“Our land. Our future. We are #GenerationRestoration.”

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Government of Nepal
Ministry of Agriculture and Livestock Development
Singhadurbar, Kathmandu
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Singhadurbar, Kathmandu

EDITORIAL

Welcome to the latest issue of the 25th volume of The Journal of Agriculture and Environment. In this edition, we feature innovative research articles, review papers and insightful studies that highlight the crucial role of agriculture and environment for sustainable development. Through these contributions, we aim to highlight the best practices that promote both agricultural productivity and ecological balance.

Agriculture forms the backbone of Nepal's economy, providing livelihoods for large population. Nepal is rich in agro-biodiversity due to diverse agro-climatic condition, with huge numbers of various accessions of genetic resources and wide range of ecosystem. However, unsustainable practices pose a significant threats to biodiversity conservation causing degradation, losses and disappearance of local and indigenous genetic resources. The complexity of agricultural systems necessitates a collaborative approach to develop solutions that address both food security and environmental resilience.

The articles featured in this edition cover wide range of topics, including 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. We encourage our readers- academics, practitioners and policy makers- to engage with the findings presented.

We extend our sincere appreciation to all authors, esteemed reviewers, who contributed their expertise and insights, enriching our understanding of this field. 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. Editorial team aim to uphold the highest standards of academic quality, originality and relevance in the articles published. Each submission is rigorously reviewed by editorial members and undergoes a peer-review process by two reviewers. Together we can foster a more sustainable and equitable relationship between agriculture and the environment.

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. 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.

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.

Dr. Matina Joshi Vaidya

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.

1. The manuscript must be an original work written in English and not published elsewhere.
2. The title should be short and specific, but it should reflect the contents in the manuscript.
3. The abstract should be in **Times New Roman** font (italics) 11-point font size, not exceeding 200 words, should concisely state major objective, methodology, findings and conclusion. It should not include diagram, footnote, equation or any parenthetical reference.
4. Key-words in alphabetical order should not exceed five standard words.
5. Main text of the technical manuscripts should include introduction, materials and methods, results and discussions, conclusions and references.
6. The manuscript should not exceed 4500 words in total. It should be in MS-word with pages set on A4 size, the top and left margins at 3 cm and the right and bottom margins at 2.5cm. The text format should be on **Times New Roman** font of, 11-point size, single space and **double columns**. Authors are encouraged to use the following **Microsoft Word-template** to prepare the manuscript;
    a. The title of the manuscript set as HEADING 1 (paragraph style) should be all capitalized in bold 12-point font size.
    b. Name of the author(s) should follow the title in new paragraph in normal 10-point font size, bold.
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    d. In tables, borders should be minimized, and text and numbers should be in 9-point font size.
    e. Bibliographic entries in the reference should be in 9-point font size.
7. Number of footnotes should be minimized, and it should not come for citation.
8. Many/big tables and figures in the text should be avoided. Supplementary figures and tables may be placed in the annexure.
9. Whenever possible, SI Units (International System of Units) should be used.
10. Images/Diagrams should be of high-quality illustrations in any of the following formats: PNG/JPEG/TIFF. Written text in an image should be clear and easy to read and located only either at the top or bottom of the page.
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    For a book:
    Manay Shakuntala, N., & Shadaksharaswami, M. (2001). Food Facts and Principles. New Age International (P) Limited, New Delhi, India.

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TABLE OF CONTENTS

ARTICLE **PAGE No.**

PATRONS......v
EDITORIAL BOARD......v
EDITORS......v
EDITORIAL......vii
GUIDELINES TO AUTHORS: MANUSCRIPT PREPARATION AND SUBMISSION...... .viii
TABLE OF CONTENTS......ix-x

ANALYSIS OF LIVELIHOOD VULNERABILITY INDEX TO CLIMATE CHANGE IN KASKI DISTRICT, NEPAL......1
Basu Dev Gajurel and Bhupendra Devkota

SILKWORM PUPAE MEAL AS AN ALTERNATIVE TO FISH MEAL IN THE COMPOUND DIETS OF ROHU FISH (*Labeo rohita* Hamilton)......13
Ganesh Belbase, Prashant Chaudhary and Shailesh Gurung

EVALUATION OF GROUNDNUT GENOTYPES FOR LEAF SPOT RESISTANCE AND HIGH YIELDING TRAITS IN TERAI CONDITIONS AT NAWALPUR, SARLAHI, NEPAL......21
Subash Subedi, Saraswati Neupane and Pramod Wagle

STUDY OF DIFFERENT SOWING DATES ON MAIZE APHID (*Rhopalosiphum maidis* (Fitch)) INFESTATION, AND YIELD OF MAIZE VARIETIES IN INNER TERAI REGION OF NEPAL......31
Saraswati Neupane, Subash Subedi, Gopal Bhandari and Ramesh Shrestha

MATHEMATICAL MODELING OF THE BIO-SORPTION OF CALCIUM IN OYSTER MUSHROOM: AN ALTERNATIVE NUTRITION SOURCE TO COMBAT CALCIUM DEFICIENCY......41
Sajan Shrestha, Sanjeev Kumar Karn and Prateek Joshi

IMPACTS OF WTO AGREEMENTS ON THE EXCHANGE OF AGRICULTURAL COMMODITIES: A REVIEW......57
Gaurab Luitel, Hari Krishna Pant, Kishor Chandra Dahal, Tara Prasad Bhusal and Krishna Prasad Timsina

OPTIMIZATION OF GERMINATION TIME OF SORGHUM VARIETIES TO ENHANCE THE BIOACTIVE COMPOUNDS, ENZYMES, AND REDUCING SUGAR AND REDUCE THE ANTINUTRITIONAL FACTORS......67
Roman Karki, Pravin Ojha, Sushma Maharjan, Utsah Manandhar, Sophi Maharjan, Ranjan Shrestha, Kabina Karki and Sanju Upadhyaya

EFFICACY OF FUNGICIDES FOR THE MANAGEMENT OF FOOT ROT DISEASE, *Sclerotium rolfsii* (SACCARDO) IN CHILLI......87
Meena Kumari Poudel, Sundar Man Shrestha, Hira Kaji Manandhar, Ritesh Kumar Yadav and Sujan Subedi

MANAGEMENT OF *Epilachna dodecatigma* (Wied.) (COLEOPTERA: COCCINELLIDAE ON BITTER GOURD *(Momordica charantia* L.*)* IN CHITWAN,NEPAL......93
Rajanish Mishra and Prashant Kumar Chaudhary

ECOLOGICAL STATUS, THREATS, AND HABITAT CHARACTERISTICS OF PANCHAULE *(Dactylorhiza hatagirea)* IN GHASA OF MUSTANG, DISTRICT..99
Aadesh Bikram Malla, Sandesh Subedi, Dhruba Bijaya G.C and Jyoti Bhandari

CHARACTERIZATION OF FUNCTIONAL AND VISCOELASTIC PROPERTIES OF FLOUR FROM RELEASED WHEAT VARIETIES IN NEPAL..109
Pravin Ojha, Kabina Karki, Roman Karki, Utsaha Maharjan, Ranjan Shrestha and Atul Upadhyay

EVALUATION OF RADISH (*Raphanus sativus* L.) VARIEITES IN DIFFERENT SOWING DATES FOR OFF-SEASON PRODUCTION AT DAILEKH...117
Binod Prasad Luitel, Yubaraj Bhusa and Bishnu Bahadur Bhandari

PERFORMANCE OF TOMATO GENOTYPES UNDER OPEN FIELD CONDITIONS IN MID HILLS OF NEPAL..127
Dhruba Raj Bhattrai, Sujan Subedi, Navin Gopal Pradhan, Meena Kumai Poudel, Arjun Subedi and Jiya Poudel

A REVIEW ON YAK AND ITS HYBRIDS FARMING PRACTICE IN NEPAL.....................................137
Mandeep Pokharel and Bhargab Dhital

CONSUMPTION OF MILLETS BY THE INDIGENOUS PEOPLE (IP): A CASE STUDY IN CHINGMEI VILLAGE, TUENSANG NAGALAND, NORTHEAST INDIA...145
Chipensangla.S. Chang, Melodynia Marpna and Laribha Dohtdong

FACTORS INFLUENCING THE ADOPTION OF CLIMATE SMART AGRICULTURAL PRACTICES IN SUNDARBAZAR, LAMJUNG...157
Sulav Neupane, Sulakshan Neupane, Sagardeep Bhusal and Sujan Mishra

EVALUATING THE EFFICACY OF POTENTIAL FUNGICIDES FOR THE MANAGEMENT OF SOUTHERN LEAF BLIGHT *(Bipolaris maydis)* OF MAIZE...169
Deepu Kumar Tiwari, Sujita Aryal and Shishir Sharma

EVALUATION OF DIFFERENT WEED MANAGEMENT PRACTICES ON THE PHONOLOGY OF SPRING MAIZE IN GAURADAHA, JHAPA...177
Karishma Khanal, Rumita Limbu Sanwa, Shwastika Baral and Goma Dhital

FARMERS' KNOWLEDGE AND MANAGEMENT PRACTICES OF FALL ARMYWORM IN MAJOR MAIZE-PRODUCING DISTRICTS OF NEPAL..185
Dipak Khanal, Mina Kunwar, Pratima Bista, Puja Kandel, Sirjana Thapa, Sila Pun Magar, Krishna Lamsal, Prashant Chaudhary and Pushpa Pandey

IMPLEMENTATION STATUS OF FOOD SAFETY MANAGEMENT SYSTEMS IN NEPALESE FOOD INDUSTRIES..195
Prateek Joshi, Pramod Koirala and Sanjeev Kumar Karn

INTEGRATED APPROACHES FOR THE MANAGEMENT OF ROOT KNOT DISEASE OF TOMATO IN KASKI, NEPAL...203
Omprakash Thakur, Chandeshwor Prasad Shrivastava, Roshan Adhikari, Tilak Raj Chaulagain, Manoj Dhakal and Arjun Dev Jnawali

ANALYSIS OF LIVELIHOOD VULNERABILITY INDEX TO CLIMATE CHANGE IN KASKI DISTRICT, NEPAL

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ABSTRACT

The research analyzed the Livelihood Vulnerability Index (LVI) from the major components (SDP, SN, LS, H, W, F, and ND & CV) on indigenous communities in the Kaski district of Nepal at different altitudinal variation, who depend primarily on agriculture for their livelihood and had been most impacted by climate change, requiring adaptation and coping strategies. Kruskal- Wallis H test and multiple comparisons showed that each community (Melache, Hudu, Dhampus Upper and Lower) of two distinct municipalities (Gaupalika) were normally distributed (H= 0.458 and P > 0.05) and communities were not significantly different from each other in terms of their distribution. Similarly, LVI had a level value from 0 to 1, in which 0 is the least vulnerable and 1 is highly vulnerable. All the communities fell under the intermediate level of vulnerabilities. However, Melache had the highest LVI (0.439) in terms of food, health, natural disaster and climatic variability followed by Hudu (0.403). The Dhampus Upper region had the LVI (0.39), whereas, the Dhampus Lower Region had the lowest LVI (0.376) among the four communities.

1. INTRODUCTION

Climate change refers to long-term alterations in the Earth's climate system, including changes in its properties and patterns, caused by both natural factors and human activities that impact the composition of the atmosphere (IPCC, 2014). With 80% of its population at risk from various impacts such as extreme heat, floods, and air pollution, Nepal is placed 10th on the Climate Risk Index (Eckstein *et al*., 2021).

1.1 Nepal's Diverse Climate and its Impacts on People's Livelihood

Nepal is geographically divided into three distinct regions, each characterized by varying altitudes and temperatures. The southern Terai belt, where the altitude is as low 60 meters, the temperature is high and with rise in altitude towards the north, reaching the awe-inspiring height of 8,848.84 meters in High Himalayas, the temperatures plunge to sub-zero degrees Celsius (Kansakar *et al.*, 2004) based on the elevations, the regions can be classified as follows: Terai, extending above 60 meters, Siwalik between (900-1,200) meters, middle mountain between (1,500-2,000) meters, high mountain between (3,500-5,000) meters, and high Himalaya ranging above 5,000 meters (Dhital, 2015; Kansakar *et al.*, 2004).

The average monthly income of rural Nepali households stands at approximately USD 170, making them particularly vulnerable to such natural disasters. The financial toll of the historic five-year flood damage amounted to around USD 293, (USD 1 = NPR 131, as of 25 June 2023) almost double the average monthly income. Furthermore, under the scenario of climate change, the total loss of 0.4% GDP and 0.87 deaths per 100000 people and estimated annual economic loss damage cost would be between USD 290 billion to

USD 580 billion by the year 2030 (IIDS, 2022).

1.2 Climate Change Livelihood Vulnerability Index (LVI)

In the context of households, livelihood encompasses four essential elements: the activities undertaken by people, the assets they possess, and the gains or outputs they achieve. These components intertwine harmoniously to shape the livelihood of a household, forming a foundation from which individuals derive their sustenance and livelihood (Chambers & Conway, 1991).

The Livelihood Vulnerability Index (LVI), as defined by IPCC (IPCC, 2001), is a comprehensive measure that combines seven factors to identify households, communities, and districts that are vulnerable to climate change. These factors include exposure (natural disasters and climatic variability), sensitivity (impacts on water, food, and health), and adaptive capacity determined by socio-demographic profiles, livelihood strategies, and social networks which are the useful indicators for allocating the proper a significance level, tools to determine the impact of climate change, and gathering accurate data (Hahn *et al*., 2009). By considering these aspects, the LVI helps assess and understand the level of vulnerability in various areas and populations.

2. MATERIALS AND METHODS

2.1 Study Area

The survey trail was formed to carried out the research at distinct elevations (meters above the sea level) of Machhapuchchhre Gaupalika (Dhampus-7 upper region and Dhampus-7 lower region at elevations of 1160m and 1360m, as well as of Annapurna Gaupalika (Melache-11 (upper region) and Hudu-10 (lower region) at elevations of 2210m and 1490 meters above the sea level (Figure 1). The GPS location the Gandaki Province of Nepal's Kaski District is 28°16'00" N and 83°58'06" E in latitude and longitude.

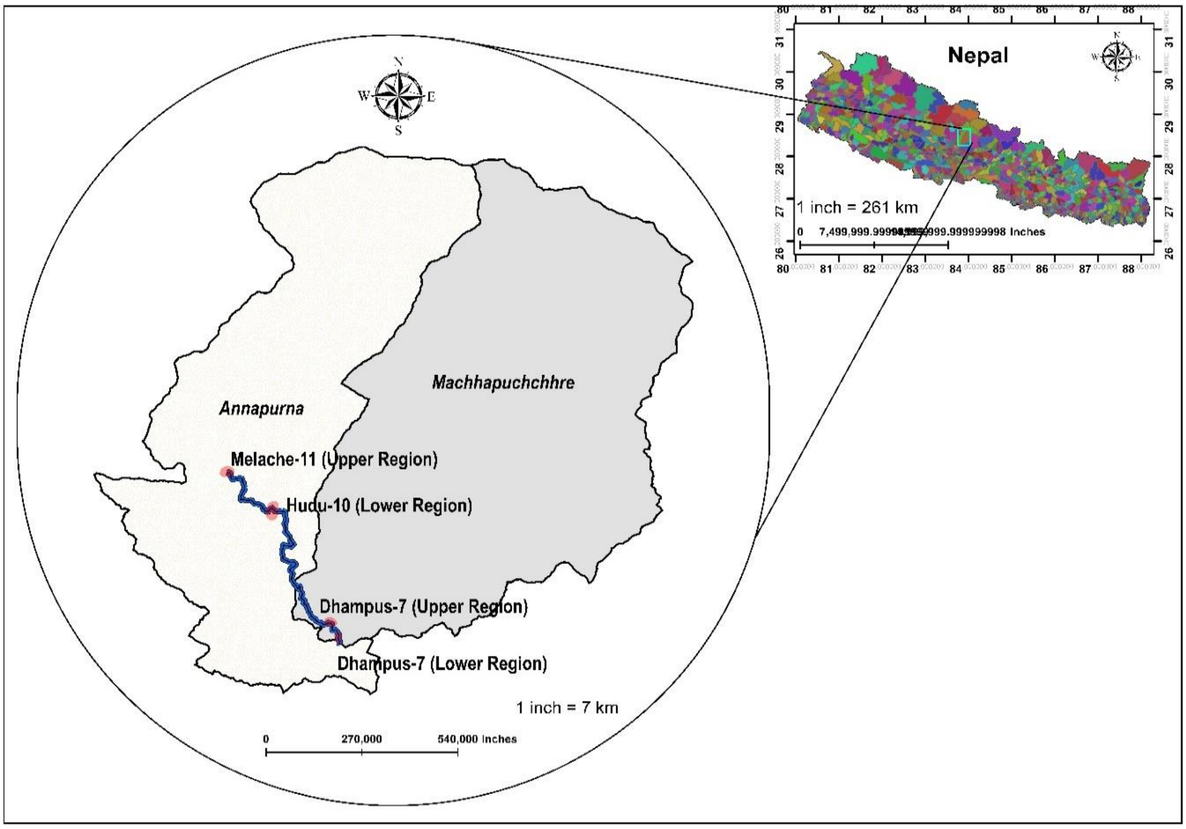

Figure 1. Map of the study area along with survey trail

2.2 Sampling process

A total of 65 households were surveyed using the lottery method for unbiases. The sample size formulae were taken from (Kothari, 2004) to compute the sample size of a respondent community, as it was a crucial step in the research with the known population size. The population size of respective wards was taken from NSO (2021), and the equations (1 & 2) below were used:

$$n = \frac{Z^2.N.p(1-p)}{(N-1).e^2+Z^2p(1-p)} \text{ -----------Equation (1)}$$

Where, N= Size of Population, n = Size of a sample, e = acceptable error (the precision) (error 5% = 0.05 which is relevant with a confidence level of 95% so the error is 5%), p = Estimate of a proportion of the Population (So, the highest proportion cannot be more than 50% = 0.50), Z = Standard variate of a confidence level at 95% (so, Z-score is 1.96).

$$s = \frac{H.R.C}{N} * n \text{ --------------------- Equation (2)}$$

Where, s = Sample size for respondent community or Sample size (n) for the community, H.R.C = Total number of households in the respondent community, N = Total finite population size of respective ward, n = Sample size of respective ward.

