* contribute to non-compliance with food safety standards, as it allows for the sale of unsafe and adulterated food products.

## 6. REGIONAL COLLABORATION FOR FOOD SAFETY: AREAS, IMPLICATIONS AND BENEFITS

According to the Global Food Security Index (GFSI), which assesses the fundamental factors of affordability, availability, quality, and safety in 113 countries worldwide, the overall food safety situation in Southeast Asia is comparatively poor when compared to other developed nations (Shrestha & Khanal, 2019). The GFSI is a comprehensive, dynamic, and multidimensional model based on 34 distinct indicators that assess these food security drivers in both developed and developing countries. As seen from Table 4, India has the highest overall and category-ranked score in the South Asian Association for Regional Cooperation (SAARC) countries, while Pakistan has the lowest. All scores are rated on a scale of 0 to 100, with 100 being the most desirable (GFSI, 2022).

Regional collaboration in the food sector can have significant implications and benefits, particularly for poor countries. Sharing of knowledge and experiences can lead to the development of a common understanding of food safety standards, which can improve the overall quality and safety of food products in the region. This can result in a reduction in the number of foodborne illnesses and related deaths, improving public health outcomes. Regional collaboration can also facilitate the exchange of goods and services, leading to economic growth and development in the region. Moreover, regional collaboration can help to address common challenges and issues faced by poor countries, such as lack of infrastructure and limited resources for monitoring and surveillance of the food sector. Collaboration can also promote the harmonization of food safety regulations and standards, which can reduce trade barriers and improve market access for poor countries (Martinez *et al.*, 2007). Additionally, regional collaboration can lead to technical assistance and capacity building for poor countries, enabling them to improve their food safety systems and comply with international standards. This can enhance the competitiveness of poor countries in the global market and increase their access to international trade opportunities.

Regional collaboration in the food sector can greatly benefit poor countries by ensuring that food safety standards are harmonized across borders, reducing trade barriers and promoting a more efficient food supply chain. One area of collaboration could be the harmonization of food standards to ensure that the same regulations are applied in all countries within a region. This could be achieved by developing a regional framework for food safety based on the Codex Alimentarius, which provides guidelines and standards for food safety and quality.

**Table 4.** Global Food Security Index of SAARC countries (GFSI, 2022)

| Country     | GFSI Rank/Score | Category Rank/ Score |              |                    |
|-------------|-----------------|----------------------|--------------|--------------------|
|             |                 | Affordability        | Availability | Quality and Safety |
| Afghanistan | NA              | NA                   | NA           | NA                 |
| Bangladesh  | 80/54           | 87/52.1              | 46/61.5      | 71/58.4            |
| Bhutan      | NA              | NA                   | NA           | NA                 |
| India       | 68/58.9         | 80/59.3              | 42/62.3      | 67/62.1            |
| Nepal       | 74/56.9         | 85/52.7              | 13/70.9      | 72/57.8            |
| Maldives    | NA              | NA                   | NA           | NA                 |
| Pakistan    | 84/52.2         | 75/59.9              | 61/58.3      | 97/49.4            |
| Sri Lanka   | 79/55.2         | 74/61                | 64/57.2      | 81/55              |

Another area of collaboration could be the development and implementation of a regional food safety strategy, which would aim to improve food safety practices and reduce the incidence of foodborne illnesses. Mutual Recognition Agreements (MRAs) could also be established to facilitate trade and recognition of food safety standards between countries.

The development and recognition of regional standards for system certification could also help to clear doubts and smoothen trade, especially for small and medium-sized enterprises. A Regional Food Safety Rapid Alert System could be established to improve communication and reduce trade losses in the event of a food safety issue. Finally, the establishment of a Regional Food Borne Disease Epidemiology Centre could help to identify and respond to foodborne disease outbreaks in a timely and effective manner. Moreover, through surveillance, investigation, risk assessment, capacity building, and collaboration, the center can contribute to improved food safety standards, enhanced public health outcomes, and ultimately protect the well-being of the population in the region. Overall, regional collaboration in the food sector has the potential to enhance food safety standards, promote trade, and improve public health in poor countries.

## 7. CONCLUSION

In conclusion, addressing policy and regulatory challenges, capacity building, regional collaboration, and private sector engagement are crucial for strengthening food safety governance in Nepal. The implementation of tools such as GMP, GHP, HACCP, and the establishment of a centralized food safety authority, risk-based inspection, and strengthening laboratory infrastructure are necessary for ensuring food safety standards. Similarly, collaborating and sharing best practices with neighboring countries can help Nepal to develop a more effective food safety regulatory framework. Additionally, private sector engagement plays a crucial role in ensuring compliance with food safety standards; however, small and medium-scale industries encounter numerous challenges that require immediate attention. Likewise, developing a regional framework for food safety based on codex, harmonization of food standards, and the formation of a Regional Food Safety Rapid Alert System can enhance regional collaboration and improve communication among countries. Furthermore, the establishment of a Regional Food Borne Disease Epidemiology Centre and the recognition of regional standards for system certification can contribute to improving food safety in the region.

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# SUSTAINABLE RICE INDUSTRY IN NEPAL: COMPARATIVE ANALYSIS WITH SOUTH ASIA

Pradyumna Raj Pandey<sup>1,\*</sup>, Hemprabha Pandey<sup>2</sup> and Aashish Shrestha<sup>1</sup>

<sup>1</sup> Ministry of Agriculture and Livestock Development, Singhadurbar, Kathmandu, Nepal

<sup>2</sup> Nepal Institute of Yoga and Management, Lalitpur, Nepal

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\*Correspondence:

pandeypr08@yahoo.com

Tel.: +977-9851125554

## ABSTRACT

*Rice, a prime crop in Nepal and other South Asian nations, plays a crucial role in ensuring food security and socio-economic development. In Nepal's agricultural sector, rice accounts for the largest share of the Agricultural Gross Domestic Product (AGDP), comprising approximately 20%. However, the absence of appropriate policies poses a significant challenge to the sustainable production of rice in Nepal. This study aims to assess the various policy dimensions of the sustainable rice industry in Nepal and compare them with the policy implications in neighboring South Asian countries such as India, Pakistan, and Bangladesh. The study draws on secondary sources of information for its analysis. The findings reveal that Nepal lacks a comprehensive rice policy, while its existing agriculture policy is characterized by confusion and contradictions. To attain self-sufficiency in food production, it is imperative to establish a comprehensive rice policy in Nepal and implement price subsidies on diverse inputs, as observed in neighboring countries which will ultimately contribute to enhancing the sustainability of the rice industry and promote the goal of food self-sufficiency in Nepal.*

## 1. INTRODUCTION

South Asia region, home for about 25% of world's population but with hardly 2% of global income, faces several challenges to end hunger, ensure food security, promote sustainable agriculture, and achieve the Sustainable Development Goals (SDGs) by 2030. In South Asia, more than 50% of the 1.8 billion people engaged in agriculture is dominated by small, resource-poor farmers with an average holding size of less than 2 hectares. South Asia's development trajectory for poverty reduction and food security continues to rely on ways in which agricultural research and policy are supported and shaped, both nationally and regionally (World Bank, 2022).

Rice is a prime crop in Nepal and South Asian countries on the basis of food security and socio-economic prospectus. Increased rice production is key to achieving food security, reducing unemployment, increasing the income of farmers, and reducing poverty. The yield of rice and other major food crops in Nepal is the lowest among South Asian countries. However, there is a large potential to increase rice yields in Nepal. Increasing

rice production can solve this food-deficit problem and save millions of rupees now spent by the government every year in importing food grain. Various factors have accounted for this stagnation in yield: small and fragmented land holdings, lack of irrigation facilities, accessibility to marketing and purchasing of inputs, appropriate technology, and land degradation. Among the various factors responsible for sustainable rice industry in Nepal, policy implication might be a crucial but an underlooked factor. And there haven't been many policy reviews pertaining to the rice sub-sector. It has been necessary to identify the key challenges and opportunities in achieving a sustainable rice industry in Nepal by comparing its status with other South Asian countries to suggest policy interventions and strategies that can enhance food security, environmental conservation and economic growth. This study was conducted with the objective to analyze the different policy dimensions of sustainable rice industry in Nepal with comparative policy implications of South Asian countries.

## 2. MATERIALS AND METHODS

This paper is based on the review of secondary sources. Agricultural policies of Nepal and three South Asian countries namely: India, Bangladesh and Pakistan were reviewed. Published reports, documents, articles of government of Nepal and other national and international organizations served as the sources of information.

### 2.1 Theoretical framework of rice industry

Rice has political, economic, and social significance for almost all countries in Asia. Millions of farmers depend on rice production for their livelihoods. Since peoples' livelihoods are tightly linked with rice production, sustainable rice production is considered as a way to achieve sustainable livelihoods, which leads to sustainable economic development. Therefore, rice technologies and policies that provide adequate income for rice producers are needed to achieve sustainability

in the rice sector, particularly in less developed rice producing countries.

Sustainable rice industry interlinks economic, ecological and social activities of farmers and the distribution of resources. It may be based on one activity (growing rice), but it may include other activities that do not conflict with respect to labor requirements, use of land area or use of other resources (Sharma *et al*, 1991). In this conceptual framework sustainable rice industry would be possible if economic activity would be profitable and productive, ecology would be balanced by climate change and biological activities, like soil, water, etc. and these activities are socially and culturally accepted by rice farmers. This concept is more related to the sustainable farming system, which is an organizational structure that interlinks the various activities of farmers and the distribution of resources.

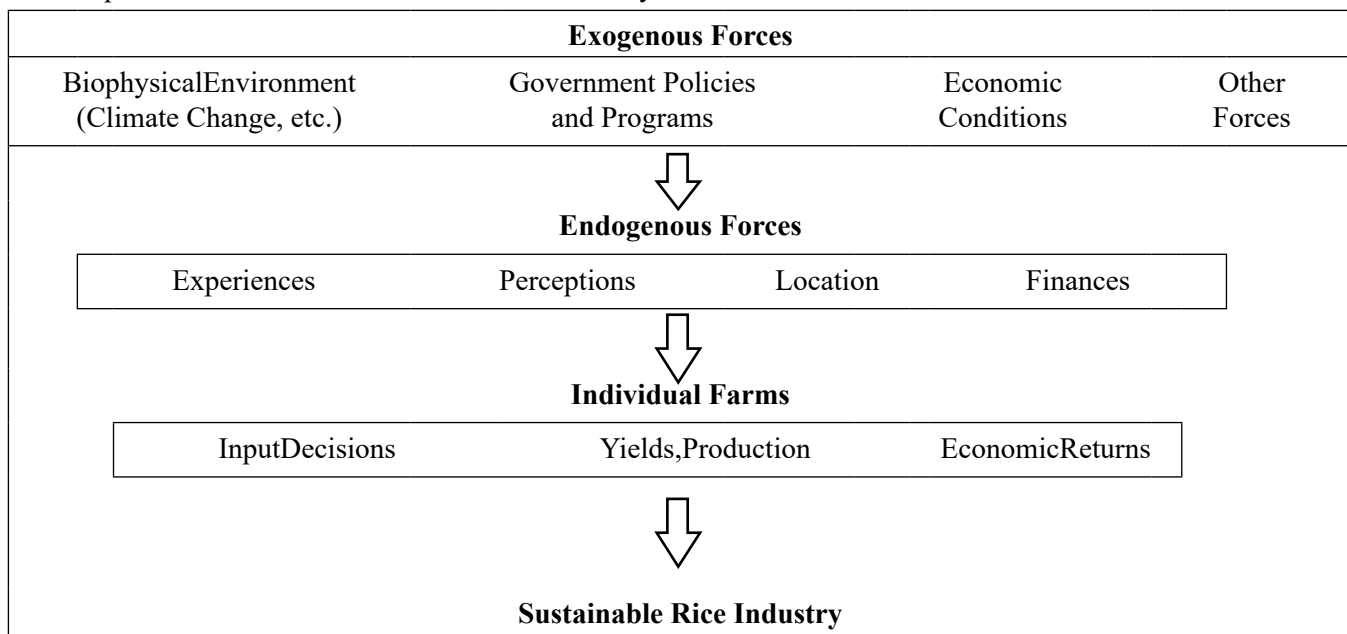


**Figure 1.** Exogenous and Endogenous Forces in Sustainable Rice Industry in Nepal, Adopted: Smit *et al.*, 1996

Sustainable rice industry in Nepal is influenced by many exogenous and endogenous forces (Figure 1). Exogenous forces, those beyond the control of the farmers, have a major influence on each agro-ecological region in Nepal. These forces include the biophysical environment, government policy, and economic conditions. The endogenous forces are those that farmers have some control over. They include the farmer's experience, perceptions, location of the farm, and finance. Combining these forces results in a vast array of individual farm decisions, and when

the results of each farm are combined, this leads to the agro-ecological regional agriculture system. The rice farming system is also part of a broader agri-food system, whereby modern commercial agriculture is one stage in a food production process linking farmers and consumers via a system of processors, distributors and retailers. The agri-food system is both complex and differentiated across the agro-ecological regions of Nepal, with some regions more dependent upon rice and other major food crop production, particularly in the plain and hilly regions; however, the mountain

region is more diversified into livestock production and/or value added processing.