2.3 Data collection

The extensive field study and household surveys were conducted in four distinct locations situated at different elevations and the scheduling method was performed. In Annapurna Gaupalika, in the upper region of Melache (2210m), 21 houses and in the lower region of Hudu (1490m), 13 houses were surveyed. Likewise, in Machhapuchchhre Gaupalika, 17 households were surveyed in the upper region (1660m) and 14 households in the lower region (1360m) of Dhampus ward number 7. The primary data collection process took approximately 12 days, between June 25, 2023, and July 6, 2023.

The primary data were gathered through household surveys and Key Informant Interviews (KII) with the ward chairperson, heath workers and the ward representatives. The secondary data were gathered from multiple sources, including the Wards and Gaupalika information portal sites. The population structure data was obtained from the National Population and Housing Census of 2021, ensuring up-to-date and accurate information. Additionally, climate-related data were taken from Lumle the nearest station operated by the Department of Hydrology and Meteorology (DHM, 2023).

2.4 Data Analysis

The Principal Component Analysis (PCA) method was employed in data processing and feature extraction to ensure that all variables within a dataset were on a consistent scale. This is particularly useful when dealing with data that are either highly correlated or contains large datasets. Composite Index Method (CIM) is a statistical approach used to condense multiple variables or indicators into a single numerical index (Hahn *et al*., 2009; Mainali & Pricope, 2017).

Importantly, no prior assumptions were made concerning the significance of individual factors, as most values exhibited a normal distribution within the composite index. Additionally, the analysis included trend analysis using linear regression, allowing for an examination of trends (Figure 2) and relationships within the data, for which Microsoft Excel software and SPSS were used.

2.5 Calculation of LVI (Livelihood Vulnerability Index): Composite Index Approach

Seven key elements make up LVI: Social Demographic Profile (SDP), Livelihood Strategies (LS), Social Networks (SN), Health (H), Food (F), Water (W), Natural Disaster (ND), and Climatological Variability (CV). These key components, sub-components, and methodologies, which were completed by Hanh *et al*. (2009), were employed as the requirements of the research.

However, UNDP (1990) has developed a mathematical equation for the Human Development Index (HDI). In this approach, maximum and minimum values are established for the sub-components, to be placed within a range and balanced against each other through weighted calculations and obtain the single digit value.

Step 1: Maximum value was set at 100 and minimum value at 0, other sub-components were set according to requirements, for example, months = 12, the ratio in 1:2 format

$$\mathbf{Index_{Sc}} = \frac{S_c - S_{min}}{S_{max} - S_{min}} \text{........... Equation (3)}$$

Where, S_c = Original sub-component for a community, S_{max} & S_{min} = Maximum and Minimum values of Sub-components from the community after a survey.

Step 2: After normalizing the value in Equation 3, the sub-components were averaged (Standardization) to calculate the major components of a respective community (M_c).

$$\mathbf{M_C} = \sum_{i=1}^{n} \frac{\mathbf{Index_{Sc}}^{i}}{n} \text{........... Equation (4)}$$

Where, M_c = Major components, n = The Total number of sub-components available in the major components, $Index_{Sc}^{i}$ = Normalized or balanced weight calculation of each sub-component indexed by i from equation 3.

Step 3: In the final stage the LVI, was measured in a scale between 0 to 1. Once the value of each of the seven major components for the community is calculated they were averaged using below Equation 5 or 6.

$$\mathbf{LVI} = \sum_{i=1}^{7} \frac{W_{mi}\, M_i}{W_{mi}} \text{.............. Equation (5)}$$

Which can also be written as:

$$\mathbf{LVI} = \frac{W_{SDP}\, SDP_c + W_{LS}\, LS_c + W_{SN} SN_c + W_H H_c + W_F F_c + W_W W_C + W_{NDVC} NDVC_c}{W_{SDP} + W_{LS} + W_{SN} + W_H + W_F + W_W + W_{NDVC}} \text{ Equation (6)}$$

Where, W_{mi} = Weight of each major component, M_i = Number of each sub-component in each major component, and others are the Weight of the major seven components and the weight of sub-components in the major components.

3. RESULTS AND DISCUSSION

3.1 Precipitation Trend Analysis of Lumle (1992-2022)

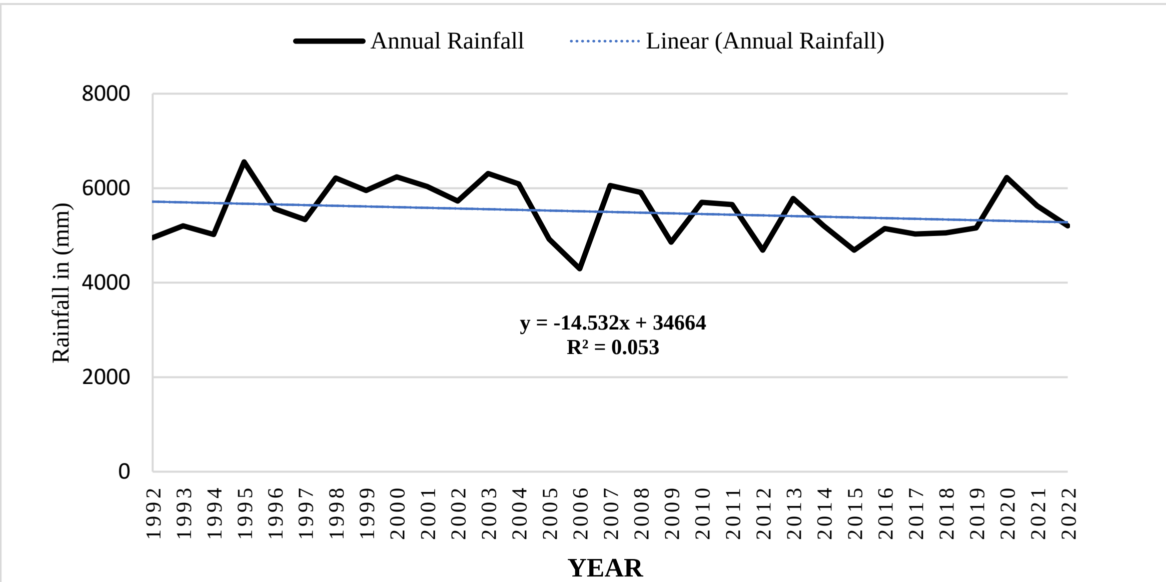

Figure 2. Annual trend analysis of annual data

Lumle, station index 0814, situated in Annapurna Gaupalika has the highest amount of rainfall in the entire country. From the analysis period of (1992-2022) the rainfall has been dropping on average at a rate of 14.532mm per year in or around the Lumle area (Figure 2). So, in a similar study of Lumle station from (2001 to 2017) analyzed the decreasing trend in rainfall, at the rate of 12.53mm per year (Basnet et. al., 2020). Around the country, precipitation is decreasing annually at a rate of 4.78 mm, with all seasons experiencing declining average precipitation, particularly during the monsoon season, where the decline is nearly 4 mm per year (Piya et. al., 2019).

A phenomenal 95% of respondents said have seen changes in the climate during the previous 30 years. However, the study also showed that these locals had difficulties correctly estimating the timing, amount, and patterns.

Nepal's distinct climatic seasons result in varying rainfall patterns throughout the year (Shrestha et al., 2019). Similar case in the Lumle with the monsoon season witnessing the highest rainfall, the post-monsoon season showing fluctuating trends, and the winter season typically experiencing lower precipitation (Figure 2).

3.2 Major Components and Sub-Major Components on LVI

Since the primary components were derived from the IPCC reports and the sub-components have been adapted from (Hahn *et al.*, 2009; IPCC, 2001) and, according to the requirements of the study area and geographical considerations, the average of sub-major components was included after the calculation of multiple factors as given in Tables 1& 2.

Table 1. LVI major components, sub-major components, and Average of sub-major components of four distinct locations.

Major Components	Sub-Major Components	Average of sub-major component			
		Melache (Upper Region)	Hudu (Lower Region)	Dhumpus (Upper Region)	Dhumpus (Lower Region)
Socio-Demographic Profile	Dependency ratio	0.18	0.311	0.235	0.408
	% of women headed HH				
	% of family heads without primary education				
	% of orphans				
Livelihood Strategies	% of HH solely depends upon Tourism	0.402	0.567	0.487	0.575
	% of HH that rely solely on agriculture for their income.				
	% of HH where members seek external sources of income.				
	The Agricultural Livelihood Diversification Index, with an average value ranging from 0.20 to 1.				
Social Network	Average receives help ratio from others (range 0-15)	0.372	0.428	0.413	0.573
	Percentage of individuals who haven't gone to help the community or engaged in local government at work.				
	The average number of HH individuals who have lent money to others in a ratio (0.5 - 2)				

Health	The average distance from the village to the nearest health post. (Minutes)	0.498	0.292	0.355	0.232
	Percentage of households with chronic diseases.				
	Percentage of students in HHs who have missed school due to illness in the past four weeks.				
Water	Average time taken to reach a water resource (in minutes).	0.33	0.247	0.204	0.127
	% of HHs dependent on natural resources.				
	Percentage of households reporting water conflicts.				
Food	The average number of HHs experiencing food scarcity per month (range: 0-12).	0.593	0.367	0.426	0.238
	% of HHs dependent on family farms.				
	Average crop diversity index ranging from 0 to 1.				
	Percentage of households that do not save seeds.				
	The average amount of crops destroyed by natural calamities in HH				
	Percentage of households that do not save harvested crops.				
Natural Disaster and Climate Variability	Average number of natural disasters experienced (range: 0-7).	0.549	0.514	0.497	0.458
	% of HHs lack access to early warning systems.				
	% of HHs that have experienced death or injury in the past 10 years.				
	The average daily maximum temperature by month is expressed in terms of mean and standard deviation.				
	The average daily minimum temperature by month is expressed in terms of mean and standard deviation.				
	The average monthly precipitation is represented by its mean and standard deviation.				

Table 2. LVI value of four communities (Melache, Hudu, Dhampus Lower, and Dhampus Upper)

	Major Components							**LVI**
	SDP	**LS**	**SN**	**H**	**W**	**F**	**ND & CV**	
Melache	0.180	0.402	0.372	0.498	0.330	0.585	0.549	0.439
Hudu	0.311	0.567	0.428	0.292	0.240	0.367	0.514	0.403
Dhampus Upper	0.235	0.487	0.413	0.355	0.204	0.426	0.497	0.391
Dhampus Lower	0.408	0.575	0.573	0.232	0.127	0.238	0.458	0.376

Calculation of sub-components and major components (Example of Dhampus Lower Region)

Step 1: (repeat for all sub-components)

$$\text{Index}_{\text{(Health)}} = \frac{\text{Observed value} - \text{minimum value}}{\text{Maximum value} - \text{Minimum value}}$$

$$= \frac{2-1}{9-1} = 0.125$$

Step 2: (repeat for all major- components)

$$\text{Health} = M_{\text{Dhampus Lower region}} = \sum_{i=1}^{n} \frac{\text{Index}_{Sc}i}{n} = \frac{0.124 + 0.429 + 0.143}{3}$$

$$= \frac{0.693}{3} = 0.232$$

Step 3: (Repeat all major values for the entire four communities) as given in Table 2

$$\text{LVI}_{\text{Dhampus Lower region}} = \sum_{i=1}^{7} \frac{W_{mi} M_i}{W_{mi}}$$

(Also written as**)**

$$\mathbf{LVI}_{\textbf{Dhampus Lower region}} = \frac{W_{SDP}\,SDP_c + W_{LS}\,LS_c + W_{SN}SN_c + W_H H_c + W_F F_c + W_W W_C + W_{NDVC}NDVC_c}{W_{SDP} + W_{LS} + W_{SN} + W_H + W_F + W_W + W_{NDVC}}$$

$$\mathbf{LVI}_{\textbf{Dhampus Lower region}} = \frac{(4)*0.408 + (4)*0.575 + (3)*0.573 + (3)*0.232 + (3)*0.127 + (6)*0.238 + (6)*0.458}{4+4+3+3+3+6+6}$$

$$\mathbf{LVI}_{\textbf{Dhampus Lower region}} = 0.37$$

LVI is the aggregate result of seven major components (SDP, LS, SN, H, F, W, and ND & CV) which are integral factors contributing to the Vulnerability Index (LVI) for four distinct communities. Melache lying at a higher altitude (2210m a.s.l) was found to have the highest LVI (0.439) among the three communities because Melache appeared to be more vulnerable to food and health issues. In addition to the heavy snowfall during the winter, people there were more susceptible to natural disasters like landslides and frost, and year-round food scarcity reached a high of 0.593, triggering people to migrate to the valleys (Table 1). Dhampus Lower region situated at 1360m with lower LVI (0.376) made it least vulnerable; the study indicated that as the elevation increased, the vulnerability of people tended to rise, primarily due to higher levels of exposure and sensitivity, while adaptive capacity diminished with elevations (Mainali & Pricope, 2017). Hudu with a LVI of 0.403 and Dhampus upper region with a LVI of 0.391 made them less vulnerable than Melache, but more vulnerable than Dhampus Lower region when compared with Mandal and Khanal (2019) in Langtang, where the mid-valley settlement was highly vulnerable because of the direct impact of earthquake-induced avalanche and the LVI was 0.377, Hudu (LVI) with 0.403 and Dhampus Upper regions (LVI) with 0.391) were found to be more vulnerable.

However, the total LVI of Seven major components for four distinct communities based on the Kruskal- Wallis H test and with multiple comparisons showed that each community (Melache, Hudu, Dhampus Upper and Lower) was normally distributed ($H= 0.458$ and $P > 0.05$) as shown in Figure 3.