### 3. RESULTS AND DISCUSSION

#### 3.1. Development of rice and food policy in nepal

Nepal started its planned agricultural programs in 1950 with the initiation of the Tribhuvan village development program. This sector has also received priority in periodic plans and various agricultural policies, plans and guidelines. However, here is no separate rice policy. The major policies affecting rice are as follows:

##### 3.1.1. The agriculture perspective plan (APP)

Nepal adopted the 20-years Agriculture Perspective Plan (APP) as a guiding strategy for the overall agricultural development of the country. The APP (1995—2015) emphasizes a few priority inputs, outputs and outcomes. Liberalization, deregulation and devolution are central to the policy regime that is aligned with the larger participation of multiple actors in service provisioning.

##### 3.1.2. National agriculture policy 2004 and other sectoral policies

The Government of Nepal (then HMG) implemented a National Agricultural Policy in 2004 as an umbrella policy of all other agriculture related policies of the country. Within the greater framework of the National Agriculture Policy of 2004 other necessary commodity policies were also formulated to create a better commercial farming environment. All these policies attempt to create favourable and sustainable growth and a competitive environment for commercial farming including rice, thus supporting export promotion and import substitution. However, none of these policies mentioned clearly about the rice policy.

##### 3.1.3. Periodic plans

Agriculture received top priority from the very beginning of periodic plans. However, in recent periodic plans social sectors and infrastructure are provided more priority than the agriculture sector. The fifteenth periodic plan focus on defining farmers and emphasising financial incentives, production based incentives and social protection programmes. It spells out to incentivize production and export of agricultural goods, mobilize concessional loan to increase investment in agriculture, provide tariff protection to domestic products to increase their consumption. It also spells to fix minimum support prices of specific crops and products and expand credit and insurance services (NPC, 2020).

#### 3.1.4. Agriculture development strategy, ADS (2015-2035)

Government of Nepal approved ADS as a 20-year vision for the agriculture development in Nepal. The strategy envisions to increase the access to quality and timely inputs by introducing voucher system in inputs, and increase access to microfinance and agricultural insurance. It focuses on tax policy that supports an efficient commercial agriculture sector. A new concept of warehouse receipt envisaged in ADS can help the farmers for the storage of their produce, fetch better price of their produce and to obtain the credit facilities on the guarantee of the stored produce (ADS, 2015).

Nepal liberalized most extensively during the 1980s and with continuity in the 1990s on both fronts, domestic and internationally. Nepal has the lowest tariffs in South Asia and has taken several steps to downsize its public distribution system and remove a host of agricultural subsidies. This twin scenario where the lowest per capita income country is perhaps also the most liberalized makes for an interesting case for policy analysis.

##### 3.1.5. Rice inputs and price policies

In Nepal, there is no crop-specific policy on credit. In order to make it more productive and effective, it is felt that proper attention needs to be paid to integrating agricultural credit with farming needs. There is a provision of subsidized agricultural loan (with or without collateral) upto ten crore rupees as per “Unified Manual Related to Interest Subsidy for Concessional Loan, 2018” (NRB, 2021). The government provides price subsidies on urea, DAP and MOP fertilizer sales. The prices of fertilizer are fixed by the government considering the landed cost of fertilizer at entry points, amount of subsidy allocation, prices of fertilizer across the border in India, and anticipated farmer reaction (APP, 1995). Fertilizer prices in Nepal are maintained at a level that is about 15 to 20% higher than in India to prevent smuggling of subsidized fertilizer to India.

As per the fertilizer use pattern of farmers, the estimated demand of the fertilizer is about 5,20,000 mt. If recommended doses of fertilizer is assumed, the actual need of fertilizer seems about 7,00,000 mt. However, in fiscal year 2020/2021, the annual sale was only 379,152 mt (MoALD, 2022). In this regard, there is a huge gap between the demand and the supply of chemical fertilizers. Inadequate and untimely availability and ineffective distribution becomes the major issue every year. Agriculture Input Company Limited (AICL)

and Salt Trading Company Limited (STCL) has been entrusted to procure and distribute subsidized fertilizer across the country. However, the fertilizer subsidy policy is a complex issue in Nepal. First, the Indian border price exerts a big influence on setting a fertilizer price policy in Nepal because of the long and open border. Second, the fiscal burden which the current subsidy places on the government is very high and it constrains fertilizer use. The quantity imported is basically dictated by the financing set by the government.

### **3.1.5.1. Minimum domestic support price**

The government began to announce minimum support prices for rice and wheat in 1976/77, with Nepal Food Corporation (NFC) (now, Food Management and Trading Company Limited) acting as the agency responsible for implementing the farm support price policy. The prices are fixed on the basis of the cost of production and a reasonable profit margin for farmers, food grain production levels and market prices in major producing areas, market prices in Indian border markets, and Indian support prices. The support price policy has not been implemented effectively in Nepal for several reasons. First, support prices are, in most cases, announced at harvest time or later, which precludes any possible impact from decisions by producers. Second, because the prices have usually been lower than the market prices they do not provide any incentive for farmers to increase production. Finally, financial and other constraints on Food Management and Trading Company Limited have resulted in ineffective enforcement of announced support prices. In addition to FMTCL, Prime Minister Agriculture Modernization Project (PMAMP), Project Implementation Units have also been utilized for the enforcement of announced MSP of rice.

The border with India and the relatively large markets of bordering Indian States limit the effectiveness of any output price support policy adopted in Nepal. Nepal can buy from, or sell to, the Indian market without affecting Indian prices. Moreover, the prices in India affect Nepalese domestic prices every year. The output prices in Nepal in such a situation are equal to the Indian border prices plus or minus transportation and transaction costs. Therefore, prices in Nepal can be controlled with price policy instruments only within upper and lower bounds defined by transport and transaction costs. In an effort to rationalize tariffs, tariff rates have been reduced from 87 to 8 basic rates. In addition, the maximum rates have also been lowered. Like previous years, currently

the government announces the support price prior to planting the rice crop (MoALD, 2021).

### **3.1.5.2. Trade policy of rice export and import**

Marketing of rice is done as per the provision of free market policy. Price is determined by the free market behaviour. As a primary agricultural product, rice is exported and imported to and from India according to the bilateral preferential trading arrangement of Indo-Nepal trade. No any tariff is applied in all primary agricultural products. But Nepal is levying around 5% tax as agricultural improvement tax for all these agri-products (ITA, 2021).

Nepal's current average applied tariff rate for agricultural products for other countries is 14%, but the average bound tariff rate for agricultural products permitted for Nepal in WTO is 42%. The permitted tariff rates are different in multilateral, regional and bilateral trading regimes. In the SAFTA and BIMSTEC, the tariff rates are set as 0-5% for most of the products, but there are amazingly high numbers of products in the sensitive list of each member. Nepal has 1257 products for other LDCs and 1295 products for Non-LDCS members in sensitive list. By this complex provision of sensitive list in both SAFTA and BIMSTEC, there is no considerable trade between and among their members on these provisions. Thus, attempt has been made to lower the sensitive list. In spite of the provisions in regional trading regimes, Nepal has plenty of scope in WTO provisions to increase its tariffs for imported agricultural products for the protection of domestic producers. But being food importing country, raising the tariff rate of rice will only raise its consumer price in the domestic market.

## **3.2. Comparative analysis of rice policies between Nepal and other South Asian countries**

The comparison of rice policy of Nepal and neighbouring South Asian countries (India, Bangladesh and Pakistan) is presented in Table 1 and is explained separately in paragraphs.

### **3.2.1. India**

India's food grain sector turned perennially large deficits into large surpluses after its independence in 1947. During the 1960s and 70s, the turnaround was achieved through rapid gains in yields combined with policies that balanced producer and consumer interest. After the 1990s, growth in yields and consumption slowed and government policy sought to sustain progress by

**Table 1.** Comparison of Rice Policies between Nepal and Other South Asian Countries.

| <b>Rice Policy Related Issues</b> | <b>Bangladesh</b>            | <b>India</b>              | <b>Nepal</b>                                                                   | <b>Pakistan</b>            |
|-----------------------------------|------------------------------|---------------------------|--------------------------------------------------------------------------------|----------------------------|
| Rice Policy                       | Yes                          | Yes                       | Not specific                                                                   | Yes                        |
| Minimum Support Price             | Yes                          | Yes                       | Yes, but less effective                                                        | Yes                        |
| Development Authority             | Bangladesh Rice Foundation   | Commission of Agriculture | Food Management and Trading Company Limited (Formerly: Nepal Food Corporation) | Rice Exporters Association |
| Input Subsidies                   | Effective Production subsidy | Production subsidy        | Not effective and very less                                                    | Production subsidy         |
| Trade Policy                      | Mostly Protected             | Mostly Protected          | Mostly Liberalized                                                             | Mostly Protected           |

Source: Pyakuryal, 2005; MOICS, 2022

increasing producer support and improving the targeting of food subsidies to low income consumers. By 2000, the Government confronted with combination of high domestic prices, declining per capita consumption, record grain surpluses and soaring budgetary costs. By 2006, lower prices, weak yield growth, and rising subsidized distribution led to the re-emergence of substantial food deficits (Jha *et al.*, 2007).

The domestic trade policy of India is governed by the Essential Commodities Act of 1995 that gives the government the right to restrict the holding of cereals by the private sector and to restrict the movement of cereals across provinces. The Indian government has been providing subsidies to farmers for inputs such as fertilizers, power, irrigation water, agricultural loans and technology.

The distribution of rice to consumers is made under the provision of the Public Distribution System of India, through which affordable prices are maintained through the Fair Price Shops (FPS). Rice is issued by the central government at the uniform central issue price (CIP) across the country for distribution of rice under the provision of the ‘Targeted Public Distribution System (TPDS)’ of 1997.

To maintain inter year price stability, the Indian government has been carrying out a buffer stock scheme for food grains. The Indian government established a Commission on Agricultural Costs and Prices in 1965 to suggest the minimum support price (MSP)

for some important crops. The minimum support price of rice is declared each year at the recommendation of the Commission of Agricultural Cost and Prices (CACP). As per the provision of MSP policy, the Food Corporation of India buys the rice from farmers if the farmers cannot get the minimum support price from traders (Radhakrishna and Indrakant, 2004).

### 3.2.2. Bangladesh

The rice farming and production system in Bangladesh is facilitated by ‘The Bangladesh Rice Foundation (BRF),’ which is a non-stock, non-profit, private organization established in 2002 to enhance and sustain the well-being of all value chain actors the producers, workers, processors, traders and consumers through research, advocacy, and dissemination of research-based information in areas related to policies on rice. BRF also recognizes the importance of preserving the rich national culture and heritage associated with rice and promoting the associated traditions and values (UNDP, 2005).

The Board of Trustees of the Bangladesh Rice Foundation (BRF) devotes time to formulating the working strategy and identifying the programmatic thrusts of the Foundation. Consistent with the mission and objectives of BRF, activities of the Foundation fall within the research, communication and advocacy, and awareness and promotion of culture and tradition are three broad program categories.

To financially help the farmers by solving the issue of low paddy prices during harvest season and ensuring rice stockpiles for the Public Food Grain Distribution System (PFDS), the government announces a minimum support price at the time of harvest (Islam *et al.*, 2023). The Government procured 37.57 percent of the targeted amount in 2020. After harvest, majority of farmers sell their rice within two months of harvest primarily to middlemen for practical reasons such as urge for cash for loan repayment as well as lack of confidence and awareness about current paddy procurement system. About two third of paddy growers are unhappy on the present procurement system due to disturbance of the middlemen and delayed payment (Rahman *et al.*, 2021).

Increased labor cost due to labor shortage during peak transplanting time and harvesting time, and increased irrigation cost are the major factor for increased cost of production of rice in Bangladesh. In addition to this, the pattern of paddy price during peak harvest has been unpredictable over the years 2009-2020 resulting in the reduced profitability. This has pushed the farmers to shift to non-rice agricultural commodities (Islam *et al.*, 2023).

### 3.2.3. Pakistan

For maintaining the goal of self-sufficiency and securing higher farmer income by ensuring price stability and increasing quality for the open competitive market, Pakistani government has formulated rice policy with various provisions. Since Pakistan is also one of the important rice producing country of the world, government has paid a great attention on different issues that affects commercial rice farming. Emphasis on agricultural price policy is an important and relatively a new phenomenon that the Pakistani government has adopted (ADB/N, 2004).

As most of the agricultural markets, rice market *also* suffers from the cobweb phenomenon where price fluctuations are caused by the time lag between making the decision to cultivate the crop and ultimate selling of the crop, Pakistani government has introduced the provision of minimum support price for rice crop to decrease the risk faced by farmers from fluctuating price.

## 4. CONCLUSION

Rice has been playing an important role in the Nepalese economy and food security. Nepal, previously rice exporting country, is now being a net importer of rice due to the two major reasons: the first slower increment in productivity of food crops but larger growth rate of population and the second is the uneven internal marketing and distribution system of food crops within the country. Nepal has opened up its trading market into the multilateral & global free and fair trading system. Since the major trading partner and closest neighbouring country India has been providing huge subsidy to producers, Nepal should also pay attention to provide the subsidy to the farmers so that they can compete in internal and external markets. Nepal has been supporting rice subsector through scattered agriculture policy and program instruments not specific for rice. Thus, Nepal does not have comprehensive rice policy so far. Crucial support on input subsidies including chemical fertilizers, credit facilities, announcement of minimum support price in time, its effective implementation in procurement, support for rice processing industries, etc. are inevitable in competitive and sustainable rice industry. Thus, formulation of separate rice policy and subsequent program instrument including these activities can help the rice subsector to grow and contribute to meet the national aspiration of self sufficiency in food grains.

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# TIME OF HARVEST AS THE DETERMINANT OF DRY MATTER PRODUCTIVITY AND CHEMICAL COMPOSITION OF FIELD PEA (*Pisum sativum*) and OAT (*Avena sativa*) AND THEIR MIXTURE IN THE ABANDONED LANDS OF SUBTROPICAL TERAI NEPAL

Shanker Raj Barsila<sup>1, \*</sup>, Ram Bahadur Bogati<sup>2</sup> and Dawa Tshiring Tamang<sup>1</sup>

<sup>1</sup> Agriculture and Forestry University, Rampur, Chitwan, Nepal

<sup>2</sup> FAO Country Office, Pulchowk, Lalitpur, Nepal

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\*Correspondence:

srbarsila@afu.edu.np

Tel: +977-9851166703

## ABSTRACT

The grass-legume mixture formulation is an important criterion to improve biomass availability with an improved balance of the nutritive components. The objective of this research was to determine the yield and forage quality traits (green fodder yield, dry matter yield) and chemical composition (protein, fibre and mineral contents) of field pea (*Pisum sativum*) and oat (*Avena sativa*) and their mixtures under subtropical conditions. The experiment was performed over 4 months (December 2018 to March 2019) at Agriculture and Forestry University Livestock Farm, Rampur, Chitwan, Nepal, using four different treatment mixture rates of pea and oat crops. Treatments were; treatment-1 - 100% Pea+0% Oat, treatment-2 - 75% Pea+25% Oat, treatment-3 - 50% Pea+50% Oat and treatment-4 - 25% Pea+75% Oat) and samples were collected in three different cutting stages (the jointing stage of oat, the stage oat in scabbard and milk dough stage of oat). The plant height was affected by the growing days while the tillers and branches in peas respectively were affected by both the time of harvest and the seed proportions. According to the results, it is suggested that the highest dry matter productivity was observed in 75% pea mix with 25% oat in the 3rd harvest, followed by 75% oat mix with 25% pea in the 3rd harvest. As well as, within intercropping, the best protein yield was obtained in a 50% oat mix with 50% pea (16.73%) at 1st harvest oat-pea intercropping. Besides, the pea + oat mixture should be harvested at the milk-dough stage of oat for better protein content and to increase the nutritive value of forage.

## 1. INTRODUCTION

Great efforts have to be made to provide adequate feed not only to increase production but also to lower the production cost but there are only limited options available to tackle this problem. Among them, the grass-legume mix--culture is one. It is commonly reported that grass-legume mixtures limit the negative effects of an excessive share of grasses in the rotation and they would assist as a good crop for succeeding crops. They enrich the soil fertility, adding nitrogen through a symbiosis of legumes with nodule bacteria and in organic matter due to the huge amount of crop residue left behind. Oat (*Avena sativa*) and pea (*Pisum sativum*) are the commonly available winter forage resources and are well adapted in combinations and fed as green whilst the surplus is converted into hay in the fodder deficit periods (Suttie and Reynolds, 2004).

Farmers of low-income countries like Nepal limitedly afford to use industry-based concentrates and

chemicals as supplements to improve the utilization of roughages. Leguminous forage crops can improve the utilization of low-quality roughages. One of the potential approaches to improve livestock feed availability in terms of quality and quantity is the use of grass-legume mixtures (Alemu *et al.*, 2007) and they are being used more extensively throughout the world. In various production systems legumes are capable of enhancing both crop production through sustained soil fertility and livestock production through increased availability of high-quality feed. Alemu *et al.*, (2007) reported that planting oats and vetch mixtures at 25% oats and 75% vetch proportion resulted in better relative yield, but only one variety of each species was tested. The potential of improved forages such as oats and peas in enhancing livestock feed availability is highly recognized mainly in intensively cultivated highlands. Sanderson *et al.*, (2012, 2013) also reported that growing mixtures of

grasses and legumes improve biomass production as compared to grass monocultures. Mixed planting of grasses and legumes was also indicated to be more productive than monocultures and the approach was thus reported to help control weeds, diseases and pests (Erla, 2011). The productivity of oats and vetch mixtures is also known to be superior to pure stands in yield and quality (Assefa and Ledin, 2001; Erol *et al.*, 2009) and in areas where market-oriented livestock production is practised.

Mixed planting of grasses and legumes was also indicated to be more productive than monocultures and the approach was thus reported to help control weeds, diseases and pests (Erla, 2011). Therefore, in the present study, it was hypothesized that varietal and seed proportion differences of oat and pea mixed stands would influence the productivity and compatibility of the mixed stands. The study further envisaged seeing the differences in forage quality as influenced by the varietal and seed rate proportion of the component species. More production in intercropping can be expected due to the higher growth rate, reduction of weeds, reduced pests and diseases and more effective use of resources due to differences in resource consumption etc. (Eskandari, 2012; Eskandari *et al.*, 2009; Watiki *et al.*, 1993). If the intercrops components have a difference together in the use of environmental resources, that are complementary in the use of these resources are more effective than pure cropping, and a resultant increase in yield (Jensen, 1996). In a review by Francis (1989) on intercropping, in 53% of the experiments intercropping reduced the pest, and in 18% increased the pest than pure cropping. In intercropping, there is a better utilization of nutrients, soil moisture, and light and fill the empty niche which leads to weed suppression (Saudy and El-Metwally, 2009; Altieri, 1995). Rhizobium bacteria have a symbiotic relationship with plants of Leguminosae family and thereby can fix atmospheric nitrogen into available nitrogen for uptake. And the resulting nitrogen is an essential element for soil fertility and plant growth. Farmers have to face fodder shortage problems in winter when they have only dry stalks of summer cereal fodders or dry summer grasses. To increase productivity with quality per unit area, there is a need to develop promising cultivars having high forage yield potential and quality (Ahmad *et al.*, 2014). The forage mixture, harvesting time and their interaction were considered major factors of analysis for dry matter productivity and chemical constituents. This research finding would help to identify the

better proportion of legume peas with oat. One of the potential approaches to improve livestock feed availability in terms of quality and quantity is the use of grass-legume mixtures (Alemu *et al.*, 2007). In this regard, the dry matter yield of grass and legume mixed stands has been reported to be superior compared with sole legume plots (Assefa & Ledin, 2001). The experiment was conducted, thus, to know the biomass production (dry matter) and chemical composition of the different ratios of oat combined pea for higher aboveground biomass productivity in the abandoned land.

## 2. MATERIALS AND METHODS

### 2.1 Study area

The field experiment was conducted at Agriculture and Forestry University Livestock Farm, Rampur, Chitwan, Nepal. The experimental site was situated between 84° 20' east longitudes to 27°39' north latitudes and was 182 masl, and was previously abandoned for cultivation at an unknown time.

### 2.2 Study design

The field experiment was conducted in a Randomized Complete Block Design (RCBD); each treatment was replicated four times. Altogether there were 16 experimental units, each with 4×4 meter square area.

### 2.3 Treatments

The following treatments were used for the experiment.

Treatment 1 (T1): 100% Oat

Treatment 2 (T2): 25% oat & 75% Pea

Treatment 3 (T3): 50% oat & 50% Pea

Treatment 4 (T4): 75% oat & 25% Pea

The variety of oat used in the experiment was Kamadhenu and that of pea was the Sikkime Local respectively.

### 2.4 Seed rate, sowing, and irrigation

The seed was sown using the broadcast method. The seed rate used was oats @ 80 kg/ha; pea @ 20 kg/ha in a mixture was used for the mono-cropping of them. The forage seeds were sown on 1<sup>st</sup> December 2018.

After sowing, the plots were irrigated once and then second irrigation was done 21 days after sowing (DAS) the seed.

## 2.5 Fertilizer application

The NPK was applied at the rate of 100:60:40 kg/ha, and farmyard manure (FYM) at the rate of 10 tons/ha was used (1/2 at the time of land preparation and the remaining half at the 21 days after seeding).

## 2.6 Sampling methods

Sampling was done using a simple random technique from 1 sq.m. in each plot. Samples were collected in three harvests from each plot. Samples taken at each plot were cut above 2 cm from the ground and weighted for fresh. The first harvest was done on January 15<sup>th</sup> 2018 and the later harvests were done at every 15 days interval. Altogether three consecutive harvests were taken.

## 2.7 Morphological study

Morphological study of the forage plants was done by measuring plant height, number of branches and number of tillers per plant, stem and root ratio, and aboveground herbage productivity.

## 2.8 Laboratory analysis

Laboratory analysis was done at AFU Animal Nutrition Lab following the standard procedure developed at the laboratory. Laboratory analysis for Crude Protein (CP), Ether Extract (EE), Total Ash (TA), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL), Hemicellulose, (HEM), Cellulose (CEL), Calcium (Ca) and Phosphorous (P) was done from each treatment. The aliquot method was used to predict the single value of the composition data from the mix of oat and pea relative to dry matter productivity following Dangi *et al.* (2020).

The sample aliquot was calculated based on DM and individual chemical composition i.e., CP, CF, EE etc. for the mix plots and a single value of composition was thus obtained by using the following formula:

$$CP = \frac{(\text{Dry weight of oat})}{(\text{Total dry weight of the mixture})} \times \% \text{ CP of oat} + \frac{(\text{Dry weight of vetch})}{(\text{Total dry weight of the mixture})} \times \% \text{ CP of vetch.}$$

## 2.9 Statistical analysis

The data were analysed using the software R version 3.5.2. The mean difference was set by Duncan's Multiple Range Test (DMRT) at a 5% level of significance.

The effect of harvesting time and forage mixture on dry matter productivity and chemical composition was determined using a linear statistical model for two factors which are given:

$$Y_{ijk} = \mu + \sigma_i + \beta_j + (\sigma\beta)_{ij} + \varepsilon_{ijk} \dots\dots\dots \text{Eq. 1.}$$

$\mu$  = constant factor

$\sigma_i$  = effect of *i*th level of harvesting

$\beta_j$  = effect of *j*th level of forage mixture

$(\sigma\beta)$  = interaction effect of harvesting time and forage mixture

$\varepsilon_{ijk}$  = random error

## 3. RESULTS

### 3.1 Morphological attributes

#### 3.1.1 Plant height

The height of peas and oat was found significant ( $p < 0.05$ ) at all harvests. The maximum height of the pea was observed in 75% pea mix with 25% oat (65cm) at 3<sup>rd</sup> harvest whereas the minimum was recorded in 50% pea mix with 50% oat (19.38cm) at 1<sup>st</sup> harvest. Similarly, the height of the oat was observed as maximum in 75% pea mix with 25% oat (41.5cm) at 3<sup>rd</sup> harvest while it was recorded as minimum in the same treatment of 75% pea mix with 25% oat (24.5cm) at 1<sup>st</sup> harvest. A detailed description of plant height in various treatment combinations is shown below in Table 1. No significant ( $p < 0.05$ ) result was seen in the interaction of harvesting time and forage mixture for plant height.