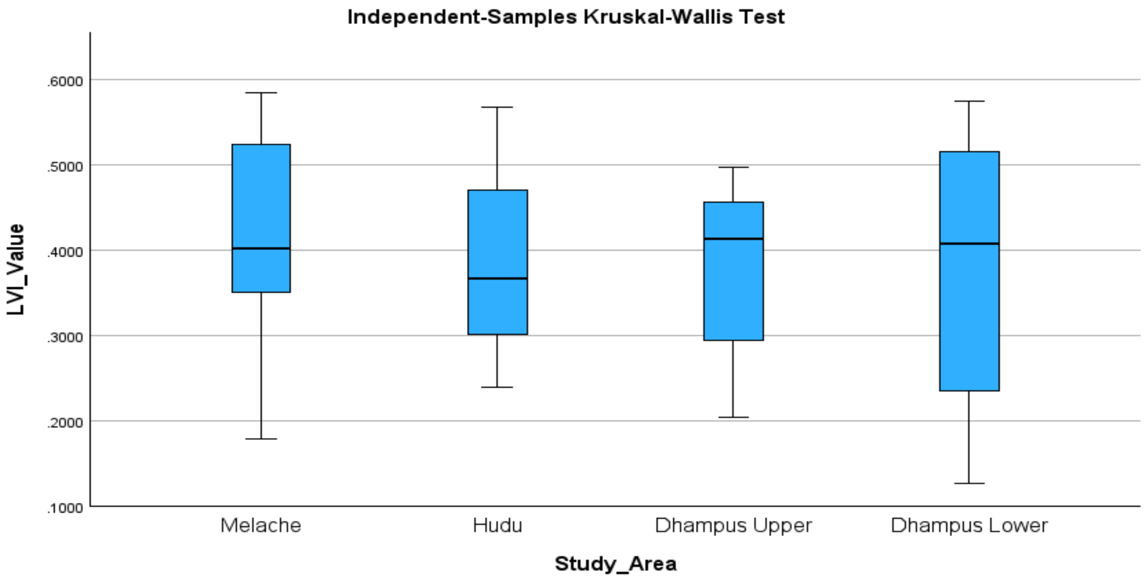

Figure 3: The boxplot with the LVI value and study area (Significant value P >0.05)

3.3 Major Components of Livelihood Vulnerability Index

The web diagram (Figure 4) provides a breakdown of each major component for each community, including SDP, LS, SN, H, W, F, ND & CV. The values associated with these components range from 0.00 to 0.600, in which 0 is the least vulnerable and 1 is highly vulnerable allowing for a comprehensive analysis of the different communities:

a. Social Demographic Profile (SDP)

The survey in four communities indicate that the female HH heads were more vulnerable, and women's workloads (earning and domestic work, as well) were higher, which accounted for high dependency ratio. In the lower region of Dhampus about 50% of women were involved in earning activities indicating that women were as active as men in the earning and managing their homes. This might explain why only 35% of residents in that community had completed their primary education. Hudu was found to have a larger percentage (about 46%) of household heads without a primary education.

b. Livelihood Strategies (LS)

The study region's primary industry was agriculture, as most of the population was employed on farms. According to the current study, over 48% of the population had left their villages and moved to lower regions, as well as abroad, in seek of better opportunities for a living. This suggests that the poor crop yield was insufficient to sustain a family for a year. Because of climate change, there were only five types of crops in the rotation and comparing to Suryanto & Rahman (2019) in Indonesia found that farmer's vulnerability index on the LSI was 0.392 in Sonorejo and 0.499 in Jiwo Wetan Village, the households with a complete reliance on agriculture were more vulnerable to the effects of climate change than those with a diverse source of income. People were also traveling overseas and looking for employment chances for outside sources of income.

c. Social Network (SN)

Out of four communities, 10% of people in Dhumpus upper region had taken business loans for tourism and agricultural upliftment but due to the natural calamities and unexpected rainfall the transportation and trekking trails had collapsed. In contrast, the research carried out by Rai *et al.*, (2022) the local government in Bhutan has not provided any necessary facilities to the people but only 37% of the people in Melache and Hudu were able to obtain agricultural loans, but the farmers were 40% less productive because they were unaware of landslides and conflicts between wildlife. To lessen the effects of the climate change-induced disaster, NAPA has prioritized eight thematic and four cross-cutting years, totaling 64 years. It has also estimated 11.2 billion USD for agriculture (MoE, 2010) although the funds are still not being subsidized in those sectors.

d. Health (H)

KII conforms that it was considerably more challenging to access medical care for mild injuries to the people of Melache and Hudu because of the transportation issue, the people of Hudu had to walk 64 minutes on average to get to the health post. The survey reveals that the lack of iodine, sufficient nutrition, and other factors contribute to numerous chronic diseases that burden the population in that region, including asthma, high and low blood pressure, diabetes, and thyroid problems in 61.90% of those over 60 years old. However, in a study of Shrestha and Aryal (2011) found that the food habits of the lower region may also be a contributing factor to chronic diseases like diabetes, high cholesterol, and asthma that affect residents.

e. Water (W)

The concern during monsoon seasons was that the water easily got polluted and sometimes people suffer from typhoid, cholera, and diarrhea (MoHP, 2022). In Melache 38% of people depended upon natural sources during winter because the water in pipes got frozen during those seasons meanwhile in Dhampus lower region only 14% used water from natural sources for irrigation purposes, compared to Rai *et al.*, (2022) in Bhutan between 50% and 100% of families reported experiencing water scarcity, sources of water running out, a lack of retention, and unstable provision of water that influence on agricultural and production when paired with the direct consequences of climate change.

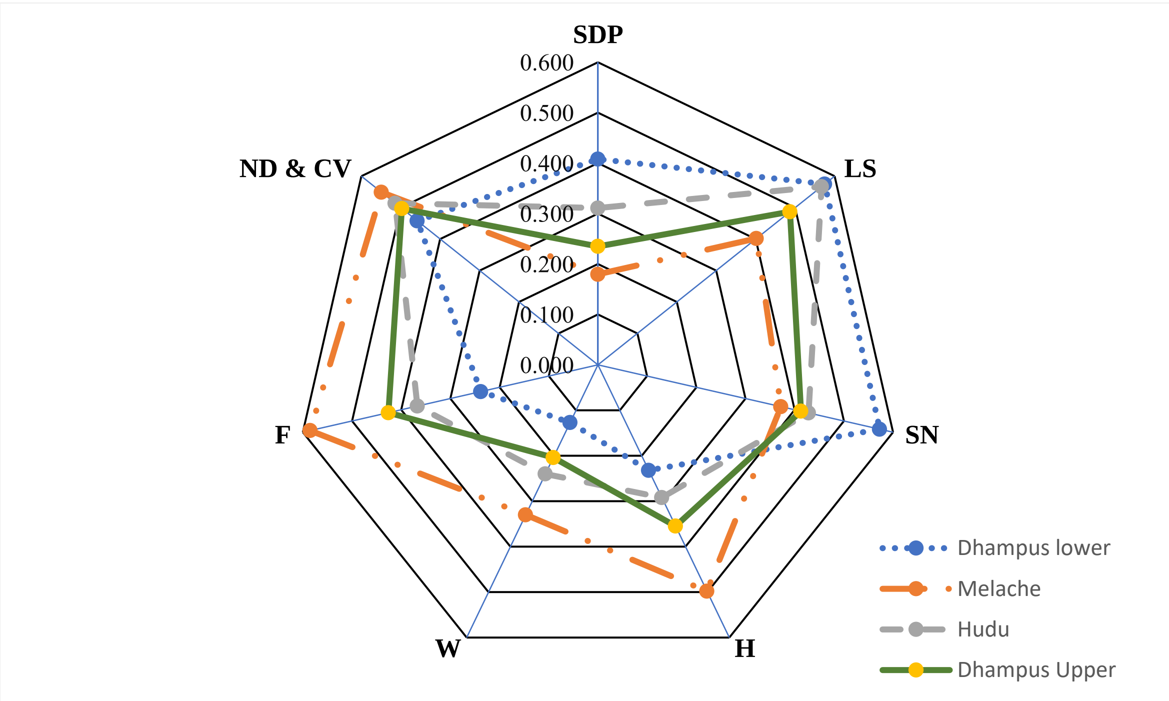

Figure 4. Vulnerability spider web diagram for major components of LVI for Annapurna and Machhapuchchhre Gaupalika

f. Food (F)

Food was the major concern in the upper part of Dhampus and Melache compared to Hudu and Dhampus lower region because due to topographic factors only limited and indigenous crops like millet, buckwheat, potato, and beans only planted in elevations more than 2000m altitude. Food shortage affected the people of Melache for approximately 6.71 months out of the year. Rainfall and unexpected snowfall brought on by the CC are the primary factors in the output. To verify this, KII was conducted, and the representative confirmed that there was very little food production in October, November and December. Due to the freezing climate, it took more than 5 months to successfully produce a potato. According to research of a similar nature done by McDowell *et al.,* (2013) in the Solukhumbu area of Nepal, where farmers have seen a reduction in agricultural yield due to an unfavorable change in rainfall, food production, and security may be compared. The productivity decline has influenced severe economic poverty since it wasn't enough to provide food security.

g. Natural Disaster and Climate Variability (ND & CV)

The climate data (temperature and precipitation) were used from a single station, Lumle, to study climate variability and determine the mean standard deviation from the data (Figure 2). After the data were analyzed, it was found that the rainfall has been dropping on average at a rate of 14.532mm per year in or around the Lumle, with the overall maximum temperature rising and rainfall also falling. Lumle was exposed to high levels of exposure like warming and an increase in rainfall unconditionally in winter season (Figure 2). About 14.9% of HH were in a high level of exposure (Pandey & Bardsley, 2015). In comparison to Hudu and Dhampus Upper and Lower areas, inhabitants in Melache experience an average of 2.38 natural disasters out of 7 every 10 years because the HH command to AC is so weak that the people were unable to cope or tackle the impacts of climate change. However, the Dhampus Upper area likewise has 47.5%, while the rate of deaths and injuries in 10 years is greater in Melache at 42.85%. Regarding early warning systems for climate change and natural disasters, residents, and representatives at KII assert that no such system is offered by the local, provincial, and federal governments.

4. CONCLUSION

According to the research, there is a positive correlation between elevation and increased susceptibility to various natural disasters such as cold waves, landslides, soil erosion, floods, and issues related to health, food security, and infrastructure, including as hospitals, schools, and transportation. The community of Melache, which is located at an elevation of 2210 meters, has witnessed its residents migrate to lower regions or overseas in seeking a better living due to its vulnerability. On the other hand, Dhampus Lower, which is the least vulnerable, is situated at a height of 1360 meters, and has better transportation and health facilities. Climate variability, however, requires adaptation and coping strategies across all four fields of study.

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SILKWORM PUPAE MEAL AS AN ALTERNATIVE TO FISH MEAL IN THE COMPOUND DIETS OF ROHU FISH (*Labeo rohita* Hamilton)

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ABSTRACT

Fish meal is a standard protein source in aqua-feed; however, its production is unsustainable. Hence, a suitable alternate protein source is essential. The study evaluated the effects of substituting fishmeal with Silkworm Pupae Meal (SPM) on the growth performances of Labeo rohita as an alternate source. The substitution rates were 10% 20% 30% for each treatment designated as, T_1, T_2, T_3 and T_4 respectively. The experiment was conducted in a pond with sixteen nylon cages (1m^3 each) in a Completely Randomized Design at the Paklihawa campus, Rupandehi. The fish were fed four iso-nitrogenous (30% CP) compound diets at 8% 5% & 3 % by body weight. The temperature, pH and DO range of the pond water were 15.57 ˚C to 24.5 ˚C, 6 to 9 and 8.03 mg/L to 10.18 mg/L respectively. A significantly ($p<0.05$) higher weight gain was observed in T_3 (13.47±0.24 g) than control (12.34±0.37 g). The highest survivability was observed in T_4 (0.70±0.02) at $p<0.05$. No significant difference was observed in relative weight gain, specific growth rate, FCR and PER ($p>0.05$). Additionally, SPM-incorporated diets performed significantly superior ($p<0.05$) to the control without any observable side effects on the fish. Overall, we can conclude that SPM can be used in partial replacement to fishmeal up to 30% without any observable detrimental effect on L. rohita.

1. INTRODUCTION

Aquaculture is the fastest-growing food sector in the world, contributing to about one-third of global fish production. In 2018, 179 million tonnes of fish were produced worldwide and only 82.1 million tonnes were produced from the aquaculture industry (FAO, 2020). Aquaculture includes the rearing of all forms of aquatic animals and plants for the production of sustainable protein sources (Liao & Chao, 2009), which

Contribute for 50% of the overall food supply (Okocha *et al.*, 2018). It is viable in a wide range of water sources such as pools, natural water, recirculating water systems, and so on (Hall, 2011). Animal protein demand is

increasing with the increase in human population. Aquaculture is considered an emergent protein production sector to fulfill the gap between demand and supply of protein for human consumption (Ibrahim *et al.*, 2020). Aquaculture uses 63% and 81% of the global supply of expensive ingredients like fishmeal (FM) and fish oil, respectively (Boyd, 2015). Feeding the cultured fish accounts for 40-75% of the production costs in aquaculture (FAO, 2018). Aquaculture feeding is dependent on FM because of its particular nutritive characteristics. FM is a significant protein source, and it is in short supply and exorbitant. Due to the depletion of fisheries and the degradation of the marine environment, FM production is decreasing; consequently, the quantity and quality of FM production varies. As a result, a rich amino acid profile and a different kind of protein are necessities for the future. (Robinson & Li, 1998). Animal-derived proteins integrated with other novel and conventional ingredients are effective sustainable alternatives (Gasco *et al*., 2016; Tran *et al.*, 2022). One of the potential animal protein sources, insects, have garnered great interest as an animal feed ingredient. One major reason for such interest is that insects have a more diverse concentration of high-quality essential amino acids than proteins of plant origin (Babji *et al.,* 2010; Hall, 1992; Hoppe *et al.,* 2008; Michaelsen *et al.,* 2009). Similarly, numerous insect species are reported to have higher protein levels than FM or soya-meal (SM) (Sanchez-Muros *et al.*, 2014). A huge gap in demand and supply of FM makes insect protein a good alternative source of feed protein. Silkworm rearing is one of the industrial insects used to produce silk. During silk yarn production, a huge amount of silkworm pupa is discarded as a byproduct. This byproduct can contain 50-70% crude protein which can go as high as 70% in defatted silkworm pupae (Karthick *et al.,* 2019; Yeruva *et al.,*2023). This makes silkworm pupae meal (SPM) a potent protein source for feed formulation (Nisha *et al.*, 2014).

The use of silkworm pupae protein as an alternate protein source has a rich history in the field of aquaculture. SPM is one of the best substitutes for FM in the diets of some cultured fish species, especially carps (Nandeesha *et al.*,2000; Rangacharyulu *et al.*,2003). A replacement rate of up to 50% of FM by SPM has been found to have no negative effects in most feed trials in broilers, layers, catfish, and common carp (Abdullo *et al.*, 2015; Fagoonee, 1983; Hong Ji *et al.*, 2013). However, numerous studies have observed adverse effects on growth and development when replacement rates were increased by more than 50% (Deshpande *et al.*, 1996; Reddy *et al,* 1991). Hence, the use of a replacement rate of less than 50% seems a safer choice.

The feeding habit of *L. rohita* has been studied by various scholars. There have been equal claims of *L. rohita* preferring phytoplanktons over zooplanktons (Dewan *et al.*, 1991) or both (Wahab *et al.,* 1994). Furthermore, Khan & Siddiqui (1973) stated that *L. rohita* prefers zooplanktons when in fingerling stage and phytoplanktons when it grows into an adult. Hence, it feeds on both plant and animal-based nutrients with varying preferences at different stages of its life cycle. Additionally, Hossain (1997) found that soybean meal had the highest actual and total protein digestibility among various plant and animal-based proteins, however, SPM was found to have a higher digestibility than that of FM in *L. rohita.* The objective of this study is to explore the potential of FM replacement with SPM in the diets of *L. rohita.* At the end of the experiment, we would be able to identify the best rate of replacement among the pre-specified treatments.

2. MATERIALS AND METHODS

2.1 Experiment details

The experiment was conducted in Cage system at Bhairahawa (110m asl; Coordinates: 27°28'48.5"N 83°26'50.2"E). The experimental set up was done in a newly constructed pond under cage culture system. Fertilizers (urea-20kg/ha and di-ammonium phosphate-15kg/ha) were applied in the pond to increase its fertility status. Underground water was pumped into the pond to maintain the depth of 1.5 meter and pond dynamics. There were 16 Nylon cages each of size 1m × 1m × 1m. Fingerlings of average size 3-4 grams were procured from a local fish hatchery, Rupandehi, and acclimatized for 20 days using the commercial feed (Crude Protein: 26.88%, Crude Fat: 8.81%, Crude Fiber: 1.50%, Ash: 10.85%) used by the source hatchery. After acclimation fingerlings were randomly distributed to each cage with densities of 12 fish/m^3. CRD was used having with 4 diets replicated 4 times. The treatments were randomized by the lottery method. Experimental pond was kept under continuous aeration using pond aerator to avoid oxygen deficiency.

Table 1. Treatment details

Treatments	Details
T_0	0% SPM
T_1	10% SPM
T_2	20% SPM
T_3	30% SPM

2.2 Feed ingredients

Most of the ingredients like soybean, mustard oil cake (MOC), rice bran, fishmeal, and vitamin premix were procured locally. Silk pupa was collected from silk processing farm at Gadhwa- Dang District and sundried for 3 days.The ingredients were cleaned, pulverized and sieved (0.5 mm sieve) to obtained fine powder.

2.3 Proximate analysis

Proximate analysis of all ingredients was done to make the feed iso-nitrogenous and iso-caloric. For proximate analysis, all the ingredients were weighed 250g each, kept in airtight plastic bags, and labeled. Those were sent to National Animal Feed and Livestock Quality Management Laboratory, Hariharbhawan, Lalitpur.

Table 2. Proximate analysis of ingredients

Ingredients	Crude Protein (%)	Crude Fiber (%)	Moisture (%)	Fat%
SPM	66.24	6.54	5.45	7.39
FM	67.47	6.28	5.45	-
SM	35.45	6.69	1.97	14.73
MOC	40.62	7.87	9.88	11.23
Rice Bran	13.45	8.42	9.90	12.87

2.4 Feed formulation

Pearson's Square method was used for the feed formulation. Crude protein (CP) of all the ingredients was taken into reference to produce an iso-nitrogenous feed containing 30% CP. All the feed ingredients were mixed thoroughly and the feed ball was made by adding 200 ml of water per kilogram feed. The ball was passed through a manually operated pellet machine to produce 1.5 mm feed pellets.

Table 3. Feed formulation details

Ingredients	T_1	T_2	T_3	T_4
FM	25	22.	20	17.
SPM	0	2.5	5	7.5
SM	6	5	5	5
MOC	7	8	8	8
Rice Bran	60	60	60	60
Vit	2	2	2	2
Total(gram	10	100	10	100

*Vit premix/Kg content: Vit A 7,00,000 I.U, Vit D3 70,000 I.U, Vit E 250mg, Co 250mg, Cu 1200mg, I 325mg, Fe 1500mg, Mg 6000mg, K 100mg, Na 5.9mg, Mn 1500mg, S 0.72%, Zn 9600mg, DL-Methionine 1000mg, Ca 25.5%, P 12.75%.

2.5 Feeding

Fishes were fed with a known amount of feed at 8% body weight for the initial month and adjusted to 5% and 3% in the following months. The total daily feed ration was divided into two equal parts, which were delivered every day at the morning (9 AM) and evening (4 PM).

2.6 Data collection

Dissolved Oxygen (DO), pH, and temperature were measured everyday using a portable water quality measurement kit. Fish weights were observed every fortnight. The final harvesting of fish was carried out after 115 days of stocking.

2.7 Growth parameters

Growth performance and feed utilization parameters were determined as follows**:**

- Mean Weight Gain (MWG) = Final Mean Weight (g) – Initial Mean Weight (g)
- Weight gain (g) = W2 – W1, where W1 (g) – the initial average (for each cage) body weight, W2 (g) – the average (for each cage) final fish weight
- Relative growth rate (g/day) = weight gain / t, where t – period in days
- Specific growth rate (SGR) (% per day): [(lnW2 –lnW51) / t] * 100, where ln – natural log
- Feed conversion ratio (FCR) **=** dry weight if feed intake (g)/wet body weight gain (g)

- Protein efficiency ratio (PER)=wet weight gain(g)/crude protein(g)
- Daily weight gain(g/day) = Average final weight (g) – Average stocked weight (g)/ culture period
- Survival Rate % = (total number of fish harvested/total number of fish stocked) × 100

2.8 Statistical Analysis

MS Excel was used for data management. R-stat was used for statistical analysis of observations. A one-way analysis of variance (ANOVA) was done to identify any significant differences among treatments. The value of α was set to 0.05. The DMRT method was used for post hoc analysis.

3. RESULTS AND DISCUSSION

3.1 Growth conditions

The average water temperature ranged between 15.57- 24.5 ˚C. In the initial two weeks, the temperature was more than 20˚C. Afterwards, the temperature dropped below 20 ˚C and started rising after the 11th week. The maximum temperature recorded was 24.5 ˚C. The water pH was in between the optimum level ranging between 6 and 9. The observed DO during the culture was between 8.03 mg/L and 10.18mg/L. All the recorded water quality parameters were in the desirable range (Kumar & Sharma, 2018; Nesara & Sheethal, 2020). During the research period, no any distinctly observable side effects were recorded.

3.2 Growth parameters

The processed data in Table 5 shows no significant difference (p>0.05) in stocking weights, relative weight gain and specific growth rate among the treatments. The weight gain of T_1 was found significantly different (p<0.05) and inferior from the rest of the treatments. Weight gains in T_2, T_3 and T_4 were statistically similar (p>0.05). Even though there was no significant difference between diets incorporated with SPM, it certainly promotes growth and development. SPM-incorporated diet exhibits higher digestibility in *L. rohita* (Hossain *et al.*, 1997) than in fishmeal. Furthermore, a mixture of protein sources is more effective than a single protein source (Samocha *et al.*, 2004; Viola *et al.*, 1988). The inclusion of 10-40% SPM in diets of *Cyprinus carpio* was reported to produce similar positive growth (Rahman *et al.*, 1996). Similar results were obtained by Olaniyi and Babasanmi (2013), who reported that the inclusion of 43.75% pupae protein in the diet of *L. rohita* yielded better growth, feed utilization and survival rate. The best survivability (0.70±0.02) was observed in T_4, which was significantly different (p<0.05) and superior to the rates in other treatments.

Table 4. Feed efficiency ratios

Treatments	FCR	PER
T_1	3.99±0.416	0.42± 0.0191
T_2	3.89 ±0.615	0.34± 0.025
T_3	3.206±0.461	0.38 ± 0.032
T_4	2.77 ±0.109	0.48± 0.064
F-test	ns	ns
CV %	21.97	16.57
LSD	1.43	0.364

FCR and PER of treatments were found non-significant (p>0.05) as mentioned in Table 3. The FCR levels are comparable to the findings (2.32 ± 0.87 to 5.00 ± 1.27) from Saeed *et al.* (2005) and higher than the range between 1.89 ± 0.01 and 2.50 ± 0.02 observed by Singh *et al.* (2006).

The observed FCR in this study seems higher because the reference studies were carried out in aqua tanks, which allows a more precise estimation of feed residue. However, in cage culture, the loss of unused feed is not accounted precisely. This also accounts for a lower PER observation in this study.

Table 5. Growth Parameter Analysis

Treatments	Mean stock weight(g)	Mean Relative Weight Gain (g)	Mean Daily Weight Gain (g)	Mean Final weight Gain (g)	Specific growth rate (SGR)	Survivability
T_1	5.14±0.52	2.45±0.283	0.098±0.002^b	12.34±0.37^b	1.55±0.058	0.56±0.05^b
T_2	5.34±0.85	2.61±0.47	0.105 ±0.01^a	13.13±0.27^a	1.59±0.090	0.63±0.09^{ab}
T_3	4.48±0.57	3.14±0.53	0.106±0.001^a	13.47±0.24^a	1.70±0.092	0.64±0.09^{ab}
T_4	3.86±0.11	3.46±0.13	0.107±0.00^a	13.33±12.31^a	1.76±0.023	0.70±0.02^a
F test	ns	ns	*	*	ns	*
CV %	21.48	22.88	3.06	3.068	7.52	7.62
LSD	1.90	1.252	0.0060	0.755	0.234	0.09

* Significant at p-value <0.05; ns= non-significant; CV=coefficient of variation; LSD= Least significant difference

4. CONCLUSION

Silkworm pupae meal is an effective alternative to fishmeal as it contains a good amount of crude protein source and other essential nutrients for the diet of *L. rohita*. The results of this study revealed that up to 30% replacement of FM by SPM has no observable adverse effects on the growth performance parameters of *L. rohita.* The replacement rate could be increased up to 50% in further research studies to replicate and test the results from previous studies. Based on the present finding, silkworm pupae can be used in aquafeed and its usage will increase the rationality of silkworm breeding and provide aqua-feed production with a high-quality source of animal protein. Therefore, the local commercial expansion of silkworm farming technology could be attributable to the sustainability of such alternative insect protein sources in aqua-feed industries.

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EVALUATION OF GROUNDNUT GENOTYPES FOR LEAF SPOT RESISTANCE AND HIGH YIELDING TRAITS IN TERAI CONDITIONS AT NAWALPUR, SARLAHI, NEPAL

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ABSTRACT

Keywords:
Groundnut,
high yielding,
leaf spot,
resistance

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ABSTRACT

Groundnut (Arachis hypogaea L.) serves as a vital summer oilseed crop in Nepal, facing significant yield constraints from Cercospora arachidicola Hori and Cercospora personatum (Berk. & Curt.) Deighton, causing early and late leaf spots, respectively. Study, conducted at the Oilseed Research Program (ORP), Nawalpur, Sarlahi, aimed to identify high-yielding, leaf spot-resistant genotypes to enhance production, productivity and reduce fungicide dependence. Over two summer seasons (2021-2022), nine genotypes of early maturing short duration set and 12 genotypes of medium duration set were evaluated using randomized complete block design with three replications. Observations on yield, yield attributing traits, and leaf spot disease severity i.e. percent disease index (PDI) were recorded. Among medium duration genotypes, ICGV 95412 (2638 kg/ha), ICGV 07220 (2633 kg/ha), and ICGV 07247 (2392 kg/ha) exhibited higher yield with moderate resistance (13-33% PDI) to late leaf spot. In case of early maturing short duration genotypes, ICGV 05155 (2559 kg/ha) and ICGV 07214 (2465 kg/ha) demonstrated high yield with moderately resistant (28-34% PDI) to early leaf spot. These identified genotypes showed promising for advanced breeding material, genetic stock and would be potential candidate for commercial cultivation, in areas prone to Cercospora leaf spots.

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop grown globally for its high oil content and protein-rich seeds. It is a major cash crop for small farmers in developing countries, and is also use for animal feed and in the manufacture of confectionery products (ORP, 2021). However, groundnut production is affected by various biotic and abiotic stresses, of which fungal diseases are the major constraints. Among the fungal diseases, early and late leaf spots caused by *Cercospora arachidicola* Hori and *Cercospora personatum* (Berk. & Curt.) deighton respectively, are the most important foliar diseases of groundnut worldwide (Subrahmanyam et al., 1985). Early leaf spot (ELS) is more prevalent in hot and humid regions, while late leaf spot (LLS) is more common in semi-arid and warm regions. Both diseases harm the plant by lowering the photosynthesis-capable leaf area and by promoting leaflet abscission, which results in severe defoliation (McDonald *et al.*, 1985). These two diseases can occur separately or together and cause significant yield losses, up to 50% or more, if left uncontrolled (Subrahmanyam *et al.,* 1985). To mitigate these losses, the most common control measures used are the application of fungicides and the use of resistant cultivars. However, the high cost of fungicides and the development of fungicide resistance in fungal pathogens have led to increased interest in the use of resistant cultivars as a sustainable and cost-effective approach to disease management.

Therefore, evaluating groundnut genotypes for resistance to ELS and LLS along with high yield contributing traits is an important step in the development of improved cultivars that are high yielder and resist1ant to these diseases. This experiment can help identify the genotypes that are highly resistant, moderately resistant, or susceptible to the diseases, and can aid in the selection of parents for breeding programs aimed at developing resistant cultivars. Moreover, the identification selection, and release of resistant varieties can also be great advantage to the farmers for cultivation in their fields, thereby reducing their dependence on chemical control measures and save time and money. This study, aimed to evaluate an early maturing short and medium duration sets of groundnut genotypes for their resistance to ELS and LLS, and to identify genotypes with high yield and levels of resistance to both diseases.

2. MATERIALS AND METHODS

Experiments were conducted with standard protocol during summer seasons of 2021 and 2022 at the Oilseed Research Program (ORP), Nawalpur, Sarlahi under natural epiphytotic conditions to identify high yielding and leaf spot resistant genotypes. A total of 9 genotypes including Baidehi and Jayanti as a check in coordinated varietal trial (CVT) early maturing short duration set and 12 genotypes including Rajarshi and B4 as a check in CVT medium duration set were tested in randomized complete block design with three replications during summer season of 2021 and 2022. Each plot area was 10.5 m^2 with row to row spacing of 30 cm and plant to plant spacing of 15 cm for both experiments and years. Application of fertilizer was done with 20:40:20NPK kg/ha and other package of agronomic practices for groundnut cultivation was followed as per the protocol developed by ORP (ORP, 2017).

Observation of leaf spot diseases severity was recorded from 10 randomly tagged plants/plot on the basis of a 1-9 scoring scale (Subrahmanyam *et al.*, 1985) during the vegetative and pod formation stage.

Description on leaf spots on modified (1-9) scale

Score	Leaf spots (Early and late leaf spot)
1	No disease
2	Lesions on lower leaves and no defoliation
3	Very few lesions on mid leaves, defoliation of some lower leaves
4	Lesions on lower and mid leaves but severe on lower leaves, defoliation of some lower leaflets
5	Lesions on lower and mid leaves over 50% defoliation of lower leaves
6	Also, lesions on top leaves, defoliation of some mid leaflets
7	Lesions on all leaves but severe on top leaves, defoliation of some mid leaves
8	Defoliation of lower and mid leaves, severe lesions on top leaves
9	Almost all leaves defoliated

The percent disease index (PDI) has been computed based on recorded data according to the formula (Wheeler, 1969). Phenological and morphological traits like days to flowering, days to maturity, plant height (cm), including pod yield (kg/ha) and its components viz. pod per plant, hundred seed weight (g) and shelling percentage were recorded. The meteorological data was recorded from the station located at ORP, Nawalpur.

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of individual ratings}}{\text{No of plants examined} \times \text{Maximum disease scale}} \times 100$$

All data were analyzed statistically using Microsoft Excel 2016 and GENSTAT 18th edition computer-based package programs. The relationship between disease index and grain yield was also calculated.

3. RESULTS AND DISCUSSION

3.1 Early maturing short duration genotypes

The combined mean performance of early maturing short duration groundnut genotypes for the pod yield, yield components and leaf spot severity during 2021-2022 shown in Table 1. Genotypes and year both varied significantly (P≤0.05) for days to flowering, days to maturity, plant height (cm), pod per plant, shelling percentage, hundred seed weight (g), pod yield (kg/ha) and leaf spot severity (both ELS and LLS) among the tested groundnut genotypes in combined analysis for two consecutive years. However, Genotype × Year interaction showed significant (P≤0.05) difference for the parameters days to maturity, plant height (cm), pod per plant, shelling percentage, hundred seed weight (g), and pod yield (kg/ha) only, and other parameters, days to flowering and leaf spot severity (both ELS and LLS), were at par (Table 1). The flowering days ranged from (25-30 days), maturity days (129-138 days), plant height (68.67-102.33 cm), pod per plant (9-54), shelling percentage (59-79 %), hundred seed weight (31-46 g), grain yield (1213-3025 kg/ha), early leaf spot severity (27.89-61.61%) and late leaf spot (22.48-59.27%). Genotype ICGV 05155 had highest mean yield (2559 kg/ha) and lowest disease severity for early (28.79%) and late (23.98%) leaf spot, followed by ICGV 07214 with a yield of 2465 kg/ha, 34.14% ELS, and 31.45% LLS severity. Similarly, ICGV 00350 had higher yield of 2258 kg/ha and lower ELS (38.36%) and LLS (36.26%) severity than the standard check Jayanti, which produced 2059 kg/ha in case of ELS and LLS severity of 38.57% and 35.59%, respectively. The lower yield (1262 kg/ha) with higher disease severity (61.11 %) for ELS and 58.44% for LLS was recorded in ICGV 00338 (Table 1).