**Table 1.** Plant height (cm) of oat and pea was measured before each harvest.

| Treatments              | Pea                 |                    |                      |                     | Oat               |                     |                      |
|-------------------------|---------------------|--------------------|----------------------|---------------------|-------------------|---------------------|----------------------|
|                         | 75%Pea+<br>25%Oat   | 50%Pea+<br>50%Oat  | 25%+Pea+<br>75 % Oat | 100%<br>Oat         | 75%Pea+<br>25%Oat | 50%Pea+<br>50%Oat   | 25% Pea +<br>75% Oat |
| 1 <sup>st</sup> harvest | 23 <sup>de</sup>    | 19.38 <sup>e</sup> | 23 <sup>de</sup>     | 25.75 <sup>bc</sup> | 24.5 <sup>c</sup> | 26.13 <sup>bc</sup> | 27.63 <sup>bc</sup>  |
| 2 <sup>nd</sup> harvest | 32.13 <sup>cd</sup> | 34.38 <sup>e</sup> | 34.88 <sup>c</sup>   | 28.13 <sup>bc</sup> | 31 <sup>b</sup>   | 28.5 <sup>bc</sup>  | 29.25 <sup>bc</sup>  |
| 3 <sup>rd</sup> harvest | 65 <sup>a</sup>     | 48.25 <sup>b</sup> | 58.25 <sup>a</sup>   | 40.63 <sup>a</sup>  | 41.5 <sup>a</sup> | 38 <sup>a</sup>     | 38.63 <sup>a</sup>   |
| p-value                 |                     |                    |                      |                     |                   |                     |                      |
| Ht                      |                     | <0.01***           |                      |                     | <0.01***          |                     |                      |
| Fm                      |                     | 0.115              |                      |                     | 0.856             |                     |                      |
| Ht * Fm                 |                     | 0.207              |                      |                     | 0.463             |                     |                      |
| sem                     |                     | 4.74               |                      |                     | 3.48              |                     |                      |

The mean difference set by Duncan's Multiple Range Test, Ht=harvesting time, Fm=forage mixture, s.em= standard error of the mean, different superscripts within the row and column indicated difference at  $p<0.05$ . sem indicated the Standard error of the mean

### 3.1.2 No. of branches and tillers per plant

The effects of harvesting time and forage mixture on the number of branches in peas and the number of tillers in oat were found significant ( $p<0.005$ ) at all harvests. The highest number of branches was observed in the 75% pea mix with 25% oat (14 branches/plant) at 2<sup>nd</sup> harvest, whilst it was lowest in the 25 % pea mix with 75% oat (4 branches/plant) at 1<sup>st</sup> harvest. Similarly, 100% oat

treatment recorded the highest number of tillers (59) at 2<sup>nd</sup> harvest, whilst it was observed lowest in 50% pea mix with 50% oat (35.38) at 1<sup>st</sup> harvest. The detailed description of no. of branches of pea per plant and tiller no. of oat per plant in various treatment combinations is shown below in Table 2. No significant ( $p<0.05$ ) result was seen in the interaction of harvesting time and forage mixture for no. of branches and tiller no. per plant.

**Table 2.** No. of branches and tiller no. per plant at AFU livestock farm.

| Treatments              | Branches (Pea)      |                     |                     |                     | Tiller (Oat)        |                      |                     |
|-------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|----------------------|---------------------|
|                         | 75%Pea+<br>25%Oat   | 50%Pea+<br>50%Oat   | 25%+Pea+<br>75% Oat | 100%<br>Oat         | 75%Pea<br>+25%Oat   | 50%Pea+<br>50%Oat    | 25%Pea<br>+75%Oat   |
| 1 <sup>st</sup> harvest | 10.5 <sup>ab</sup>  | 6.625 <sup>bc</sup> | 4.38 <sup>c</sup>   | 43.5 <sup>bc</sup>  | 44.63 <sup>bc</sup> | 35.38 <sup>c</sup>   | 54.13 <sup>ab</sup> |
| 2 <sup>nd</sup> harvest | 14.5 <sup>a</sup>   | 12.25 <sup>ab</sup> | 9.25 <sup>abc</sup> | 59 <sup>a</sup>     | 50.25 <sup>ab</sup> | 47.25 <sup>abc</sup> | 54 <sup>ab</sup>    |
| 3 <sup>rd</sup> harvest | 11.88 <sup>ab</sup> | 13.63 <sup>a</sup>  | 12.13 <sup>ab</sup> | 48.8 <sup>abc</sup> | 44.25 <sup>bc</sup> | 42.25 <sup>bc</sup>  | 47 <sup>abc</sup>   |
| p-value                 |                     |                     |                     |                     |                     |                      |                     |
| Ht                      |                     | 0.00198**           |                     |                     | 0.7178              |                      |                     |
| Fm                      |                     | 0.07844             |                     |                     | 0.0385*             |                      |                     |
| Ht * Fm                 |                     | 0.217               |                     |                     | 0.4053              |                      |                     |
| sem                     |                     | 2.85                |                     |                     | 7.75                |                      |                     |

The mean difference set by Duncan's Multiple Range Test, Ht=harvesting time, Fm=forage mixture, sem= standard error of the mean, different superscripts within the row and column indicated difference at  $p<0.05$ .

### 3.2 Stem and root ratio

The effects of harvesting time and forage mixture on the stem and root ratio were found significant ( $p < 0.005$ ) at both harvests. 50% pea mix with 50% oat had the highest stem: root ratio (15.23), whilst 25% oat mix with

75% pea recorded the lowest stem: root ratio (7.34). A detailed description of plant height in various treatment combinations is shown below in Table 3. No significant ( $p < 0.05$ ) result was seen in the interaction of harvesting time and forage mixture for stem and root ratio.

**Table 3.** Stem and root ratio of oat and pea mixture at AFU livestock farm.

| Treatments              | Stem: root Pea     |                    |                    |                    | Stem: root Oat     |                    |                      |
|-------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|----------------------|
|                         | 75%Pea+<br>25%Oat  | 50%Pea+<br>50%Oat  | 25%Pea+<br>75% Oat | 100%<br>Oat        | 75%Pea<br>+25%Oat  | 50%Pea+<br>50%Oat  | 25% Pea +<br>75% Oat |
| 1 <sup>st</sup> harvest | ---                | ---                | ---                | ---                | ---                | ---                | ---                  |
| 2 <sup>nd</sup> harvest | 7.34 <sup>b</sup>  | 8.02 <sup>b</sup>  | 8.19 <sup>b</sup>  | 2.07 <sup>bc</sup> | 1.81 <sup>c</sup>  | 2.89 <sup>ab</sup> | 2.26 <sup>abc</sup>  |
| 3 <sup>rd</sup> harvest | 13.68 <sup>a</sup> | 15.28 <sup>a</sup> | 12.96 <sup>a</sup> | 2.78 <sup>ab</sup> | 2.71 <sup>ab</sup> | 3.01 <sup>a</sup>  | 2.64 <sup>abc</sup>  |
| p-value                 |                    |                    |                    |                    |                    |                    |                      |
| Ht                      |                    | <0.001***          |                    |                    | 0.0118*            |                    |                      |
| Fm                      |                    | 0.521              |                    |                    | 0.0979             |                    |                      |
| Ht * Fm                 |                    | 0.537              |                    |                    | 0.5172             |                    |                      |
| sem                     |                    | 1.99               |                    |                    | 0.47               |                    |                      |

The mean difference set by Duncan's Multiple Range Test, Ht=harvesting time, Fm=forage mixture, s.em= standard error of the mean, different superscripts within the row and column indicated difference at  $p < 0.05$ . sem indicated Standard error of the mean.

### 3.3 Above-ground herbage mass productivity

The harvesting time and forage mixture had a significant effect ( $p < 0.05$ ) on productivity. The highest dry matter productivity was observed as 5.61 tons/ha in 75% pea mix with 25% oat at 3<sup>rd</sup> harvest, followed by 75% oat mix with 25% pea at 3<sup>rd</sup> harvest. The lowest Dry Matter (DM) yield was recorded for 100% oat (1.09 tons/ha),

followed by 50% oat mix with 50% pea at 1<sup>st</sup> harvest. The detail of forage species and their mixture used in this experiment to measure productivity has been prescribed in Table 4. No significant ( $p < 0.05$ ) result was seen in the interaction of harvesting time and forage mixture to the productivity of forage and forage mixture.

**Table 4.** The productivity of oat and pea and their mixture (t/ha)

| Treatments              | 100% Oat          | 75%Pea+25%Oat     | 50%Pea+25%Oat      | 25%Pea+75%Oat      |
|-------------------------|-------------------|-------------------|--------------------|--------------------|
| 1 <sup>st</sup> harvest | 1.09 <sup>f</sup> | 1.67 <sup>f</sup> | 1.43 <sup>f</sup>  | 1.63 <sup>f</sup>  |
| 2 <sup>nd</sup> harvest | 2.64 <sup>c</sup> | 3.28 <sup>d</sup> | 2.86 <sup>de</sup> | 3.12 <sup>de</sup> |
| 3 <sup>rd</sup> harvest | 4.7 <sup>bc</sup> | 5.61 <sup>a</sup> | 4.60 <sup>c</sup>  | 5.22 <sup>ab</sup> |
| p-value                 |                   |                   |                    |                    |
| Ht                      | <0.001***         |                   |                    |                    |
| Fm                      | <0.001***         |                   |                    |                    |
| Ht * fm                 | 0.3274            |                   |                    |                    |
| sem                     | 0.357             |                   |                    |                    |

The mean difference set by Duncan's Multiple Range test, Ht=harvesting time, Fm=forage mixture, different superscripts within the row and column indicated difference at  $p < 0.05$ . sem indicated the Standard error of the mean.

### 3.4 Chemical Composition of forage species and their mixture

#### 3.4.1 Crude protein

The harvesting time and forage mixture had a significant effect ( $p<0.05$ ) on the crude protein content of the herbage. The highest crude protein (CP) content was found in a 50% oat mix with 50% pea (16.73%) at 1<sup>st</sup>

harvest. The trend of CP content was found to be in decline from the first to the third harvest, as expected from the plant maturity point of view. No significant ( $p<0.05$ ) result was seen in the interaction of harvesting time and forage mixture for crude protein content of the forage mixture. The details of the CP content of herbage harvested for legumes and grasses at mix culture have been shown in Table 5.

**Table 5.** CP content of the herbage harvests of oat and pea

| Treatments              | CP                  |                     |                      |                      |
|-------------------------|---------------------|---------------------|----------------------|----------------------|
|                         | 100%Oat             | 75%Pea+<br>25%Oat   | 50%Pea+<br>50%Oat    | 75%Oat+<br>25%Pea    |
| 1 <sup>st</sup> harvest | 16.27 <sup>a</sup>  | 16.14 <sup>a</sup>  | 16.73 <sup>a</sup>   | 15.94 <sup>a</sup>   |
| 2 <sup>nd</sup> harvest | 13.14 <sup>bc</sup> | 12.9 <sup>bcd</sup> | 12.25 <sup>bcd</sup> | 13.4 <sup>b</sup>    |
| 3 <sup>rd</sup> harvest | 11.02 <sup>cd</sup> | 10.96 <sup>cd</sup> | 10.75 <sup>d</sup>   | 12.81 <sup>bcd</sup> |
| p-value                 |                     |                     |                      |                      |
| Ht                      |                     | <0.001***           |                      |                      |
| Fm                      |                     | 0.494               |                      |                      |
| ht × fm                 |                     | 0.237               |                      |                      |
| sem                     |                     | 0.788               |                      |                      |

The mean difference set by Duncan's Multiple Range test, ht=harvesting time, fm=forage mixture, and different superscripts within the row and column indicated a difference at  $p<0.05$ .

sem indicated the Standard error of the mean.

#### 3.4.2 Ether extract

The harvesting time and forage mixture had a significant effect ( $p<0.05$ ) on the chemical composition of forage species and their mixture (table 8). The highest ether extract observed was 3.85% in 50% oat mix with 50% pea at 1<sup>st</sup> harvest, while the lowest ether extract of 2.9% was recorded in 75% oat mix with 25% pea at 3<sup>rd</sup> harvest as shown in Table 8. No significant ( $p<0.05$ ) result was seen in the interaction of harvesting time and forage mixture for the ether extract content of forage and forage mixture.

#### 3.4.3 Total Ash

The harvesting time had a significant effect ( $p<0.005$ ) on the total ash (TA) content of forage species and their mixture (table 6). The highest total ash content observed was 6.20% in 75% oat mix with 25% pea at 3<sup>rd</sup> harvest. This was followed by a 50% oat and 50% pea

combination at the same harvest (5.90%). The lowest total ash content observed was 5.25% in 50% oat & 50% pea at 1<sup>st</sup> harvest (Table 8). No significant ( $p<0.05$ ) result was seen in the interaction of harvesting time and forage mixture for the total ash content of forage and forage mixture.

#### 3.4.4 Neutral Detergent Fiber (NDF)

The harvesting time and forage mixture had a significant effect ( $p<0.05$ ) on Neutral Detergent Fiber (NDF). The highest NDF content was observed as 66.5% in 75% oat mix with 25% pea at 1<sup>st</sup> harvest, while the lowest NDF content recorded was 55.12% in the 50% oat mix with 50% pea at 3<sup>rd</sup> harvest (Table 7). No significant

( $p<0.05$ ) result was seen in the interaction of harvesting time and forage mixture for the NDF content of forage and forage mixture.

**Table 6.** Ether extract and the total ash content of the forage mixture

| Treatments              | %EE                |                     |                     |                    | %TA                |                    |                   |                   |
|-------------------------|--------------------|---------------------|---------------------|--------------------|--------------------|--------------------|-------------------|-------------------|
|                         | 100%Oat            | 75%Pea+<br>25%Oat   | 50%Pea+<br>50%Oat   | 75%Oat+<br>25%Pea  | 100%Oat            | 75%Pea+<br>25%Oat  | 50%Pea+<br>50%Oat | 75%Oat+<br>25%Pea |
| 1 <sup>st</sup> harvest | 3.4 <sup>bc</sup>  | 3.1 <sup>bcd</sup>  | 3.85 <sup>a</sup>   | 3.2 <sup>bcd</sup> | 5.45               | 5.5 <sup>ab</sup>  | 5.25 <sup>b</sup> | 5.35 <sup>b</sup> |
| 2 <sup>nd</sup> harvest | 3.1 <sup>bcd</sup> | 3.35 <sup>bcd</sup> | 3.5 <sup>ab</sup>   | 3.2 <sup>bcd</sup> | 5.8 <sup>ab</sup>  | 5.35 <sup>b</sup>  | 5.35 <sup>b</sup> | 5.5 <sup>ab</sup> |
| 3 <sup>rd</sup> harvest | 2.9 <sup>d</sup>   | 3 <sup>cd</sup>     | 3.05 <sup>bcd</sup> | 2.9 <sup>d</sup>   | 5.75 <sup>ab</sup> | 5.75 <sup>ab</sup> | 5.9 <sup>ab</sup> | 6.2 <sup>a</sup>  |
| p-value                 |                    |                     |                     |                    |                    |                    |                   |                   |
| harvesting              |                    | <0.001              |                     |                    |                    | 0.003              |                   |                   |
| Fm                      |                    | 0.017*              |                     |                    |                    | 0.620              |                   |                   |
| ht × fm                 |                    | 0.113               |                     |                    |                    | 0.431              |                   |                   |
| sem                     |                    | 0.150               |                     |                    |                    | 0.239              |                   |                   |

The mean difference set by Duncan's Multiple Range tests, ht=harvesting time, fm=forage mixture, and different superscripts within the row and column indicated a difference at  $p<0.05$ . sem indicated the Standard error of the mean.

**Table 7.** The NDF content of the herbage harvests of oat and pea grown at AFU livestock farm

| Treatments              | % NDF               |                     |                     |                     |
|-------------------------|---------------------|---------------------|---------------------|---------------------|
|                         | 100%Oat             | 75%Pea+25%Oat       | 50%Pea+50%Oat       | 75%Oat+25%Pea       |
| 1 <sup>st</sup> harvest | 65.58 <sup>ab</sup> | 65.49 <sup>ab</sup> | 61.92 <sup>ab</sup> | 66.5 <sup>a</sup>   |
| 2 <sup>nd</sup> harvest | 60.22 <sup>ab</sup> | 62.4 <sup>ab</sup>  | 61.41 <sup>ab</sup> | 66.35 <sup>a</sup>  |
| 3 <sup>rd</sup> harvest | 61.80 <sup>ab</sup> | 64.15 <sup>ab</sup> | 55.12 <sup>b</sup>  | 58.95 <sup>ab</sup> |
| p-value                 |                     |                     |                     |                     |
| Ht                      |                     | 0.0309              |                     |                     |
| Fm                      |                     | 0.2308              |                     |                     |
| ht * fm                 |                     | 0.6983              |                     |                     |
| sem                     |                     | 3.79                |                     |                     |

The mean difference set by Duncan's Multiple Range tests, ht=harvesting time, fm=forage mixture, and different superscripts within the row and column indicated a difference at  $p<0.05$ . sem indicated the Standard error of the mean.

### 3.4.5 Acid Detergent Fiber (ADF)

The harvesting time and forage mixture showed no significant effect ( $p<0.05$ ) on Acid Detergent Fiber (ADF). The monoculture of oat had the highest ADF content (51.29%) at 1<sup>st</sup> harvest, whereas 25% oat

mix with 75% pea recorded the lowest ADF content (46.46%) at 1<sup>st</sup> harvest (Table 8). No significant ( $p<0.05$ ) result was seen in the interaction of harvesting time and forage mixture for the ADF content of forage and forage mixture.

### 3.4.6 Acid Detergent Lignin (ADL)

The harvesting time and forage mixture had a non-significant effect ( $p < 0.05$ ) on Acid Detergent Lignin (ADL) content. 100 % oat recorded the highest ADL content (27.2%) at 1<sup>st</sup> harvest, while the lowest ADL content (23.13%) was recorded in 25% peas with 75% oat at 3<sup>rd</sup> harvest (Table 8).

### 3.4.7 Hemicellulose

The harvesting time and forage mixture had a non-significant effect ( $p < 0.05$ ) on hemicellulose content. The maximum hemicellulose content (19.93%) was recorded in 75% oat mix with 25% pea at 1<sup>st</sup> harvest, while the

minimum hemicellulose content (8.42%) was observed in 50% oat mix with 50% pea at 3<sup>rd</sup> harvest. No significant ( $p < 0.05$ ) result was seen in the interaction of harvesting time and forage mixture for the hemicellulose of forage and forage mixture (Table 8).

### 3.4.8 Cellulose

The non-significant effect ( $p < 0.05$ ) of harvesting time and forage mixture was seen on the cellulose content. The maximum cellulose content (24.89%) was recorded in 75% oat mix with 25% pea at 3<sup>rd</sup> harvest, while the minimum cellulose content (22.24%) was observed in 50% oat mix with 50% pea at 1<sup>st</sup> harvest (Table 8).

**Table 8.** ADL, ADF, HC and Cell content (%) of the herbage harvests of oat and pea grown at AFU Livestock Farm.

| Treatment               | ADL                |                    |                    |                    | ADF                |                    |                    |                    | HC                 |                    |                    |                    | CEL                |                    |                    |                    |
|-------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|                         | 100%<br>oat        | 75%                | 50%                | 25%                | 100%<br>oat        | 75%                | 50%                | 25%                | 100%<br>oat        | 75%                | 50%                | 25%                | 100%<br>oat        | 75%                | 50%                | 25%                |
|                         |                    | pea                | pea +              | pea +              |                    | pea                | pea +              | pea +              |                    | pea                | pea +              | pea +              |                    | pea                | pea +              | pea +              |
|                         |                    | +25%<br>Oat        | 50%<br>0at         | 75%<br>oat         |                    | +25%<br>Oat        | 50%<br>0at         | 75%<br>oat         |                    | +25%<br>Oat        | 50%<br>0at         | 75%<br>oat         |                    | +25%<br>Oat        | 50%<br>0at         | 75%<br>oat         |
| 1 <sup>st</sup> harvest | 27.20 <sup>a</sup> | 23.85 <sup>a</sup> | 25.73 <sup>a</sup> | 23.3 <sup>a</sup>  | 51.29 <sup>a</sup> | 46.46 <sup>a</sup> | 47.97 <sup>a</sup> | 46.57 <sup>a</sup> | 14.29 <sup>a</sup> | 19.03 <sup>a</sup> | 13.9 <sup>a</sup>  | 19.93 <sup>a</sup> | 24.08 <sup>a</sup> | 23.10 <sup>a</sup> | 22.24 <sup>a</sup> | 23.27 <sup>a</sup> |
| 2 <sup>nd</sup> harvest | 26.06 <sup>a</sup> | 24.14 <sup>a</sup> | 26.41 <sup>a</sup> | 25.88 <sup>a</sup> | 48.31 <sup>a</sup> | 47.86 <sup>a</sup> | 50.31 <sup>a</sup> | 50.57 <sup>a</sup> | 11.9 <sup>a</sup>  | 14.53 <sup>a</sup> | 11.09 <sup>a</sup> | 15.78 <sup>a</sup> | 22.25 <sup>a</sup> | 23.72 <sup>a</sup> | 23.89 <sup>a</sup> | 24.68 <sup>a</sup> |
| 3 <sup>rd</sup> harvest | 26.46 <sup>a</sup> | 26.76 <sup>a</sup> | 22.92 <sup>a</sup> | 23.13 <sup>a</sup> | 51.07 <sup>a</sup> | 50.15 <sup>a</sup> | 46.69 <sup>a</sup> | 48.03 <sup>a</sup> | 10.72 <sup>a</sup> | 13.99 <sup>a</sup> | 8.42 <sup>a</sup>  | 10.97 <sup>a</sup> | 24.6 <sup>a</sup>  | 23.39 <sup>a</sup> | 23.7 <sup>a</sup>  | 24.89 <sup>a</sup> |
| <i>p</i> -value         |                    |                    |                    |                    |                    |                    |                    |                    |                    |                    |                    |                    |                    |                    |                    |                    |
| ht                      | 0.951              |                    | 0.720              |                    |                    |                    | 0.0124             |                    |                    | 0.6251             |                    |                    |                    |                    |                    |                    |
| fm                      | 0.381              |                    | 0.613              |                    |                    |                    | 0.161              |                    |                    | 0.973              |                    |                    |                    |                    |                    |                    |
| ht×fm                   | 0.375              |                    | 0.773              |                    |                    |                    | 0.8136             |                    |                    | 0.993              |                    |                    |                    |                    |                    |                    |
| sem                     | 2.29               |                    | 3.36               |                    |                    |                    | 3.39               |                    |                    | 3.72               |                    |                    |                    |                    |                    |                    |

Mean difference set by Duncan's multiple range test, ht=harvesting time, fm=forage mixture, NDF= Neutral Detergent Fibre, ADF=Acid Detergent Fibre, ADL=Acid Detergent Lignin, CEL= Cellulose(ADF-ADL), HC=Hemicellulose (NDF-ADF). Different superscripts within the row and column indicated a difference at  $p < 0.05$ . sem indicated Standard error of the mean.

### 3.4.9 Calcium

The harvesting time and forage mixture had a non-significant effect ( $p < 0.05$ ) on the calcium content. Fifty percent (50%) oat mix with 50% pea recorded the highest calcium content (0.81%) at 1<sup>st</sup> harvest, while the lowest calcium content was recorded in the 25% oat with 75% pea at 1<sup>st</sup> harvest (Table 9).

### 3.4.10 Phosphorus

The harvesting time and forage mixture had a non-significant effect ( $p < 0.05$ ) on the phosphorus content, 25% oat mix with 75% pea recorded the highest phosphorus content (0.24%) in 2<sup>nd</sup> harvest, while the lowest phosphorus content was recorded in 50% oat with 50% pea at 3<sup>rd</sup> harvest. No significant ( $p < 0.05$ ) result was seen in the interaction of harvesting time and forage mixture for the phosphorus of forage and forage mixture (Table 9).

## 4. DISCUSSION

### 4.1 Morphological attributes of mixed grass-legume forage

In the study, it was found that the heights of peas and oat were found significant in all harvests. The maximum height of pea & oat was observed in 75% pea mix with 25% oat at 3<sup>rd</sup> harvest. The maximum height gives more yield biomass per unit area. Jaballa (1995) also reported that intercropped treatments gave higher biomass yield per unit area than sole crops. Our results were in close agreement with Buyukburc *et al.* (1989) and Hatipoglu *et al.* (1990) in terms of herbage mass production.

However, some studies have indicated that the legume ratio increased gradually with the growth stage in the mixture (Acikgoz and Cakmakci, 1986; Tukul and Yilmaz, 1987; Tan and Serin, 1996). The research results further indicated that several branches in peas and the number of tillers in oat were found significant at all harvests. The highest number of branches in pea were observed in treatment with 75% pea mix with 25% oat at 2<sup>nd</sup> harvest & highest number of tillers were recorded in treatment with 100% oat at 2<sup>nd</sup> harvest. The increase in the number of branches also attributes to the higher yield in biomass.