Table 1. Combined analysis of pod yield, yield components and leaf spot severity of groundnut genotypes from early maturing short duration set during year 2021 and 2022 at ORP, Nawalpur, Sarlahi

Treatments	**FL_ days**	**Mat_ days**	**Pht (cm)**	**P /PL**	**Shell %**	**HSW (g)**	**PY (kg/ha)**	**LS severity (PDI %)**	
								ELS	**LLS**
Genotype (G)									
ICGV 00338	27	134	86.50	16	65.17	44.33	1262	61.11	58.44
ICGV 07213	27	134	86.67	24	69.67	44.17	1643	59.33	54.21
ICGV 07214	27	135	88.00	33	74.83	37.17	2465	34.14	31.45
ICGV 00350	28	136	84.67	31	75.00	36.17	2258	38.36	36.26
ICGV 05155	28	135	90.33	36	77.83	37.83	2559	28.79	23.98
ICGV 06319	27	135	87.00	25	69.50	36.33	1861	54.09	51.97
ICGV 99089	29	132	71.17	19	66.00	41.33	1605	60.37	55.29
BAIDEHI (Check)	28	134	82.83	28	70.83	43.83	1962	51.43	46.10
JAYANTI (Check)	27	137	86.50	29	72.83	31.67	2059	38.57	35.59
P-value	0.018	<.001	0.004	<.001	<.001	<.001	<.001	<.001	<.001
LSD 0.05	1.14	1.43	8.42	2.32	3.06	1.79	421.40	1.07	1.11
Year (Y)									
Year 2021 (Y1)	26	135	82.22	14	68.37	38.22	1671	48.04	42.56
Year 2022 (Y2)	29	134	87.48	39	74.19	40.19	2256	46.67	44.84
P-value	<.001	<.001	0.011	<.001	<.001	<.001	<.001	<.001	<.001
LSD 0.05 (Y)	0.54	0.68	3.97	1.09	1.44	0.84	198.70	0.51	0.52
Genotype ×Year									

ICGV 00338 × Y1	25	134	83.33	9	59	43.00	1311	61.61	57.60
ICGV 00338 × Y2	29	133	89.67	23	71	45.67	1213	60.61	59.27
ICGV 07213 × Y1	25	135	71.00	12	68	43.00	1352	60.29	52.61
ICGV 07213 × Y2	29	133	102.33	36	71	45.33	1933	58.37	55.81
ICGV 07214 × Y1	26	136	89.67	17	73	37.00	1962	34.64	30.61
ICGV 07214 × Y2	28	133	86.33	49	77	37.33	2968	33.63	32.29
ICGV 00350 × Y1	27	136	84.00	15	72	35.67	1937	38.76	35.60
ICGV 00350 × Y2	29	137	85.33	46	78	36.67	2578	37.97	36.92
ICGV 05155 × Y1	26	136	90.67	18	77	38.00	2092	29.69	22.48
ICGV 05155 × Y2	29	135	90.00	54	79	37.67	3025	27.89	25.49
ICGV 06319 × Y1	25	135	85.33	12	67	35.67	1629	54.48	51.31
ICGV 06319 × Y2	29	135	88.67	38	72	37.00	2092	53.69	52.63
ICGV 99089 × Y1	27	135	68.67	12	60	38.67	1321	61.33	53.70
ICGV 99089 × Y2	30	129	73.67	25	72	44.00	1889	59.42	56.88
BAIDEHI × Y1	27	134	76.33	14	69	41.67	1676	52.43	44.43
BAIDEHI × Y2	28	134	89.33	41	73	46.00	2248	50.43	47.76
JAYANTI × Y1	25	138	91.00	15	71	31.33	1759	39.13	34.65
JAYANTI × Y2	28	135	82.00	43	75	32.00	2359	38.01	36.52
Grand mean	27	135	84.85	27	71.28	39.20	1963	47.35	43.70
Min	25	129	68.67	9	59.00	31.33	1213	27.89	22.48
Max	30	138	102.33	54	78.67	46.00	3025	61.61	59.27
P-value (G×Y)	0.122	0.003	0.002	<.001	<.001	0.043	<.001	0.855	0.295
LSD 0.05 (G×Y)	1.61	2.03	11.90	3.28	4.33	2.53	596.00	1.52	1.57
CV,%	3.50	0.90	8.50	7.40	3.70	3.90	18.30	1.90	2.20

Note: FL_days- days to flowering, Mat_days- days to maturity, Pht- plant height, P/PL- pod per plant, Shell%- Shelling percentage, HSW- hundred seed weight, PY- pod yield, LS- leaf spot, ELS- early leaf spot and LLS- late leaf spot, PDI- percent disease index, g-gram, cm-centimeter, kg/ha- kilogram per hectare, ICGV-ICARDA groundnut variety

Relationship between pod yield and leaf spot severity in early maturing short duration groundnut genotypes

The best fit, with adjusted R^2 = 88%, and negative correlation coefficient (r = -0.95) for early leaf spot (Figure 1) while adjusted R^2 = 90% with r = -0.96 for late leaf spot (Figure 2) showed a substantial linear negative association between pod yield and leaf spot severity in early maturing short duration groundnut genotypes. Consequently, as disease severity (PDI) of early leaf spot (Figure 1) and late leaf spot (Figure 2) increased, the yield decreased. The projected linear regression line has a decreasing slope as well, i.e y = -0.027x+102, with a regression coefficient R^2 = 0.89 in case of early leaf spot and y = -0.027x+97.81, with a regression coefficient R^2 = 0.91 in case of late leaf spot, where "y" denoted predicted pod yield (kg/ha) of early maturing short duration groundnut genotypes and "x" stood for percent disease index of early and late leaf spot of groundnut genotypes respectively (Figure 1 and 2).

3.2 Medium duration genotypes

The combined mean performance of medium duration groundnut genotypes for the pod yield, yield components and leaf spot severity during 2021-2022 shown in Table 2. Genotypes and year both varied significantly P≤0.05) for days to flowering, days to maturity, plant height (cm), pod per plant, shelling percentage, hundred seed weight (g), pod yield (kg/ha) and leaf spot severity (both ELS and LLS) among the tested groundnut genotypes in

combined analysis for two consecutive years. However, Genotype × Year interaction showed statistically significant (P≤0.05) difference for the parameters days to flowering, days to maturity, plant height (cm), pod per plant, shelling percentage, and leaf spot severity (both ELS and LLS) only, and other parameters, hundred seed weight (g), and pod yield (kg/ha) were at par (Table 2).

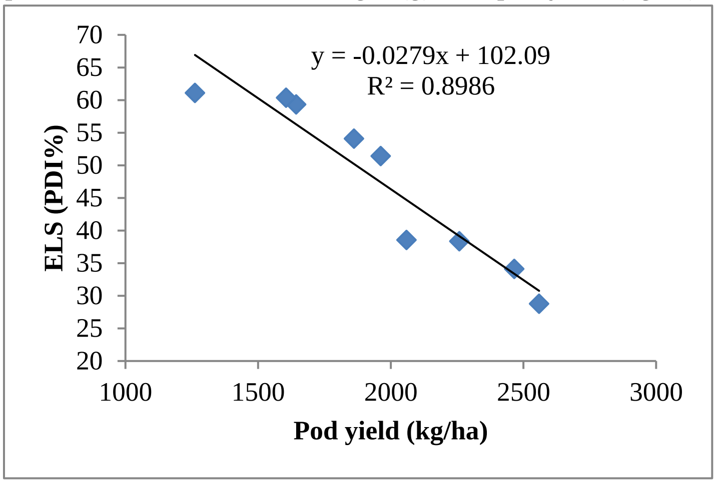

Figure 1. Relationship between pod yield and early leaf spot severity in early maturing short duration groundnut genotypes during 2021 and 2022 at ORP, Nawalpur, Sarlahi

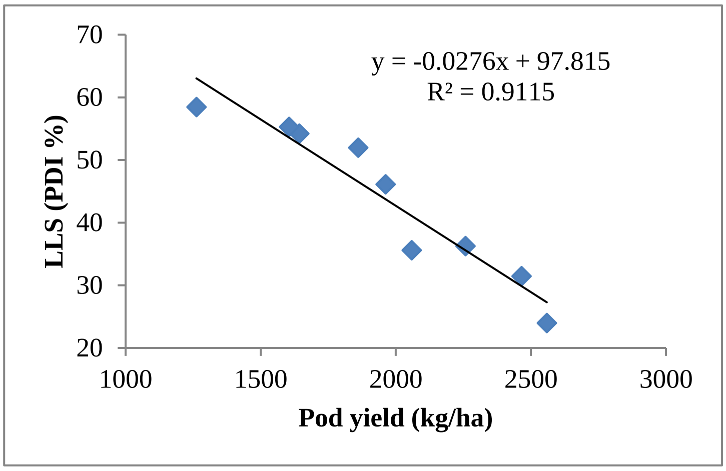

Figure 2. Relationship between pod yield and late leaf spot severity in early maturing short duration groundnut genotypes during 2021 and 2022 at ORP, Nawalpur, Sarlahi

The flowering days ranged from (26-34 days), maturity days (136-144 days), plant height (64-90 cm), pod per plant (15-46), shelling percentage (64-82 %), hundred seed weight (36-50 g), grain yield (1251-3070 kg/ha), early leaf spot severity (12.21-62.16%) and late leaf spot (13.04-63.27%). Genotype ICGV 95412 had the highest mean pod yield (2638 kg/ha) and the lowest disease severity for early (12.49%) and late (13.18%) leaf spot, followed by ICGV 07220 with a yield of 2633 kg/ha, 21.83% ELS, and 23.68% LLS severity. In addition, ICGV 07247 was recorded with a yield of 2392 kg/ha and lower ELS (27.29%) and LLS (33.35%) severity. The lower yield (1403 kg/ha) with higher disease severity (61.61 %) for ELS and 62.99% for LLS was recorded in standard check variety B-4 (Table 2).

Table 2. Combined analysis of pod yield, yield attributing traits and leaf spot severity of groundnut genotypes from medium duration set during year 2021 and 2022 at ORP, Nawalpur, Sarlahi

Treatments	FL_ days	Mat_ days	Pht (cm)	P /PL	Shell %	HSW (g)	PY (kg/ha)	LS severity (PDI %)	
								ELS	LLS
Genotype (G)									
ICGV 98180	31	139	67.50	26	68	39.83	2029	42.83	46.20
ICGV 97171	30	140	77.33	26	74	37.67	2137	34.78	42.18
ICGV 06227	31	139	84.50	23	69	37.50	1548	58.83	61.61
ICGV 98184	31	142	69.67	25	70	40.83	1922	43.01	46.11
ICGV 06423	30	141	75.50	32	74	41.83	2333	31.82	31.08
ICGV 07220	30	141	74.17	41	77	44.34	2633	21.83	23.68
ICGV 95412	30	140	72.17	45	79	49.84	2638	12.49	13.18
ICGV 07247	29	140	71.83	39	77	43.00	2392	27.29	33.35
ICGV 06211	30	140	87.17	21	68	37.67	1582	53.19	55.73
ICGV 99171	32	141	75.33	26	69	38.17	2129	36.08	43.01
RAJARSHI (Check)	30	139	73.83	25	73	41.17	1992	35.71	41.72
B4 (Check)	30	140	78.50	24	68	38.33	1403	61.61	62.99
P-value	0.008	0.01	<.001	<.001	0.007	<.001	<.001	<.001	<.001
LSD 0.05	1.26	1.57	7.02	3.07	5.57	4.62	452.90	1.54	1.38
Year (Y)									
Year 2021 (Y1)	28	141	74.86	25	69	40.14	1811	36.91	42.43
Year 2022 (Y2)	33	139	76.39	34	75	41.56	2312	39.67	41.05
P-value	<.001	<.001	0.289	<.001	<.001	0.137	<.001	<.001	<.001
LSD 0.05 (Y)	0.51	0.64	2.87	1.25	2.28	1.89	184.90	0.63	0.56
Genotype ×Year									
ICGV 98180 × Y1	29	141	64.00	19	65	38.00	1600	49.95	55.50
ICGV 98180 × Y2	34	137	71.00	33	70	41.67	2457	35.71	36.91
ICGV 97171 × Y1	27	140	82.00	21	67	38.33	1740	35.15	47.73
ICGV 97171 × Y2	33	140	72.67	30	80	37.00	2533	34.41	36.63
ICGV 06227× Y1	27	139	79.67	16	65	36.33	1381	57.72	62.16
ICGV 06227× Y2	34	138	89.33	30	72	38.67	1714	59.94	61.05
ICGV 98184× Y1	29	144	74.00	22	67	40.00	1924	33.30	38.11
ICGV 98184 × Y2	33	139	65.33	27	73	41.67	1921	52.73	54.11
ICGV 06423× Y1	28	141	70.33	23	69	40.00	1930	31.45	26.64
ICGV 06423 × Y2	33	141	80.67	41	78	43.67	2737	32.19	35.52
ICGV 07220× Y1	27	141	76.67	38	74	43.67	2197	21.09	24.05
ICGV 07220 × Y2	33	140	71.67	43	81	45.00	3070	22.57	23.31
ICGV 95412× Y1	27	142	72.33	46	76	50.00	2362	12.21	13.32
ICGV 95412 × Y2	33	139	72.00	43	82	49.67	2914	12.77	13.04
ICGV 07247× Y1	26	139	69.00	36	73	42.00	2038	25.53	38.85
ICGV 07247 × Y2	32	142	74.67	43	81	44.00	2746	29.05	27.84
ICGV 06211 × Y1	29	141	84.33	16	65	37.00	1444	52.17	56.24
ICGV 06211 × Y2	31	138	90.00	26	72	38.33	1721	54.21	55.22
ICGV 99171 × Y1	30	142	71.67	22	67	39.00	1854	33.30	44.40
ICGV 99171 × Y2	33	140	79.00	31	71	37.33	2403	38.85	41.63

RAJARSHI × Y1	26	142	71.00	25	71	41.33	2016	29.97	38.85
RAJARSHI × Y2	33	136	76.67	25	74	41.00	1968	41.44	44.59
B4 × Y1	26	141	83.33	15	64	36.00	1251	61.05	63.27
B4 × Y2	34	140	73.67	33	72	40.67	1556	62.16	62.72
Grand mean	30	140	75.63	29	72	40.85	2062	38.29	41.74
Min	26	136	64.00	15	64	36.00	1251	12.21	13.04
Max	34	144	90.00	46	82	50.00	3070	62.16	63.27
P-value (G×Y)	<.001	<.001	0.017	<.001	0.05	1.00	0.149	<.001	0.05
LSD 0.05 (G×Y)	1.78	2.22	9.93	4.34	7.88	6.53	640.50	2.18	1.95
CV,%	3.60	1.00	8.00	9.00	6.70	9.70	18.90	3.50	2.80

Note: FL_days- days to flowering, Mat_days- days to maturity, Pht- plant height, P/PL- pod per plant, Shell%- Shelling percentage, HSW- hundred seed weight, PY- pod yield, LS- leaf spot, ELS- early leaf spot and LLS- late leaf spot, PDI- percent disease index, g-gram, cm-centimeter, kg/ha- kilogram per hectare, ICGV-ICARDA groundnut variety

Relationship between pod yield and leaf spot severity in medium duration groundnut genotypes

The best fit, with adjusted R^2 = 98%, and negative correlation coefficient (r = -0.99) for early leaf spot (Figure 3) while adjusted R^2 = 89% with r = -0.95 for late leaf spot (Figure 4) showed a substantial linear negative association between pod yield and leaf spot severity in medium duration groundnut genotypes. Consequently, as disease severity (PDI) of early leaf spot (Figure 3) and late leaf spot (Figure 4) increased, the yield decreased. The projected linear regression line has a decreasing slope as well, i.e y = -0.044x + 117.0, with a regression coefficient R^2 = 0.98 in case of early leaf spot and y = y = -0.044x + 122.1, with a regression coefficient R^2 = 0.90 in case of late leaf spot , where "y" denoted predicted pod yield (kg/ha) of medium duration groundnut genotypes and "x" stood for percent disease index of early and late leaf spot of groundnut genotypes respectively (Figure 3 and 4).

The climatic factors during crop growing period (June to November) at ORP, Nawalpur, Sarlahi for both consecutive years 2021 and 2022 is shown in Figure 5.