**Table 9.** Ca and P content of the herbage harvests of oat and pea

| Treatments              | Ca                |                   |                   |                   | P                 |                   |                   |                   |
|-------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                         | 100%              | 75%Pea+           | 50%Pea+           | 75%Oat+           | 100%              | 75%Pea+           | 50%Pea+           | 75%Oat+           |
|                         | Oat               | 25%Oat            | 50%Oat            | 25%Pea            | Oat               | 25%Oat            | 50%Oat            | 25%Pea            |
| 1 <sup>st</sup> harvest | 0.61 <sup>a</sup> | 0.39 <sup>a</sup> | 0.81 <sup>a</sup> | 0.68 <sup>a</sup> | 0.15 <sup>a</sup> | 0.2 <sup>a</sup>  | 0.13 <sup>a</sup> | 0.08 <sup>a</sup> |
| 2 <sup>nd</sup> harvest | 0.46 <sup>a</sup> | 0.61 <sup>a</sup> | 0.71 <sup>a</sup> | 0.58 <sup>a</sup> | 0.06 <sup>a</sup> | 0.10 <sup>a</sup> | 0.22 <sup>a</sup> | 0.24 <sup>a</sup> |
| 3 <sup>rd</sup> harvest | 0.45 <sup>a</sup> | 0.55 <sup>a</sup> | 0.56 <sup>a</sup> | 0.5 <sup>a</sup>  | 0.15 <sup>a</sup> | 0.21 <sup>a</sup> | 0.05 <sup>a</sup> | 0.1 <sup>a</sup>  |
| p-value                 |                   |                   |                   |                   |                   |                   |                   |                   |
| Ht                      |                   | 0.343             |                   |                   |                   | 0.815             |                   |                   |
| Fm                      |                   | 0.45              |                   |                   |                   | 0.817             |                   |                   |
| ht * fm                 |                   | 0.58              |                   |                   |                   | 0.854             |                   |                   |
| sem                     |                   | 0.18              |                   |                   |                   | 0.058             |                   |                   |

Mean difference set by Duncan's Multiple Range test, ht=harvesting time, fm=forage mixture, different superscripts within the row and column indicated difference at  $p < 0.05$ .

### 4.2 Above-ground herbage mass productivity

In the present study, it was found that the harvesting time and forage mixture had a significant effect on dry matter productivity. The highest dry matter productivity was observed in the treatment of 75% pea mix with 25% oat at 3<sup>rd</sup> harvest, followed by 75% oat mix with 25% pea at 3<sup>rd</sup> harvest. Several studies resemble our research that the dry matter yield increased with the increasing rate of oat in mixtures of oat with annual legumes (Walton, 1975; Osman and Nersoyan, 1985; Droushiotis, 1989). Furthermore, Mitchell (1983) indicated that the oat physically supported the pea plants in such mixtures and provided most of the dry matter production. As expected, there was an increase in the dry matter yield due to the increasing dry matter production of the plants with the delay of cutting stages. Similarly, in comparison to pea mixed with oat and pea monoculture, the oat-pea mixture dry matter yield was

higher than pea monoculture at all harvests.

### 4.3 Chemical composition of forage species and their mixture

The crude protein content is one of the very important criteria in forage quality evaluation (Geleti, 2000; Lithourgidis *et al.*, 2006). As the growth stage of plants progresses, the crude protein ratio in the plant decreases, but the dry matter yield increases. Hence, the crude protein yields increased depending on the growth stage (Acikgoz and Cakmakci, 1986; Garnsworthy and Stokes, 1993; Tan and Serin, 1996).

The significantly highest amount of CP content was found in the treatment of 50% oat mix with 50% pea at 1<sup>st</sup> harvest in the present study. The result is almost similar to the findings of the study that confirms when interactions of the mixture, the highest crude protein yield (1.91 t ha<sup>-1</sup>) was determined at the first cutting

stages of the 50% pea + 50% oat mixtures (Acikgoz and Cakmakci, 1986; Garnsworthy and Stokes, 1993; Tan and Serin, 1996). Uzun & Asik, 2012 also indicated that CP content increased as the percentage of peas in mixtures increased because peas had higher CP content. The highest ether extract observed in treatment was 50% oat mix with 50% pea at 1<sup>st</sup> harvest, while the lowest ether extract was in treatment with 75% oat mix with 25% pea at 3<sup>rd</sup> harvest. The highest total ash content was observed in the treatment of 75% oat mix with 25% pea at 3<sup>rd</sup> harvest. This was followed by a 50% oat and 50% pea combination at the same harvest (5.90%). The lowest total ash content was observed in oat monoculture at 1<sup>st</sup> and 2<sup>nd</sup> harvest.

The total ash content was found higher in the oat-pea mixture than oat alone. The ash content is the concentration of minerals in the mix forages most probably due to the higher concentration of minerals in legumes (peas) as expected (Assefa & Ledin, 2001). Composition of Fibrous residues; the highest NDF content was observed as 66.5% in 75% oat mix with 25% pea at the first harvest. It is most probable that the forage quality is associated with the progressing stage of maturity and that comes early to grasses species that in legumes as has been shown from the almost unchanged. Adding pea to oat or barley not only increases forage CP concentration but also decreases NDF and acid detergent fibre (ADF) (Brundage and Klebesadel, 1969; Cole, 1989). The soil's physical and chemical properties might have attenuated such tendency which however was not measured in the present study.

Twenty-five per cent (25%) oat mix with 75% pea recorded the highest phosphorus content (0.25%) in 2<sup>nd</sup> harvest, while the lowest phosphorus content was

recorded in 50% oat with 50% pea at 3<sup>rd</sup> harvest. The result is similar to that of McDonald *et al.* (2002) also reported that mineral concentration declines with age and is also influenced by soil type, soil nutrient levels and seasonal conditions. The concentration of minerals in forages may be induced by factors like varieties of plant developmental stages, morphological fractions, climatic conditions, soil characteristics and fertilization (Gezahegn *et al.*, 2014).

## 5. CONCLUSION

The present study's findings indicated that pea-oat mix cropping had the potential to increase herbage productivity in subtropical locations and is expected to be crucial in the livestock industry's ability to supply quality and quantity of feed. Oats and peas together could serve as a viable intercropping model to increase forage DM yield and nutritional content, which would help to alleviate the DM scarcity problem and enhance the quality of fodder. The findings of the current study demonstrated that intercropping peas and oats could increase herbage yield in subtropical regions and decrease bulkiness at a later stage of harvest. This study recommends 50% oats + 50% pea and 75% pea + 25% oat-based on production, quality, and nutrient for use by farmers in the subtropical zone and other regions with similar agro-ecological conditions in winter with minimal tillage and irrigation for mass output with better CP content. For more precise advice, additional evaluation of the oats-pea variety mixes' performance over time, over the hills and terai, and on abandoned lands is essential. It is necessary to specify the best practices for managing the grass-legume species in unused land, including the selection of grass and legume species, sowing rates, and sowing times.

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# TRICHODERMA: ITS ECOPHYSIOLOGY, MECHANISM OF BIOCONTROL AND APPLICATION METHODS

Pratistha Adhikari <sup>1</sup>

<sup>1</sup> Agriculture and Forestry University, Rampur, Chitwan, Nepal

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\*Correspondence:  
padhikari@afu.edu.np  
Tel: +977-9845392625

## ABSTRACT

Chemically managed plants impose environmental risks to humans and environment. Using biological approaches to control plant diseases is a more effective and environment friendly alternative. The biological approach to plant disease refers to controlling disease by using organisms like fungi, bacteria and viruses. This can be done by introduction or utilization of resident antagonistic living organisms. Biological control can be achieved through different forms of interactions between organisms and the interactions includes hyperparasitism, antibiosis, commensalism, neutralism, and competition. Various biocontrol agents commonly used against plant pathogens are *Trichoderma harzianum*, *Trichoderma hamatum*, *Trichoderma viride*, *Trichoderma koningii*, *Gliocladium virens*, *Gliocladium roseum*, *Paecilomyces liacinus*, *Coniothyrium minitans*, *Bacillus subtilis*, *Bacillus polymyxa*, and *Pseudomonas fluorescens*.

## 1. INTRODUCTION

Chemically managed plants impose environmental risks to humans and environment. Using biological approaches to control plant diseases is a more effective and environment friendly alternative. The biological approach to plant disease refers to controlling disease by using organisms like fungi, bacteria and viruses (O'Brien, 2017). This can be done by introduction or utilization of resident antagonistic living organisms. Biological control can be achieved through different forms of interactions between organisms and the interactions includes hyperparasitism, antibiosis, commensalism, neutralism, and competition. Various biocontrol agents commonly used against plant pathogens are *Trichoderma harzianum*, *Trichoderma hamatum*, *Trichoderma viride*, *Trichoderma koningii*, *Gliocladium virens*, *Gliocladium roseum*, *Paecilomyces liacinus*, *Coniothyrium minitans*, *Bacillus subtilis*, *Bacillus polymyxa*, and *Pseudomonas fluorescens* (Tyśkiewicz *et al.*, 2022).

The most important genera against soil pathogens are *Trichoderma*, *Pseudomonas* and *Bacillus* (Guo *et al.*, 2004; Huang *et al.*, 2015). *Trichoderma* is found to be an effective biocontrol agent especially for soil borne

pathogens like *Rhizoctonia solani*, *Sclerotium rolfsii*, *Phythium aphanidermatum*, *Fusarium oxysporum*, and *Gaeumannomyces graminis* under both field and greenhouse conditions (Chet & Inbar, 1994; Basim *et al.*, 1999). The *Trichoderma* spp. have a positive effect on plant growth through hydrolysis of cellulose in soil, increases plant defense mechanism, mineral solubilization and improvement in root morphology, enabling roots to cover large volume of soil (Junaid *et al.*, 2013; Timila *et al.*, 2015).

## 2. TRICHODERMA: ITS BIOCONTROL POTENTIAL AND ECOPHYSIOLOGY

*Trichoderma* spp., the promising antagonistic fungi are established in agriculture use. These are classified as an anamorphic Hypocreals, belonging to Ascomycetes (Esposito & Da Silva, 1998). Conidiophores are highly branched and produce lateral side branches that may be paired or not. Phialides are formed on the conidiophore main axis or at the tip. The conidiophore with paired branches takes a shape of pyramid in some species (Samuels & Hebbbar, 2015). Watanabe (1985) found *T. hamatum*, *T. harzianum*, *T. koningii*, *T. pseudokoningii*

and *T. viride* having strong antagonistic potential against soil borne pathogens. They show antagonistic activity under both *in vitro* and *in vivo* conditions by competing for nutrients and space, antibiosis, mycoparasitism, promoting plant growth and plant defense responses (Brotman *et al.*, 2010). Seema and Devaki (2012) reported antagonistic effects of *T. harzianum* and *T. viride* and found significant suppression of mycelial growth and sclerotia formation of pathogen, *R. solani*. Similarly, the seedling mortality of groundnut was also significantly reduced by seed treatment with various isolates of *Trichoderma* spp. (Biswas & Sen, 2000). Freeman *et al.* (2004) reported various isolates of *Trichoderma*, including *T. harzianum* isolate T-39 significantly reduced anthracnose (*Colletotrichum acutatum*) and grey mould (*Botrytis cinerea*) in strawberry under greenhouse and *in vitro* conditions. Pastrana *et al.* (2016) reported preventive and curative application of *T. asperellum* totally avoided incidence of crown and root rot caused by *F. solani* at the same level as application of carbendazim. Similarly, incidence of charcoal rot caused by *M. phaseolina* reduced up to 65% under field conditions and up to 44% in a growth chamber with application of *Trichoderma*. Amin *et al.* (2010) tested six isolates of *Trichoderma* against three different soil borne pathogens namely *R. solani* (isolates from tomato), *Sclerotinia sclerotiorum* (causing web blight of beans) and *S. rolfsii* (causing collar rot of tomato) under *in vitro* conditions and found that maximum inhibition (71.41%) in *R. solani* by *T. viride* (Tv-2) followed by *T. viride* (Tv-1) and *T. harzianum* (Th-1). *T. viride* (Tv-1) showed best antagonist inhibiting 67.91 and 66.21% over control in *S. rolfsii* and *S. sclerotiorum*, respectively. They also found that all the *Trichoderma* isolates significantly inhibited sclerotia production in all three tested pathogens. *Trichoderma* is also effective against Stemphylium blight of lentil. Subedi *et al.* (2014) reported percentage disease control and percent yield increase were higher in *T. viride* i.e. 42.14% and 58.80% respectively. *T. viride* also showed high compatibility with fungicides (potassium phosphonate and fosetyl aluminium) and incompatibility with fungicides (carbendazim, hexaconazole, potassium phosphonate + hexaconazole mixture and captan + hexaconazole mixture) (Dhanya *et al.*, 2016).