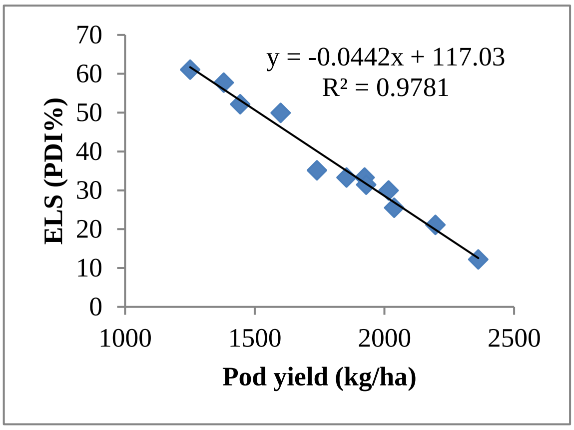

Figure 3. Relationship between pod yield and early leaf spot severity in medium duration groundnut genotypes during 2021 and 2022 at ORP, Nawalpur, Sarlahi

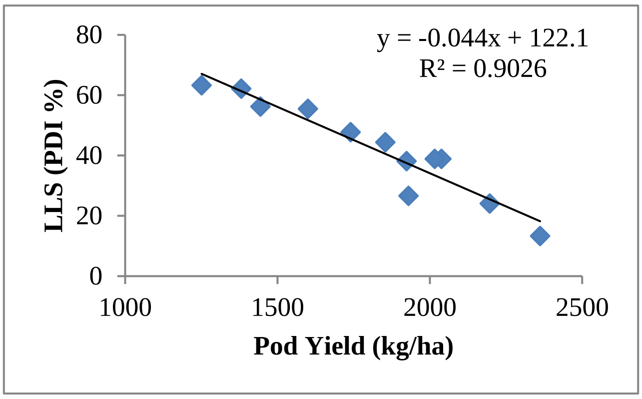

Figure 4. Relationship between pod yield and late leaf spot severity in medium duration groundnut genotypes during 2021 and 2022 at ORP, Nawalpur, Sarlahi

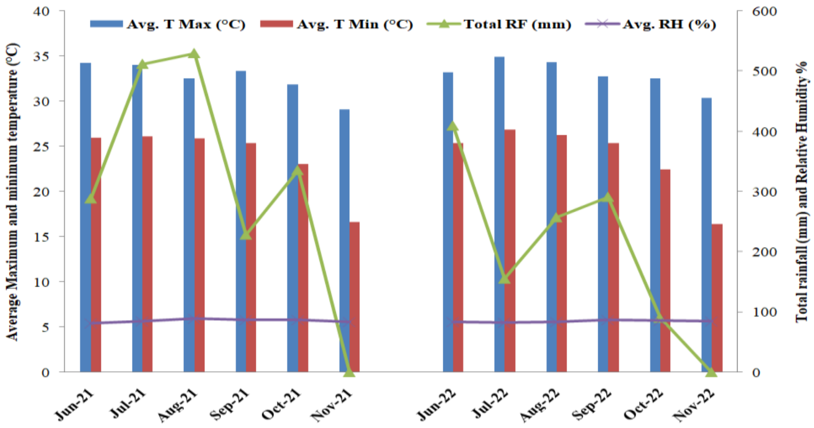

Figure 5: Weather parameters during crop growing period (June-November) at ORP, Nawalpur, Sarlahi Nepal during year 2021 and 2022.

The leaf spot disease has historically been one of the main biotic challenges restricting groundnut production with yield loss on a global scale. One of the most successful methods for developing varieties resistant to leaf spot is breeding for host plant resistance (Denwar *et al.*, 2021, Mohammed *et al.*, 2018). The results from many earlier trials in the field have shown that resistant groundnut varieties yield 55–60% more than native cultivars that are susceptible to the leaf spot disease (Desmae & Sones, 2017, Kankam *et al.*, 2020, Mohammed *et al.*, 2018, Rani *et al.*, 2017). This suggests that growing resistant varieties has been most practical and cost-effective way to manage groundnut leaf spot diseases while italso increases crop yield. The results of this study suggest that there is genetic variability among groundnut genotypes for resistance to leaf spot diseases and yield potential. This finding has been consistent with previous studies that reported significant differences among groundnut genotypes for disease resistance and yield. findings of this study has been similar with the result of Thakur *et al.* (2012), who reported that ICGV 05155, ICGV 00350 and ICGV 07214 showed resistance to leaf spot diseases and high yield potential. Similarly, ORP (2018) reported that medium duration genotypes ICGV 95412, ICGV 07220 and ICGV 07247 had high yield potential and moderate resistance to leaf spot diseases.

Identification of resistant genetic stocks is important for the development of effective breeding programs that aim to develop cultivars with improved resistance to leaf spot diseases. Resistance to leaf spot disease is a complex trait that is controlled by multiple genes, and therefore, the identification of resistant genotypes is an important step towards the development of molecular markers for resistance genes (Kankam *et al.,* 2020, Mohammed *et al.,* 2018). In addition, the identification of high-yielding genotypes is important for increasing groundnut production and improving food security in developing countries. Pandey *et al.* (2017) found that groundnut plants infected with *Cercospora personatum* exhibited a significant increase in the activity of defense-related enzymes such as peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase. This suggests that groundnut activates its defense mechanisms in response to *C. personatum* infection. In another study by Sivasakthi *et al.* (2014), it was observed that groundnut plants treated with a plant growth-promoting rhizobacterium (PGPR) showed enhanced resistance to early leaf spot disease. The researchers suggested that this enhanced resistance was due to the activation of the plant's systemic defense response, which was mediated by the production of phytohormones such as salicylic acid and jasmonic acid. A study by Khedikar *et al.* (2010) illustrated the expression of defense-related genes in groundnut plants infected with *C. personatum*. The researchers found that several defense-related genes, such as those encoding chitinases and β-1,3-glucanases, were unregulated in response to the pathogen. These genes are involved in the production of pathogenesis-related proteins, which have antifungal properties. In a review by Sharma *et al.* (2017), the authors discussed the role of plant secondary metabolites in groundnut's defense against *C. personatum*. The review highlighted the role of flavonoids, phenolics, and lignins in inhibiting fungal growth and development.

4. CONCLUSION

There is a significant variation in both resistance to leaf spot and yield potential among different genotypes. The results of this study suggest that selection of groundnut genotypes resistance to leaf spots and higher yield has been attainable. Genotypes ICGV 05155 and ICGV 07214 from early maturing short duration set and ICGV 95412, ICGV 07220 and ICGV 07247 from medium duration set had exhibited both moderately resistant to leaf spots and high yield potential, suggesting that these genotypes could be useful in breeding program aimed for varietal improvement of groundnut resistance to leaf spots and higher yield.

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STUDY OF DIFFERENT SOWING DATES ON MAIZE APHID (*Rhopalosiphum maidis* (Fitch)) INFESTATION, AND YIELD OF MAIZE VARIETIES IN INNER TERAI REGION OF NEPAL

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ABSTRACT

Maize aphid (Rhopalosiphum maidis Fitch) is a polyphagous pest threatening maize yield and quality globally. Field experiments at the National Maize Research Program (NMRP) in Rampur, Chitwan, evaluated the effect of varied sowing dates on maize aphid infestation and yield during 2019 and 2020. The study used a randomized complete block design with three replications. Plot sizes were 6 m², with rows spaced 60 cm apart and plants 25 cm apart. Two maize varieties, Manakamana 9 (ZM-401) and Rampur Hybrid-14 (RML-86/RML-96), were tested across eight sowing dates from October to December. Data collected included aphid incidence, severity, plant height (cm), grain yield (t/ha), and thousand seed weight (g). The highest aphid colony per plant (10.38) and lowest yield (1.78 t/ha) were found in Manakamana-9 sown on October 12, while the lowest aphid colony (0.23) and highest yield (6.94 t/ha) were noted in Rampur Hybrid-14 sown on December 11. Aphid incidence began in January and peaked in late February, correlating with temperatures of 26.99°C (max), 14.68°C (min), relative humidity (≥80%), and no rainfall. These results guide farmers in selecting optimal sowing dates to reduce aphid infestation and enhance maize yield.

1. INTRODUCTION

Maize is one of the top cereal crops in Nepal contributing significantly to the food security and livelihoods of rural Nepali people and accounts for 27% of the country's total production of edible cereal grains, 1.64% of overall GDP, and 6.83% of agricultural GDP (MoALD, 2022).The maize cultivated area in Nepal is 979,776 ha, with the mid-hill accounting for more than 70% of the total (MoALD, 2022). The maize aphid (*Rhopalosiphum maidis* (Fitch)) is a widespread, polyphagous pest that feeds on more than 182 plant species (Alam et al., 2014). It is a significant economic pest related with the production of maize (Neupane et al., 2022). All parts of maize are infested by *Rhopalosiphum maidis*, but the tassel sustains the most damage, resulting in different degrees of barrenness, decreased grain yields, and virus transmission (Carena & Glogoza, 2004).The pest causes direct damage to plant by sucking the phloem nutrients and hindering photosynthesis as a result of sooty mould. It is reported to cause yield loss as high as 40 percent (Everly, 1960).The factors that hinder the production of maize include numerous biotic and abiotic stresses that occur at various crop stages (Neupane & Subedi, 2019). This pest has become a severe threat and emerging pest of maize during the last 3-4 years in Nepal that the aphid infestations hang around maize from the last week of January to the third week of March (NMRP, 2020). Aphid infestation in maize causes defective pollination and transmit persistent and non-persistent viral diseases with the yield loss of about 10-20% annually in maize crop (Subedi, 2015). Most of the maize farmers in Nepal are facing a yield loss ranging from 10 to 20% (NMRP, 2019). The colonies of maize aphids can be found on or near tassels or leaf whorl in most maize fields where, particularly during winter season, up to 50% plant infestation is a common scene at the mid hill and terai region of Nepal (NMRP, 2020). The infestation of maize aphids is influenced by various factors, including sowing date, weather conditions, and the maize variety. Therefore, it is important to study the effect of different sowing dates on maize aphid infestation and its percussion on the yield of maize varieties. In this study, we aimed to evaluate the effect of different sowing dates on maize aphid infestation and yield of different maize varieties. The study contributes to the understanding of the relationship within sowing dates, maize aphid infestation, and yield of different maize varieties, which can help farmers in deciding the optimal sowing time and selecting maize variety for better pest management to get higher maize yield. This study presents an opportunity to develop an alternative aphid management strategy that can be integrated into an integrated pest management program.

2. MATERIALS AND METHODS

The experiments were organized following a randomized complete block design (RCBD) with three replications of treatments during the winter season of 2019 and 2020 under the natural maize aphid infestation at maize research field in the premises of National Maize Research Program (NMRP) Rampur, Chitwan. The geographical location of NMRP is 27°40' N latitude, 84°19' E longitude and an altitude of 228 meters above sea level. It has a humid and subtropical climate with cool winter and hot summer. The soil is generally acidic (pH 4.6-5.7), light-textured and sandy loam. The average total annual rainfall was 2215.30 mm with a distinct monsoon period (>75% of annual rainfall) from mid-June to mid-September. Two maize varieties (Manakamana-9- open pollinated) and (Rampur Hybrid-14 hybrid) were sown on eight different sowing dates viz. October 2(S1), October 12(S2), October 22(S3), November 1(S4), November 11(S5), November 21(S6), December 1(S7) and December 11of two consecutive years 2019 and 2020. The plot size was two rows of five-meter long with a spacing of 60 cm × 25 cm i.e. 6 m^2. The source of both released maize varieties was the maize breeding program of NMRP. The recommended dose of fertilizers ($N:P_2O_5:K_2O$ kg/ha) for the full season open-pollinated and hybrid maize, respectively, was 120:60:40 and 180:60:40 with farm yard manure 10 t/ha and seed rate was 20 kg/ha. Most of the cultural practices were followed as recommended (NMRP, 2017).

Data on aphid incidence (aphid colony per plant), aphid-infested plant per plot, plant height (cm), thousand-grain weight (g) and grain yield (t/ha) were recorded (NMRP, 2018). The maize grain yield was recorded from each plot and adjusted at a 15% moisture level and then converted to t/ha. The meteorological data was recorded from the weather station located at the NMRP, Rampur, Chitwan. The daily meteorological parameters like maximum temperature, minimum temperature, relative humidity, and rainfall were recorded and then converted on a weekly basis against the standard meteorological week (SMW) with correspondence to the weekly colony count of aphids. The pooled data of aphid colony per plant over the years was taken for the correlation and regression analysis. The weekly population of aphids was considered as a dependent variable and correlated with corresponding weekly weather parameters as independent variables. On the basis of significant correlation coefficients between aphid population and weather variables, a stepwise regression study was performed to develop the statistical forewarning model. All data were analyzed statistically using Microsoft Excel 2016 and

GENSTAT 18thedition computer package programs.

3. RESULTS AND DISCUSSION

The combined mean performance of maize varieties for the aphid colony per plant, aphid-infested plant percentage, grain yield, and yield parameters during 2019-2020 are shown in Table 1. Varieties, sowing dates and their interaction (Varieties × Sowing dates) varied significantly (P≤0.05) for aphid colony per plant, aphid-infested plant percentage, plant height (cm), grain yield (t/ha) and thousand seed weight (g) in combined analysis for two consecutive years. Except for plant height (cm) and thousand seed weight (g), the rest of the variables for aphid incidence and infestation with grain yield (t/ha) showed significant differences in two consecutive years (Table 1).Year × Varieties, Year × Sowing dates, and Year× Varieties × Sowing dates interactions, were statistically at par with all measured variables. The aphid colony per plant ranged from (0.18-10.87), aphid infested plant (4.14-49.33%), plant height (163.17-206.42 cm), grain yield (1.45-7.11 t/ha) and thousand seed weight (253-415.33 g).The combined analysis of two year's results showed that the higher aphid colony per plant (10.38) with lower yield (1.78 t/ha) was recorded in Manakamana-9 shown on October 12 while lower aphid colony per plant (0.23) with higher yield (6.94 t/ha) was recorded in Rampur Hybrid-14 maize shown on December 11 (Table 1).

Table 1. Combined analysis of aphid colony, aphid infested plant, grain yield and yield parameters of maize varieties in different sowing dates during year 2019 and 2020 at NMRP, Rampur, Chitwan

Treatments	Aphid colony/ plant†	Aphid infested Plant (%)†	Plant height (cm) †	Grain yield (t/ha) †	Tousand seed weight(g) †
Year					
2019	3.85	26.20	187.94	4.22	326.69
2020	3.32	24.86	186.88	4.80	335.73
P-value	0.005	<.001	0.642	0.005	0.075
LSD (0.05)	0.37	0.56	4.55	0.40	9.98
Varieties					
Manakamana 9 (ZM-401)	4.59	30.66	176.64	3.58	312.48
Rampur Hybrid-14 (RML-86/RML-96)	2.59	20.41	198.18	5.45	349.94
P-value	<.001	<.001	<.001	<.001	<.001
LSD (0.05)	0.37	0.56	4.55	0.40	9.98
Sowing dates					
Oct. 2	4.79	33.12	190.21	5.03	329.00
Oct. 12	8.54	42.72	178.29	2.59	335.17
Oct. 22	7.08	35.78	186.00	5.37	326.33
Nov.1	5.86	31.64	187.58	5.39	309.33
Nov. 11	0.83	24.11	189.08	4.11	285.83
Nov. 21	0.68	17.18	178.25	3.40	344.83
Dec. 1	0.60	12.61	201.58	4.51	390.50
Dec. 11	0.33	7.09	188.25	5.70	328.67
P-value	<.001	<.001	<.001	<.001	<.001
LSD (0.05)	0.73	1.12	9.09	0.80	19.96
Year × Variety					
2019 × Manakamana-9	4.77	31.32	177.17	3.29	308.92
2019 × Rampur Hybrid-14	2.94	21.07	198.71	5.16	344.46
2020 × Manakamana-9	4.40	29.99	176.10	3.87	316.04

2020 × Rampur Hybrid-14	2.24	19.74	197.65	5.74	355.42
P-value	0.36	1.00	1.00	1.00	0.702
LSD (0.05)	0.52	0.79	6.43	0.57	14.11
Year × Sowing date					
2019 × Oct. 2	5.02	33.87	189.33	4.60	327.50
2019 × Oct. 12	9.30	42.30	179.17	2.27	330.00
2019 × Oct. 22	7.77	36.30	187.50	5.42	314.67
2019 × Nov. 1	5.90	32.52	185.83	5.22	305.33
2019 × Nov. 11	0.87	25.08	190.83	4.03	284.67
2019 × Nov. 21	0.37	18.10	180.00	2.65	334.67
2019 × Dec. 1	0.67	13.36	203.33	4.04	390.00
2019 × Dec. 11	0.93	8.07	187.50	5.54	326.67
2020× Oct. 2	4.57	32.37	191.08	5.45	330.50
2020 × Oct. 12	7.78	43.15	177.42	4.15	340.33
2020 × Oct. 22	6.38	35.25	184.50	5.19	338.00
2020× Nov. 1	5.82	30.77	189.33	5.57	313.33
2020 × Nov. 11	0.78	23.13	187.33	4.18	287.00
2020× Nov. 21	0.28	16.25	176.50	4.99	355.00
2020 × Dec. 1	0.52	11.86	199.83	2.92	391.00
2020× Dec. 11	0.43	6.12	189.00	5.97	330.67
P-value	0.26	0.22	0.99	0.47	0.92
LSD (0.05)	1.03	1.58	12.86	1.13	28.23
Variety × Sowing date					
Manakamana-9 × Oct. 2	5.98	37.92	174.88	4.38	291.50
Manakamana-9 × Oct. 12	10.38	48.91	165.79	1.78	303.50
Manakamana-9× Oct. 22	9.66	47.87	178.50	4.91	314.33
Manakamana-9 × Nov. 1	7.63	41.70	175.08	3.85	320.17
Manakamana-9 × Nov. 11	0.98	26.22	178.25	2.22	268.50
Manakamana-9 × Nov. 21	0.43	19.60	164.92	2.33	350.50
Manakamana-9 × Dec. 1	0.77	13.95	201.58	3.66	366.67
Manakamana-9 × Dec. 11	0.88	9.08	174.08	5.49	284.67
RampurHybrid-14 × Oct. 2	3.61	28.32	205.54	5.67	366.50
RampurHybrid-14 × Oct. 12	6.71	36.54	190.79	3.40	366.83
RampurHybrid-14 × Oct. 22	4.49	23.68	193.50	6.48	338.33
RampurHybrid-14 × Nov. 1	4.09	21.59	200.08	4.48	298.50
RampurHybrid-14 × Nov. 11	0.68	22.00	199.92	5.24	303.17
RampurHybrid-14 × Nov. 21	0.48	14.75	191.58	5.99	339.17
RampurHybrid-14× Dec. 1	0.42	11.27	201.58	5.37	414.33
RampurHybrid-14× Dec/ 11	0.23	5.11	202.42	6.94	372.67
P-value	<.001	<.001	0.033	<.001	<.001
LSD (0.05)	1.03	1.58	12.86	1.13	28.23
Year × Variety ×Sowing date					
2019 × Manakamana-9 × Oct. 2	6.20	38.67	174.00	3.96	290.00
2019 × Manakamana-9 × Oct. 12	10.87	48.48	166.67	1.45	300.00
2019 × Manakamana-9 × Oct. 22	9.93	48.40	180.00	4.64	302.67
2019 × Manakamana-9 × Nov. 1	7.67	42.58	173.33	3.67	306.67
2019 × Manakamana-9 × Nov. 11	1.00	27.19	180.00	2.14	284.00

2019 × Manakamana-9 × Nov. 21	0.47	20.53	166.67	1.58	338.67
2019 × Manakamana-9 × Dec. 1	0.87	14.70	203.33	3.18	366.67
2019 × Manakamana-9 × Dec. 11	1.13	10.05	173.33	5.67	282.67
2019 × RampurHybrid-14 × Oct. 2	3.83	29.07	204.67	5.24	365.00
2019× RampurHybrid-14 × Oct. 12	7.73	36.11	191.67	3.08	360.00
2019 × RampurHybrid-14 × Oct. 22	5.60	24.20	195.00	6.20	326.67
2019 × RampurHybrid-14 × Nov. 1	4.13	22.46	198.33	5.41	304.00
2019 × RampurHybrid-14 × Nov. 11	0.73	22.97	201.67	5.92	285.33
2019 × RampurHybrid-14 × Nov. 21	0.27	15.67	193.33	3.73	330.67
2019 × RampurHybrid-14 × Dec. 1	0.47	12.02	203.33	4.89	413.33
2019 × RampurHybrid-14 × Dec. 11	0.73	6.09	201.67	6.76	370.67
2020 × Manakamana-9 × Oct. 2	5.75	37.17	175.75	4.81	293.00
2020 × Manakamana-9 × Oct. 12	9.88	49.33	164.92	2.10	307.00
2020 × Manakamana-9 × Oct. 22	9.38	47.35	177.00	5.19	326.00
2020 × Manakamana-9 × Nov. 1	7.58	40.83	176.83	4.02	333.67
2020 × Manakamana-9 × Nov. 11	0.95	25.24	176.50	2.29	253.00
2020 × Manakamana-9 × Nov. 21	0.38	18.68	163.17	3.08	362.33
2020 × Manakamana-9 × Dec. 1	0.67	13.20	199.83	4.13	366.67
2020 × Manakamana-9 × Dec. 11	0.63	8.10	174.83	5.32	286.67
2020 × RampurHybrid-14 × Oct. 2	3.38	27.57	206.42	6.09	368.00
2020× RampurHybrid-14 × Oct. 12	5.68	36.96	189.92	3.73	373.67
2020 × RampurHybrid-14 × Oct. 22	3.38	23.15	192.00	6.75	350.00
2020 × RampurHybrid-14 × Nov. 1	4.05	20.71	201.83	5.06	293.00
2020 × RampurHybrid-14 × Nov. 11	0.62	21.02	198.17	6.07	321.00
2020 × RampurHybrid-14 × Nov. 21	0.18	13.82	189.83	5.23	347.67
2020 × RampurHybrid-14 × Dec. 1	0.37	10.52	199.83	5.84	415.33
2020 × RampurHybrid-14 × Dec. 11	0.23	4.14	203.17	7.11	374.67
Grand mean	3.59	25.53	187.41	4.51	331.21
Min	0.18	4.14	163.17	1.45	253.00
Max	10.87	49.33	206.42	7.11	415.33
P-value	0.90	1.00	1.00	1.00	0.406
LSD (0.05)	1.46	2.23	18.19	1.60	39.92
CV%	25.00	5.40	5.90	21.80	7.40

†means of three replications, ZM-Zimbabwe maize, RML- Rampur maize line

3.1 Population dynamics of maize aphid

The incidence of aphids started from the second standard meteorological week (SMW) (0.60 aphid colony per plant) during years, 2019 and 2020. Initially, the population was low and gradually increased and reached its peak (8.54 aphids colonies/plant) on the 8th standard meteorological week. So far as the effect of weather parameters is considered, the maximum aphid colony was recorded during the 8th SMW when corresponding weather parameters viz. average maximum and minimum mean temperature (°C), average relative humidity (%) and total rainfall (mm) were 26.99, 14.68, 79.90 and 0, respectively over 2019 and 2020 (Table 3). The meteorological data during the crop period (2019- 2021) are shown in Table 2.

Table 2. Meteorological data during crop period (2019- 2021) at NMRP, Rampur, Chitwan

Month/Year	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)	Rainfall (mm)
December2019	22.80	10.60	91.90	24.80
January2020	20.30	10.30	91.00	67.10
February2020	24.80	11.50	85.00	7.30
March2020	30.00	17.10	68.50	20.30
April2020	33.50	21.20	61.50	97.10
December2020	24.00	10.70	88.10	0.00
January2021	21.80	10.80	87.70	0.00
February2021	27.40	12.30	73.30	0.00
March2021	31.60	17.70	66.00	0.00
April2021	35.50	21.80	52.50	33.80

Table 3. Population dynamics of aphids on maize varieties in relation to meteorological parameters during the crop growing season, 2019-2021

2019-2021 (Month)	Standard meteorological week	Aphid colony/ Plant	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)	Rainfall (mm)
January	1	0.00	20.49	10.71	31.50	89.35
January	2	0.60	20.59	11.73	23.10	90.50
January	3	0.68	21.14	11.21	10.50	91.10
January	4	0.83	21.88	9.24	7.30	87.40
February	5	4.80	24.07	8.93	2.00	76.05
February	6	5.86	25.81	9.75	0.00	81.45
February	7	7.08	26.84	13.05	0.00	79.30
February	8	8.54	26.99	14.68	0.00	79.90

3.2 Correlation between maize aphid and weather parameters

The combined correlation analysis of the aphid population with meteorological factors over the years 2019 and 2020 showed that the aphid population had a highly significant (P=0.000), strong positive correlation with maximum temperature (r= 0.99) while the minimum temperature showed non-significant (P=0.213) weak positive correlation (r=0.49). The relative humidity was highly significant (P=0.005), strong negative correlation (r= - 0.86) and rainfall showed significant (P=0.02) moderate negative correlation (r= - 0.80) with aphid colony per plant (Table 4).

Table 4. Correlation coefficient of aphid/plant population on maize in relation to meteorological factors during crop season, 2019-2021

Variables	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)	Rainfall (mm)	Aphid Colony/plant
Maximum temperature(°C)	1.00	0.44	-0.85	-0.84	0.99
Minimum temperature (°C)	0.44	1.00	-0.09	-0.12	0.49
Relative Humidity (%)	-0.85	-0.09	1.00	0.76	-0.86

Rainfall (mm)	-0.84	-0.12	0.76	1.00	-0.80
Aphid colony/ plant	0.99**	0.49	-0.86**	-0.80*	1.00

**-highly significant, *-significant

3.3 Forecasting model

Aphid colony/plant = -5.361 + 0.81 × Avg. Tmax (°C) + 0.31 × Avg. Tmin (°C) - 0.004 × total Rf (mm) -0.16 × Avg. RH (%)

Here, the aphid colony per plant is a response variable whereas average Tmax, Tmin, RH and total RF is the predictor variables. The forecasting model relies on average max and min. temperature, total rainfall and average relative humidity to assign aphid colony per plant and the model has an adjusted R^2 value of 0.98 and a standard error is 0.52 (n=8).The P-value for the full model is 0.0025 which is small enough at both 5% and 1% level of significance to suggest that at least one of the predictor variables may be useful for the prediction (Table 5). The adjusted R^2 is 0.98 which indicates that the predictor variables explain 98% of the variance in the response variable (Figure 1, Table 6).

Table 5. Parameters of aphid colony per plant as affected by average atmospheric temperature (maximum and minimum °C), total rainfall (mm) and average relative humidity (%)

Variable	Coefficient	SE	t Stat	P-value	Lower 95%, CI	Upper 95%, CI
Intercept	-5.36	11.19	-0.48	0.66	-40.97	30.25
Maximum temperature (°C)	0.81	0.26	3.10	0.05*	-0.02	1.63
Minimum temperature (°C)	0.31	0.16	1.93	0.15	-0.20	0.83
Rainfall (mm)	0.00	0.04	-0.11	0.92	-0.12	0.11
Relative humidity (%)	-0.16	0.08	-1.93	0.15	-0.42	0.10

SE-standard error, CI-confidence interval, P-probability *-significant

Table 6. Residual output of the forecasting model for maize aphid

Month/week	Observed aphid colony/ plant	Predicted aphid colony/ plant	Residuals
January first	0.00	0.12	-0.12
January second	0.60	0.37	0.23
January third	0.68	0.60	0.08
January fourth	0.83	1.19	-0.36
February first	4.80	4.69	0.11
February second	5.86	5.49	0.37
February third	7.08	7.70	-0.62
February fourth	8.54	8.24	0.30

The study found that sowing dates significantly affected the infestation and incidence of maize aphids. The highest infestation (42.72%) was observed in the maize plant shown on October 12 with a mean colony of 8.54 per plant followed by October 22 sowing date with a mean colony of 7.08 per plant with 35.78% infestation. The maize varieties sown on December 12 had the lowest infestation with a mean colony of 0.33 per plant with 7.09% infestation. This finding is consistent with previous studies that reported a higher infestation of maize aphid in mid-October sown maize (NMRP, 2018, Alam et al., 2020).

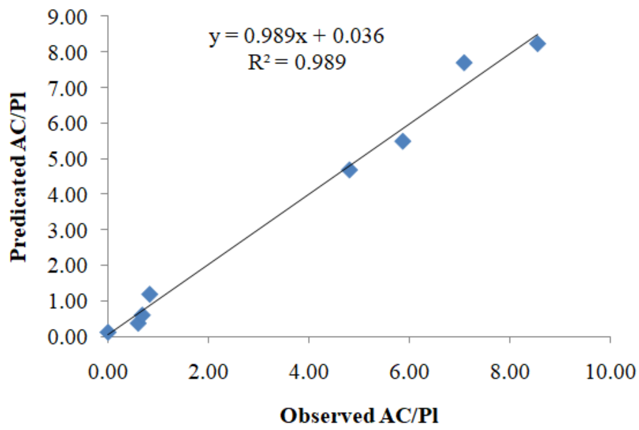

Figure 1. Observed versus predicted aphid colony per plant as affected by meteorological parameters in Rampur, Chitwan, Nepal, 2019-2021.

The higher infestation in maize varieties could be attributed to the availability of suitable host plants for aphids, as well as favorable environmental conditions for their reproduction and development. The study also revealed that sowing dates significantly affected the yield of maize varieties. The highest grain yield was obtained in the maize varieties sown on December 12 with an average yield of 5.70 t/ha, followed by the sowing date (November 1) with an average yield of 5.39 t/ha. The lowest grain yield was obtained in the maize varieties sown in October 12 with an average yield of 2.59 t/ha. This finding is consistent with previous studies that reported higher maize yield in mid-December sown maize (Shrestha et al., 2016, NMRP, 2017). The higher yield in mid-December sown maize could be attributed to the availability of favorable environmental conditions for maize growth and development, as well as a lower infestation of maize aphid. The finding of this study also indicated that maize varieties significantly affected the yield of maize. The highest grain yield was obtained from the hybrid variety Rampur Hybrid-14 with an average yield of 5.45 t/ha. The lowest grain yield was obtained from the open-pollinated (OP) variety Manakamana-9 with an average yield of 3.58 t/ha. This finding is in line with the previous experiments that reported differences in yield among maize varieties (NMRP, 2018, Neupane et al., 2022, Koirala et al., 2021). The differences in yield among maize varieties could be due to variations in genetic makeup, adaptability to different environmental conditions, and resistance to pests and diseases.

Phenotypic and genotypic responses of landraces, pure lines, and hybrids of maize indicated that the resistance to maize aphid was inherited (Carena & Glogoza, 2004). Breeding for aphid resistance in maize continued to be a challenge due to the difficulty in obtaining reliable natural infestations and the presence of genotype by environment interactions. Environmental condition highly governed the rate of colony development and grain yield reduction. Carena & Glogoza (2004) mentioned that resistance of maize to aphids was predominantly governed by additive gene effects which implied resistance through multiple genes with large environmental influence. Narang et al. (1997) demonstrated that phenols and leaf surface wax contributed towards resistance as genotypes having high amounts of these two constituents supported fewer maize aphids per plant. The previous reports supported present findings indicated a correlation between weather parameters and pest population, and the finding, also, reported that maximum and minimum temperatures had significant positive correlation with aphids population whereas relative humidity and rainfall showed negative association (Neupane & Subedi, 2019, Dalwadi et al., 2007). Prasad et al. (2008) reported that the aphid population had a significant positive association with maximum temperature, minimum temperature and a negative association with relative humidity and rainfall. In the earlier work of Hasan et al. (2009) reported that among the different environmental factors, maximum temperature and minimum temperature positively correlated with aphid population while rainfall and relative humidity negatively correlated with aphid population.

4. CONCLUSION

The sowing dates significantly affected the infestation of maize aphids and the yield of maize varieties. The maize varieties sown in the mid-December was found to be the most suitable for obtaining higher maize yield with a lower infestation of maize aphids. Moreover, the hybrid variety Rampur Hybrid-14 was found to be the most suitable than the OP variety, Manakamana-9 to obtaining higher maize yield. These findings could be used to develop appropriate management strategies for the maize production, considering the impact of sowing dates and maize varieties on maize aphids.