### 2.1 Isolation from soil

In 1981, Papavizas used V-8 juice agar as the basal medium for isolation of *Trichoderma* from soil (Papavizas, 1982). Later Papavizas and Lumsden,

(1982) developed *Trichoderma* medium E (TME) for isolation of *Trichoderma* from soil. Elad *et al.* (1981) developed a *Trichoderma* selective medium (TSM) for isolation of *Trichoderma* from soil. They used chloramphenicol, pentachloronitrobenzene, p-dimethylaminobenzenediazo, sodium sulfonate and rose-bengal as selective fungal inhibitors. Elad and Chet (1983) further improved TSM medium by adding Captan 50% 2µl/ml which helped to avoid *Fusarium* species. Later, Askew and Laing (1993) modified TSM by adding metalaxyl which suppressed oomycete. However, Williams *et al.* (2003) found captan, was inhibitory to conidial germination of *T. harzianum*.

### 2.2 Survival in the soil

*Trichoderma* can abundantly proliferate in various soils and can degrade various organic matters (Papavizas, 1982; Lewis & Papavizas, 1984). They observe that survival of chlamydospore was better than conidia in soil. Lewis and Papavizas (1984) reported that even freshly added conidia declined in the soil and the stable population densities of *Trichoderma* remained is due to survival of chlamydospores. According to Kim *et al.* (1992) with increase in soil depth, the rhizosphere colonizing ability of propagules get reduced. According to Poosapati *et al.* (2014) to adapt to extreme conditions such as high temperatures, some *Trichoderma* produce trehalose, mannose, and raffinose. These sugars are stress protectant and thus *Trichoderma* are able to grow well up to 37-40°C. Some *Trichoderma* spp. are reported to grow in extreme high or low pH, low oxygen (Chovanec *et al.*, 2005) and high salinity conditions (Gal-Hemed *et al.*, 2011).

### 2.3 Establishment and proliferation in the soil or rhizosphere

The plant root exudates various organic compounds that affect ecological and biological processes in rhizosphere (Barman *et al.*, 2021). Several *Trichoderma* species compete with pathogens for nutrients, space and infection sites on plant roots (Błaszczuk *et al.*, 2014). The effectiveness of *Trichoderma* is affected by their biocontrol qualities and their competency in the rhizosphere and plant root colonization (Barman *et al.*, 2021). *Trichoderma* introduced on seed must multiply first in the rhizosphere of the host plant to inhibit the pathogen. Biswas and Sen (2000) reported seed treatment with conidial suspension was more effective in reducing disease incidence than soil application. Juliatti *et al.* (2019) stated that seed microbiolization is an important method of application of biocontrol agents

since it requires significantly less amount of biological material as compared to the quantity needed for soil application.

## 2.4 Environmental effects on growth of *Trichoderma*

### 2.4.1 Effect of soil type

*Trichoderma* species are affected by soil pH, moisture and electrical conductivity. According to Wong *et al.* (2002); Singh *et al.* (1998) moist soil conditions is favorable for the antagonistic activity and growth of *Trichoderma*. Similarly, *Trichoderma* spp. are favored by the acidic soils condition. Amir-Ahmadi *et al.* (2017) loam and clay loam reported the best performance of *T. harzianum* in sandy loam soil and loam soil containing 2% organic matter. Soil texture affect the ability of antagonistic fungi to suppress the plant pathogens (Moosavi & Zare, 2015).

### 2.4.2 Effect of temperature

Temperature is among the important parameters that affect the biomass production of *Trichoderma*. According to Sharma *et al.* (2005) incubation temperature had profound effect on growth of *Trichoderma*. Domingues *et al.* (2016) isolates of *Trichoderma asperellum* (IBLF 897, IBLF 904 and IBLF 914) found that the mycelium of *Trichoderma* grew well at 27 to 32°C and inhibited at the temperature of 7°C and 42°C. Carro-Huerga *et al.* (2021); Adhikari *et al.* (2022) observed that the studied *Trichoderma* strains grew well at 25°C while, some strains showed good response at 35-40°C as well. Di Lelio *et al.* (2021) observed different growth for *T. afroharzianum* and *T. atroviride* at 20 and 25°C, both in *in vitro* and *in vivo* conditions. The lower temperature i.e. 20°C encouraged *T. afroharzianum* growth, whereas *T. atroviride* was abundant at 25°C. According to Klein and Eveleigh (2002); Carro-Huerga *et al.* (2021) the optimum growth temperature for most *Trichoderma* spp. is in the range between 25-30°C and thus are mesophilic in nature. Poosapati *et al.* (2014) observed *T. asperellum*, and *T. harzianum* survived the heat stress, however, with increase of temperature to 37°C, the germination rates of all isolates reduced. The tolerance of *Trichoderma* towards high temperature can be attributed to its ability to accumulate stress protectants. Under stress conditions accumulation of trehalose, mannose and raffinose increase in the cells exposed to stress conditions (Poosapati *et al.*, 2014). Under heat stress condition, accumulation of trehalose and mannitol are reported to be necessary for fungal survival and cell protein and structure stability (Daryaei

*et al.*, 2016) trehalose is accumulated in response to a heat shock or to an oxidative shock. The authors have characterized the *A. nidulans* tpsA gene encoding trehalose-6-phosphate synthase, which catalyses the first step in trehalose biosynthesis. Expression of tpsA in a *Saccharomyces cerevisiae* tps1 mutant revealed that the tpsA gene product is a functional equivalent of the yeast Tps1 trehalose-6-phosphate synthase. The *A. nidulans* tpsA-null mutant does not produce trehalose during conidiation or in response to various stress conditions. While germlings of the tpsA mutant show an increased sensitivity to moderate stress conditions (growth at 45 °C or in the presence of 2 mM H<sub>2</sub>O<sub>2</sub>).

## 3. MODE OF ACTION BY *TRICHODERMA*

*Trichoderma* spp. are free-living, commonly growing fungi in soil and rhizosphere of various crops (Harman *et al.*, 2004). *Trichoderma* can control plant pathogens directly by competition for nutrients, mycoparasitism, production of cell wall degrading enzymes, and production of antibiotics (Harman, 2006) and indirectly by stimulation of plant defense systems (Benítez *et al.*, 2004).

### 3.1. Direct mechanism

#### 3.1.1 Competition for nutrients and space

According to Chet (1987) competition between antagonist and plant pathogen for space and nutrients is a classical mechanism of biological control. One of the most common causes of death of microorganisms are due to starvation. *Trichoderma* are competent for getting limited nutrients resulting in control of fungal plant pathogens (Benítez *et al.*, 2004). Generally, filamentous fungi require iron for spore viability and under iron starvation condition, low-molecular-weight ferric-iron specific chelators are excreted to mobilize environmental iron (Eisendle *et al.*, 2004). These ferric-iron specific chelators are known as siderophores. Some *Trichoderma* species produce high amount of effective siderophores that chelate iron and absorb them causing starvation of iron to other fungi and stopping their growth (Benítez *et al.*, 2004). Based on the chemical nature siderophores are divided into three major groups i.e hydroxamate, catecholate, and carboxylate (Tyśkiewicz *et al.*, 2022).

*Trichoderma* are proficient in mobilization of immobile nutrients and their utilization (Singh *et al.*, 2018). Root exude excess amount of sugar, amino acids, iron, vitamins, organic acids and *Trichoderma* compete for these carbon source with other fungi such as *R. solani*

and *F. oxysporum* (Sarrocco *et al.*, 2009). *Trichoderma* can displace pathogens from a common habitat such as plant tissues, rhizospheres, and phyllospheres by colonizing them (Ghorbanpour *et al.*, 2018). The degree of colonization of the host plant by the biocontrol agents depend upon their adaptation to the environmental conditions in which they live and plant colonization strategies (Ghorbanpour *et al.*, 2018). Rapid growth and proliferation of *Trichoderma* on substrate determine their aggressiveness (Tyśkiewicz *et al.*, 2022). The strain quick in colonizing substrate can quickly eliminate slow growing pathogens (Oszust *et al.*, 2020).

### 3.1.2 Mycoparasitism and cell wall degrading enzymes

*Trichoderma* is a mycoparasite and has ability to parasitize other fungi such as *R. solani*, *S. rolfsii*, *S. sclerotiorum* etc. The mycoparasitic activity of *Trichoderma* on fungal pathogens starts with prey sensing and growing toward them, adhesion to the host, and intense branching and coiling around them. Also, they can form appressoria-like structure for penetration of the host cells (Moreno-Ruiz *et al.*, 2020; Mukherjee *et al.*, 2012). After penetration of the host mycelium, *Trichoderma* utilize the intracellular contents of the host (Saba *et al.*, 2012). *Trichoderma* species when come in contact with the pathogen cell wall, are triggered to produce many chitinolytic enzymes such as endochitinases, 1,4- $\beta$ -acetylglucosaminidases, and exochitinases (Benítez *et al.*, 2004).  $\beta$  1,3 glucanases produced by *Trichoderma* hydrolyse  $\beta$  1,3 glucan present in cell wall of fungal pathogens (Druzhinina *et al.*, 2011). According to Vinale *et al.* (2008) volatile secondary metabolites produced by *Trichoderma* species also play a key role in mycoparasitism of fungal pathogens. Contreras-Cornejo *et al.* (2016) reported *T. atroviride* produced 6-pentyl- $\alpha$ -pyrone and *T. virens* produced mono- and sesquiterpenes with antimicrobial functions. According to Karlsson *et al.* (2017) lectins from the fungus cell wall and release of secondary metabolites play important roles in recognition and signaling pathways such as MAPK and cAMP pathway in *Trichoderma*. After recognition of hosts, expression of molecular weapons involved in host lysis and parasitization occur. During mycoparasitism genes families such as *ech42* and *prb1* are upregulated in *Trichoderma* (Köhl *et al.*, 2019). Nature of antagonism by *Trichoderma* species also dependent upon target pathogen. Against *R. solani*, parasitism is more common mode of antagonism while, against *Fusarium*, antibiosis is the predominant one.

### 3.1.3 Antibiosis

The antagonistic organisms release low molecular weight diffusible secondary metabolites and antifungal antibiotics that are detrimental for the pathogen and inhibit their growth. Majority of *Trichoderma* species produce different types of volatile and nonvolatile toxic such as harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-pentyl- $\alpha$ -pyrone, massoialactone, viridin, gliovirin, glisoprenins and heptelidic acid (Benítez *et al.*, 2004). In general, antibiotic production is directly correlated with biocontrol ability of *Trichoderma* strains. According to Shi *et al.* (2012) *T. pseudokoningii* SMF2, exhibited antibiotic activities by producing TrichokoninsVI, a type of peptaibol causing extensive programmed cell death in fungal pathogens. Siddiquee (2017) found *T. harzianum* synthesized trichorzins, harzianins, trichotoxin, and trichokindins. Several *Trichoderma* species are also capable of producing polyketides, tetracyclines, macrolides and mycotoxins (Zeilinger *et al.*, 2015) terpenoids, pyrones and anthraquinones (Siddiquee, 2014). However, a particular antibiotic may play important role in antibiosis of one strain and another strain may not even produce it.

### 3.1.4 Sclerotial parasitization

The soil borne pathogens *R. solani*, *S. rolfsii* and *S. sclerotiorum*, have the ability to survive in soil for prolonged duration due to formation of sclerotia, a resting structure. These sclerotia play important role in disease cycle of the pathogen. *Trichoderma* species have ability to parasitize the mycelium and sclerotia of these soil borne plant pathogens. Papavizas and Lewis (1989); Bhagat and Pan (2011); Sarrocco *et al.* (2006); Adhikari *et al.* (2022) reported that the different strains of *Trichoderma* spp. varied in their ability to colonize the sclerotia of *S. rolfsii*. According to Sarrocco *et al.* (2006); Köhl *et al.* (2019), some *Trichoderma* species are able to penetrate the rind and colonize the inner cell layers of sclerotia, leading to destroying and leaving them not viable. Rawat and Tewari (2010) reported *T. harzianum* parasitization on sclerotia of *S. rolfsii* caused deformation, lysis and degradation and disappearance of cytoplasmic granules of cell wall causing loss of cellular integrity in sclerotia. Sarrocco *et al.* (2006) reported the uniformly distribution of *T. virens* mycelium beneath the rind of sclerotia but not in cortex and medulla. *Trichoderma* entered the sclerotia without any preferential point and caused infection. *Trichoderma* can induce enzymatic degradation of rind

walls and degrade melanin of the sclerotia (Sarrocchio *et al.*, 2006). Liu *et al.* (2009) found the uniform distribution of *T. virens* mycelium in inner and outer layer of sclerotia as intercellular fungal growth. Tsahouridou and Thanassouloupoulos (2001) observed the presence of hyphae, conidia and chlamydospores of *T. koningii* in the medullar tissues of the sclerotia. Ibarra-Medina *et al.* (2010) reported deformations, collapsing, cracking and increment or diminishing sclerotia size of sclerotia by different strains of *Trichoderma*. According to Butler *et al.* (2005) among different mechanism of action in biocontrol, destruction of the melanin or inhibition of its synthesis is an important one.

### 3.2 Indirect action of biocontrol agents

#### 3.2.1 Induction of resistance in plants

Plants have ability to protect themselves from range of deleterious microorganisms. They are enable to recognize the invaders and produce an arsenal of antimicrobial compounds and thereby reduce the impact of pathogens invasion (Djonović *et al.*, 2007). Induction of defense response is one of the most effective resistance mechanisms and are activated only upon pathogen invasion. The induction of plant resistance to pathogens are the consequences of the action of various elicitors released from the microorganisms cells (Tyśkiewicz *et al.*, 2022). *Trichoderma* strains have ability to induce plant defense response and systemic resistance in plant. During plant - *Trichoderma* interaction, *Trichoderma* release numerous elicitors which induce salicylic acid (SA), jasmonic acid (JA) or reactive oxygen species (ROS) mediated signals in plant triggering defense proteins expression (Nawrocka & Małolepsza, 2013). After gene activation, the plant produces different enzymes that suppress the pathogen and enhance biochemical and structural barriers against the intruders (Nawrocka & Małolepsza, 2013). Perazzolli *et al.* (2011) showed that *Trichoderma* strain, T39 reduced downy mildew severity on susceptible grapevines by a direct modulation of defense-related genes and enhanced their expression after pathogen inoculation. They suggested that jasmonic acid and ethylene signals were induced by T39. Vinale *et al.* (2008) reported plant defence mechanisms were activated and plant growth in pea, tomato and canola were regulated when harzianolide, 6-pentyl-a-pyrone, and harzianopyridone were applied. Alizadeh *et al.* (2013) reported *T. harzianum* Tr6, elicit induced resistance in cucumber against *F. oxysporum* f.sp. *radicis cucumerinum* and in *A. thaliana* against *B. cinerea*. Plants immune system are trigger via

microbe-associated molecular patterns (MAMPs) when *Trichoderma* colonize root of plants (Hermosa *et al.*, 2012). According to Guo *et al.* (2021) *T. asperellum* ACCC30536 secreted xylanase that stimulated the systemic resistance in *Populus davidiana* and *P. alba* var. *pyramidalis* Louche seedlings against *Alternaria alternata*, *R. solani*, and *F. oxysporum*.

#### 3.2.2 Growth promotion

*Trichoderma* spp. are hyperparasite of other fungi as well as avirulent plant symbionts (Saba *et al.*, 2012). Some strains of *Trichoderma* colonize root surfaces of plants and penetrate into the epidermal cells. Root colonization by *Trichoderma* suppress deleterious root microflora, produce growth stimulating factors and increase nutrient uptake. Mastouri, (2010) reported increase in vigour and emergence of tomato by *T. afroharzianum*. Mayo-Prieto *et al.* (2020) observed *Trichoderma* isolated from soil increased hypocotyl diameter and length of root system of bean plants. Degani *et al.* (2021) caused by *Magnaportheiopsis maydis*, is considered a major threat to commercial fields in Israel, Egypt, Spain, and India. Today's control methods include chemical and agronomical intervention but rely almost solely on resistant maize cultivars. In recent years, LWD research focused on eco-friendly biological approaches to restrain the pathogen. The current study conducted during two growing seasons explores the potential of three *Trichoderma* species as bioprotective treatments against LWD. These species excelled in preliminary assays performed previously under controlled conditions and were applied here in the field by directly adding them to each seed with the sowing. In the first field experiment, *Trichoderma longibrachiatum* successfully rescued the plants' growth indices (weight and height also reported reduction in incidence of *Magnaportheiopsis maydis* in maize and improved growth and yield by application of *T. longibrachiatum*. Nieto-Jacobo *et al.* (2017) reported increase in *Arabidopsis* fresh biomass by 72% and the number of secondary roots by 64 by *Trichoderma atroviride*. However, they found that *T. asperellum* significantly inhibited *Arabidopsis* growth up to 74% than in non-inoculated soil. They stated that not all *Trichoderma* strains promote plant growth. Baazeem *et al.* (2021) revealed that inoculation of *T. hamatum* on maize, cowpea, small millet, green gram, and black gram resulted in improved seedlings growth. They also reported that the enzyme activity of rhizosphere soil improved by 12-69%. *T. hamatum* soil treatment promote enzyme mediated nutrient recycling activity.

Bader *et al.* (2020) found that *Trichoderma* strains were able to produce IAA and solubilize inorganic phosphorous resulting in improved growth of tomato plant. They also found increased photosynthesis, and dry roots weight indicating profound effect of *Trichoderma* inoculation on root development and modification of root architecture. According to Contreras-Cornejo *et al.* (2009) *Trichoderma* spp. can induce growth promotion in *A. thaliana* by synthesizing IAA.

### 3.2.3 Germination stimulation

Seed treatment with *Trichoderma* spp. trigger seed to produce enzymes and phytohormones required for seed germination and also enhance seed germination and seedling vigor. Several researchers have observed enhanced germination percent in tomato, pea beans, chickpea, etc. (Mastouri, 2010; Singh *et al.*, 2015; Mayo-Prieto *et al.*, 2020). Bezuidenhout *et al.* (2012) observed that seeds treated with *Trichoderma* had early germination indicating the effect similar to gibberellic acid. Upon evaluation of secondary metabolites secreted by *T. harzianum*, gliotoxin was found to be mimicking the plant growth hormone. According to Howell (2002) germinating seeds release nutrients and addition of *Trichoderma*, metabolizes these nutrients rendering them unavailable to the pathogens.

### 3.2.4 Responses of *Trichoderma* under drought stress

Generally, plants face several abiotic stresses that affect seed germination, seedling vigor, plant establishment, growth and development, and ultimately seed yield. Several *Trichoderma* species are competent to alleviate abiotic stress and improve plant growth and vigor (Hermosa *et al.*, 2012). Bae *et al.* (2009) reported that *T. hamatum* increasing root growth of cocoa plants resulting in tolerance towards water stress condition. Mastouri *et al.* (2010) reported that *Trichoderma* strains are known to offer plant tolerance against physiological stress such as seed aging. The researcher found that peroxide levels were reduced when physiologically stressed seed of tomato treated with *T. harzianum*. Under continuous stress, Reactive oxygen species (ROS) production is increased (Schafer & Buettner, 2001). Production of ROS is important as they play a crucial role in signaling during stress condition. In response to produced ROS, plants activate the antioxidant defense systems and increased activities of ascorbate and glutathione-recycling enzymes. Mastouri *et al.* (2010) demonstrated that *T. harzianum* increases seedling vigor and ameliorates stress by inducing

physiological protection in plants against oxidative damage. Navazio *et al.* (2007) observed that when soybean cell culture was treated with culture filtrate of *T. atroviride*, intracellular ROS accumulation was detected. Such signals can induce plant ROS scavenging mechanisms which results in protection against the oxidative damage (Mastouri *et al.*, 2010). Along with the previously mentioned mechanism, *Trichoderma* can ameliorate plant growth by lowering deleterious elevated ethylene levels under abiotic stress conditions (Brotman *et al.*, 2013). In recent studies, it has been observed that *Trichoderma* spp. can reprogram plant gene expression which is a primary method of pathogen control. As a result, induced systemic resistance (ISR) occurs (Shoresh *et al.*, 2010). According to Mishra *et al.* (2020) *Trichoderma* has the ability to produce high level of phenols and proline in drought stressed rice plants leading to accumulation of chlorophyll in the plant. Ma *et al.* (2020) whereas the underlying mechanisms of *Trichoderma*-induced drought resistance of host plants remain largely elusive. Herein, the effects of a *Trichoderma harzianum* isolate on maize's responses to drought stress were investigated. Inoculation with *T. harzianum* significantly promoted the growth and enhanced drought tolerance of maize plants. The whole genome expression profiles of the *Trichoderma*-inoculated plants were examined by RNA-sequencing, showing that several differentially expressed genes were positively associated with the process of ethanol-acetic acid metabolism. Compared with non-inoculated (control reported increment in ABA level with drought stress, however, the levels of ABA was remarkably high in *T. harzianum* inoculated maize plants than the controls. Increased levels of ABA responsive gene transcripts were observed in transgenic Arabidopsis plants when exposed to abiotic and biotic stresses (Shi *et al.*, 2017).

## 4. MODE OF APPLICATION

There are several methods for application of *Trichoderma* for the successful control of plant diseases. Mode of application of *Trichoderma* play a vital role in establishment and site of action of the antagonist.

### 4.1 Seed treatment

Seed treatment is one of the effective method for application of biocontrol agents. In this method seed is coated with conidia of *Trichoderma* before sowing. The ability of the antagonist to proliferate and colonize the rhizosphere determine the efficacy of seed treatment

(Nakkeeran *et al.*, 2016). Juliatti *et al.* (2019) suggested that soon after treatment, seeds should be sown to avoid drying of spore and to enhance spore germination. Seed microbiolization not only promote seed germination, seedling emergence and disease control but also protect them from other fungi during storage (Juliatti *et al.*, 2019). Biswas and Sen (2000) reported seed treatment with conidial suspension of *Trichoderma* was effective in reducing collar rot of groundnut caused by *S. rolfsii*. Under saline condition, tomato seeds treated with *Trichoderma* spp. germinated faster than control (Contreras-Cornejo *et al.*, 2016; Mastouri, 2010). Juliatti *et al.* (2019) stated that seed microbiolization as an important method of application of biocontrol agents since it requires a small amount of biological material compared to the quantity needed for soil application.

#### 4.2 Soil treatment

For the biocontrol of soil borne plant pathogens, soil application of *Trichoderma* are also recommended. But for their effective management, high population of the antagonist is required (Nakkeeran *et al.*, 2016). Jegathambigai *et al.* (2010) reported that soil amendment with *Trichoderma* spp. provided control of Pigeonpea wilt caused by *Fusarium udum* and collar rot of Zamiodulcas caused by *S. rolfsii* by 22-30.9% whereas seed treatment was ineffective at high level of pathogen and disease control was less than 7%. As *Trichoderma* sp. is a soil inhabitant, it has an opportunity to establish and multiply more quickly in soil than on seed surface. Although soil application of bio agents is effective against plant pathogens, their feasibility for field application is low because of the cost of production and higher volume requirements.

#### 4.3 Foliar application

It is difficult for biocontrol agents to establish and proliferate in phyllosphere as the foliar environment

is frequently fluctuating and harsh (Sawant, 2014). However, the success of antagonists on foliage depends on its ability to colonize the surfaces (Lo *et al.*, 1997) brown patch, and dollar spot of creeping bentgrass was investigated. Spray applications of conidial suspensions (SA. Panwar *et al.* (2014) reported that the foliar spray of antagonist reduced the fusarium head blight of wheat caused by *F. graminearum* under greenhouse condition. Oros and Naar (2017) also reported that the liquid formulations of *T. harzianum* were efficient against rose black spot disease caused by *Diplocarpon rosae*. According to Sawant (2014), *Trichoderma* have been successfully utilized to control foliar diseases such as *B. cinerea* in strawberry, *C. gloeosporioides* and *Plasmopara viticola* in grape, *Cladosporium fulvum* in tomato and *S. sclerotiorum* in cucumber. Foliar application of bio agents is preferred during evening hours as there is least environmental effect on colonization and efficacy (Avila & Gutierrez, 1992).

### 5. CONCLUSION

*Trichoderma* is an important component in integrated disease management strategies. It does not lead to development of resistance in plant pathogens, do not enter into the food chain and do not create any pollution problems in the environment. Along with controlling plant pathogens, *Trichoderma* strains also stimulate growth and resistance in plants. *Trichoderma* is a soil resident, thus it may quickly colonize new soil, which has a positive impact on the health of the soil as well. It can be used in a variety of ways to effectively control plant diseases. *Trichoderma* application method is crucial for the establishment and location of the antagonist's site of action.

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