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MATHEMATICAL MODELING OF THE BIO-SORPTION OF CALCIUM IN OYSTER MUSHROOM: AN ALTERNATIVE NUTRITION SOURCE TO COMBAT CALCIUM DEFICIENCY

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ABSTRACT

A possible nutrition intervention of bio-fortification of calcium in oyster mushroom was conducted. For this, calcium assimilated by edible oyster mushroom cultivated on paddy straw supplemented with eggshell (ES) of chicken at different ratios along with calcium hydroxide, as a calcium-rich substrate, at different concentration were investigated in winter season around early March 2022. Quantitative calcification of the investigation was assessed through a volumetric analysis and a computational analysis in Complex Pathway Simulator (COPASI). Pleurotus sajor caju cultivated on substrate (paddy straw + ES + 0.4% $Ca(OH)_2$) have the highest calcium content of 168.72 mg/100gm after 9 days of pinhead formation. Similarly, Pleurotus sajor caju harvested from non-calcium supplemented substrate have the lowest calcium content of 32.23 mg/100gm after 3 days of pinhead formation. Based on these data, a multiple linear regression model predicted that for 1mg of calcium growth in 3 days fruiting body of mushroom, it is likely to assimilate 1.82 mg of calcium in 9 days fruiting body. Meanwhile, a mathematical modeling of calcium absorption in COPASI predicted that mushroom's biomass formed could absorb 10.87 mmol or 434.8 milligrams of calcium in approximately 360 hours, i.e 15 days. Hence, fortification of lignocellulosic wastes with animal wastes that are rich in calcium is a good substrate mixture to produce calcium-enriched mushrooms, which will pacify calcium deficiency aliments.

1. INTRODUCTION

Bioremediation generalized as a process of containing organic and inorganic pollutant, through fungi is known as myco-remediation. The process bio-sorption within this is a cleaner remedy to pollution involving heavy metals (Barh et al., 2019). The process can also be well suited to enrich a mushroom with desired metals in the form of mineral. The general principle of which is the interaction between a sorbate (mineral ion) and a biosorbent (surface of the biological agent), which results in the sorption and accumulation of sorbate through sorbate-biosorbent interface via mechanisms like complexation, chelation, reduction, precipitation and ion exchange (Ramírez Calderón et al., 2020). Here, mushroom enrichment of calcium with formative benefit of bones and cartilage, muscle contractor during heart-beat, absorbent of vitamin B12 and a threat due to daily loss of 100mg looping osteoporosis and rickets as deficient causality can be a fruitful biosorptive process (Ogidi et al., 2020). Besides, calcium compounds are often used during cultivation of mushrooms to regulate pH, increase organic matter, create porosity for air flow in substrate, improve phosphorus uptake, enhance substrate gradient, reduce the rate of bacterial contamination during cultivation and extend postharvest storage of mushroom (Choi et al., 2011; Naraian et al., 2014).

Nutrient and mineral analysis of sporophore of *Pleurotus geesteranus* mushroom per 100g showed protein (31.80g), lipid (3.6g), potassium (1.3 mg), phosphorus (0.8 mg), calcium (32 mg), iron (43 mg), magnesium (12 mg), copper (3.5 mcg), zinc (12.5 mcg) and manganese (2.3 mcg) (Ahmed et al., 2016). This leaves calcium below recommended daily requirement of 1000mg/day which tends to be even more less to 2.1 mg in a portion size of cooked mushroom (Ware et al., 2014). Hence, in view of the popularity of mushroom cultivation in the part of the world as a way of producing medicinal foods, fortification of edible fungi with calcium from different sources is another option that could be adopted, in order to increase calcium content in mushroom (Ogidi et al., 2020). Considering a bio-sorptive potential of oyster mushroom as such, amount of calcium that can be taken up and time required for it is a matter of pertinence to reach a desired goal. The research sorts out this dependency dilemma of calcium uptake and time through a modeling approach via complex pathway simulator application. Through an increment of pH in mycelial matrix to enhance bio-sorptive capability in the process, it suggests a constant proportional calcification depending on substrate choice (Tang et al., 2003).

2. MATERIALS AND METHODS

2.1. Model construction

A computational analysis of biochemical networks created in oyster mushroom through COPASI made use of software's functionalities. Within a COPASI user interface, model is entered by adding its component reactions. After entering the reaction equations, appropriate rate law is selected for these reactions. Rate laws selected here are mass action reversible and irreversible of the interface. After entering all reactions, initial concentration of reactant species can be changed.

In the created model, event function of the software was utilized to allow biomass of biochemical network to calculate entered calcium amount absorbed unless completely absorbed by surface adsorption biochemical reaction in the model. Once the model created is loaded, it is ready to be used for simulation. Time Course is used in the model to calculate estimated time for an entered calcium content to be completely absorbed by mushroom biomass (Mendes et al., 2009). To study how certain parameters affect several aspects of the biochemical network, several time-course simulations at different values of the parameters of interest was carried out. Parameter estimation functionality of COPASI was then used to predict parameter Keq(calcium absorption), a reaction constant for a reaction(calcium absorption) in the model, based on absorption data revealed from experiment in different time course (Mendes et al., 2009).

The model with associated reactions, kinetic parameters and order of reactions are tabulated below.

Table 1. Equations, parameters and orders of Biochemical reactions in constructed model

Reaction Name	Reaction equation	Order of reaction	Reaction Parameter
Aconitase	Citrate = Isocitrate	1	K1 = 0 .001 K2 = 1e-6
Biomass Formation	ATP + Protein + FattyAcid + AMP + GMP + dAMP + dGMP + UMP + CMP + dTMP + dCMP -> Biomass	high	K = 0.001
Calcium Absorption	Biomass + FreeCalcium = CalciumAb	2	Keq
Citrate Synthase	AcetylCoA + H2O + Oxaloacetate = Citrate + CoA + H	high	K1 = 0.001 K2 = 1e-6
Enolase	Phospho2glycerate = Phosphoenolpyruvate	1	K1 = 0.001 K2 = 1e-6
FattyAcid Synthesis	AcetylCoA = FattyAcid	1	K1 = 0.001 K2 = 1e-6
Fumarase	Fumarate + H2O = Malate	2	K1 = 0.001 K2 = 1e-6
Glutamine Synthetase	ATP + Glutamate + NH3 -> ADP + Glutamine + Phosphate	High	K = 0.001
Glyceraldehyde3p Dehydrogenase	Glyceraldehyde3p + NAD = Diphosphoglycerate + NADH	2	K1 = 0.001 K2 = 1e-6
Isocitrate Dehydrogenase(NADP)_1	NADP + Isocitrate -> NADPH_H + Oxalosuccinate	2	K = 0.001
Isocitrate Dehydrogenase(NADP)_2	Oxalosuccinate -> Oxo2glutarate + CO2	1	K = 0.001
NADH Oxidase	NADH -> NAD	1	K = 0.001
Nucleotide Synthesis	Phosphoribosylpryophosphate + Glutamine + ATP -> AMP + GMP + dAMP + dGMP + UMP + CMP + dTMP + dCMP + ADP	High	K = 0.001
Oxoglutarate Dehydrogenase(Dihydrolipoamide S-succinyltransferase)	Ssuccinyldihydrolipoamide + CoA = SuccinylCoA + Dihydrolipoamide	2	K1 = 0.001 K2 = 1e-6

Oxoglutarate Dehydrogenase(Lipoamide)	Oxo2glutarate + Lipoamide + H = Ssuccinyldihydrolipoamide + CO2	High	K1 = 0.001 K2 = 1e-6
Phosphoglycerate Kinase	ADP + Diphosphoglycerate = ATP + Phospho3glycerate	2	K1 = 0.001 K2 = 1e-6
Phosphoglycerate Mutase	Phospho3glycerate = Phospho2glycerate	1	K1 = 0.001 K2 = 1e-6
Protein Synthesis	Aspartate + Asparagine + Serine + Glycine + Cysteine + Alanine + Arginine + Tyrosine + Methionine + Valine + Phenylalanine + Tryptophan + Isoleucine + Leucine + Lysine + Proline -> Protein	High	K = 0.001
Pyruvate Dehydrogenase	NAD + Pyruvate + CoA -> NADH + AcetylCoA + CO2	High	K = 0.001
Pyruvate Kinase	ADP + Phosphoenolpyruvate = ATP + Pyruvate	2	K1 = 0.001 K2 = 1e-6
Reaction Intermediate 2	NADH -> 3 * ATP	1	K = 0.001
Ribose Phosphate Diphosphokinase	Ribose5p + ATP = Phosphoribosylpryophosphate + AMP	2	K1 = 0.001 K2 = 1e-6
Ribose Phosphate Epimerase	Xylulose5p = Ribulose5p	1	K1= 0.001 K2 = 1e-6
Ribose Phosphate Isomerase	Ribulose5p = Ribose5p	1	K1= 0.001 K2 = 1e-6
Succinate CoA Ligase	ADP + SuccinylCoA + Phosphate = ATP + CoA + Succinate	High	K1= 0.001 K2 = 1e-6
Succinate Dehydrogenase	FAD + Succinate = FADH2 + Fumarate	2	K1= 0.001 K2 = 1e-6
Transketolase	Xylulose5p + Ribose5p = Glyceraldehyde3p + Sedoheptulose7p	2	K1= 0.001 K2 = 1e-6
Xylitol Dehydrogenase	Xylitol + NAD = Xylulose + NADH	2	K1= 0.001 K2 = 1e-6
Xylose Reductase	Xylose + NADPH_H = Xylitol + NADP	2	K1= 0.001 K2 = 1e-6
Xylulokinase	Xylulose + ATP -> Xylulose5p + ADP	2	K = 0.001

2.2. *Mother culture preparation of oyster mushroom*

Mushroom cap of fruiting body of oyster mushroom was pull apart to expose the inside uncontaminated tissues. Tiny pieces of the inside tissue was then plucked using sterile fine-tip tweezers and transferred onto the Potato Dextrose Agar media. This was done inside a laminar flow hood to avoid airborne contamination. When new hyphae became evident growing out of the transferred mushroom pieces without any contamination, then the new isolate was sub-cultured onto new growth medium under aseptic conditions. These new cultures were maintained as mother culture at 4°C. The process of subculture was carried to a liquid medium with a combined nutrition source of xylose and corn steep liquor. This was to allow growth of mycelial biomass in accordance with the requirement for computational analysis of biochemical networks through COPASI.

2.3. *Spawn preparation and inoculation*

Wheat seed was preferred for producing oyster mushroom spawn. Prior to use, they were soaked for about twenty-four hours. It was then drained and transferred into polypropylene spawn bags and sterilized in a 20 litre vertical stainless steel autoclave produced by Bhavya Group, New Delhi, India at 121°C for 3 hours. Following a safety measure as in prior processes, healthy growing cultures that had just filled the surface of a liquid culture in conical flask was used to inoculate 400–450 g spawn substrate. The inoculated spawn bag was shaken to get the culture pieces distributed inside the substrate. Now the inoculated spawn bag was left to grow at a room temperature of 23–25°C.

2.4. *Cultivation of oyster mushroom*

Low-cost thatched house made up of bamboo mats, allowing diffused sunlight, proper cross ventilation with a clean surrounding, was used for mushroom cultivation. Cultivation of oyster mushroom was then done by Polythene bag method (Rahman et al., 2019). Here, good quality paddy straw was chopped, soaked overnight, put to hot water treatment (70-80°C) for 30 minutes and cooled. Poly bags (mushroom bags) of 40 cm x 20 cm size was unfolded and filled with cooled straw after partial preparation. Spawn culture was sprinkled uniformly. The bag was then sealed and perforated with a punch machine.

2.5. *Experimental design*

During preparation of spawned polythene bag, ready to use spawn culture was sprinkled on 6 mushroom bags. To allow surface adsorption of calcium in growing mycelium, well macerated and ground eggshells in mortar and pestle was mixed with calcium hydroxide where 40 grams of egg shell was contained in a 500 ml solution. Percentage increment in calcium hydroxide was maintained with respect to final volume of solution prepared(500ml). This solution was uniformly spread along with spawn for spawned polythene bag preparation. This way 40 grams of egg shell was added per 7 kg of poly bag with calcium hydroxide added to the bags as follows.

1. Spawned polythene bag with no egg shell and calcium hydroxide,

2. Spawned polythene bag with egg shell but no calcium hydroxide,

3. Spawned polythene bag with egg shell and 0.2% calcium hydroxide,

4. Spawned polythene bag with egg shell and 0.4% calcium hydroxide,

5. Spawned polythene bag with egg shell and 0.6% calcium hydroxide,

6. Spawned polythene bag with egg shell and 0.8% calcium hydroxide.

2.6. *Mushroom bed opening*

The mushroom bags were placed in a cool and dark place, safe from rodents and other insects in mushroom house, for spawn run.

Completion of spawn run was indicated with white to cream coloured mycelium mat covering the entire straw. A slit was made with a clean blade in the area of these lumps and then pinheads initiated from these regions in consecutive days. These pin heads grew in size to take of form consumable fruiting body.

2.7. Analysis of calcium absorption

For the analysis of calcium absorption, dry ashing followed by volumetric analysis was adopted as descripted in Official method of Analysis, Association of Officiating Analytical Chemists (AOAC) (2005).

3. RESULTS AND DISCUSSION

3.1. Preparation of mother culture and oyster mushroom cultivation

The success of the preparation of mother culture was reflected in formation of white lawn of mycelium across the surface of culture medium. Quantitative representation of the result is tabulated below.

Table 2. Quantitation of cultures adapted for growth.

Tissue culture media	Number of cultures
PDA culture (contaminated)	12
PDA culture (with mycelium lawn)	4
PDA culture (Sub-cultured)	8
Sub-culture with growth	8
Sub-culture without growth	0

In due process of oyster mushroom cultivation, the sub-culture with growth which was 8 in quantity produced a healthy spawn of 200gram, 6 in number. These healthy spawns took a 47 days long period to fully grow. They were inoculated for substrate preparation in a mushroom house which gave rise to healthy mushroom pileus in 4 mushroom bags with the exception of 2 mushroom bags which were wasted for undesirable fungal contamination after 60 days.

3.2. Time course analysis and parameter scan of created model

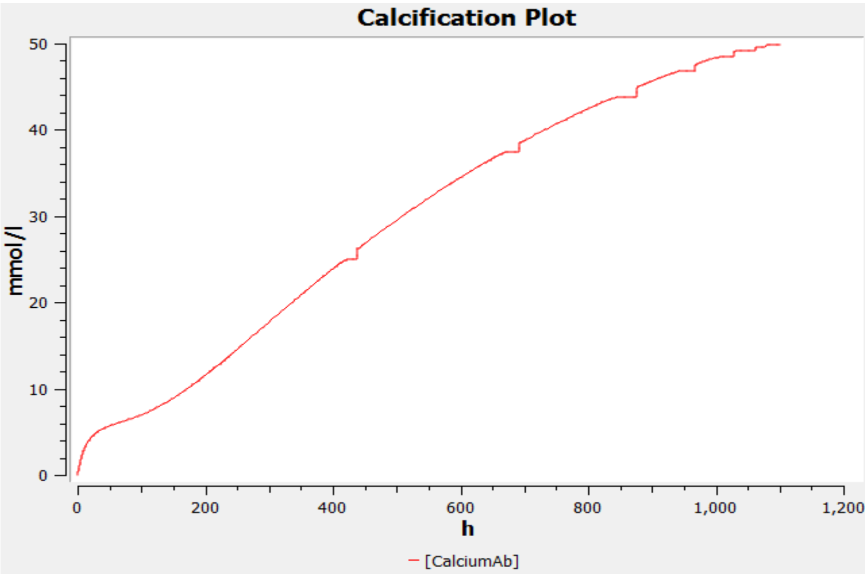

Figure 1. Time course analysis of absorption of calcium (50 mmol/l) by mushroom biomass

Based on time course analysis tool in COPASI, mushroom's biomass formed through the created model could absorb 21.43 mmol or 857.2 milligrams of calcium in approximately 360 hours, i.e 15 days. Utilizing a layout of the model where implication of parameter fluctuation could only be studied with calcium absorption reaction parameter as other reaction set were either reversible or irreversible in the model where model parameters k1= 0.001 and k2= 1e-6 was used for reversible reactions and k1= 0.001 was used for irreversible reaction, parameter scan tool showed the extent by which increment in rate constant of a reaction in the model for calcium absorption increased calcium absorption rate in oyster mushroom as follows.

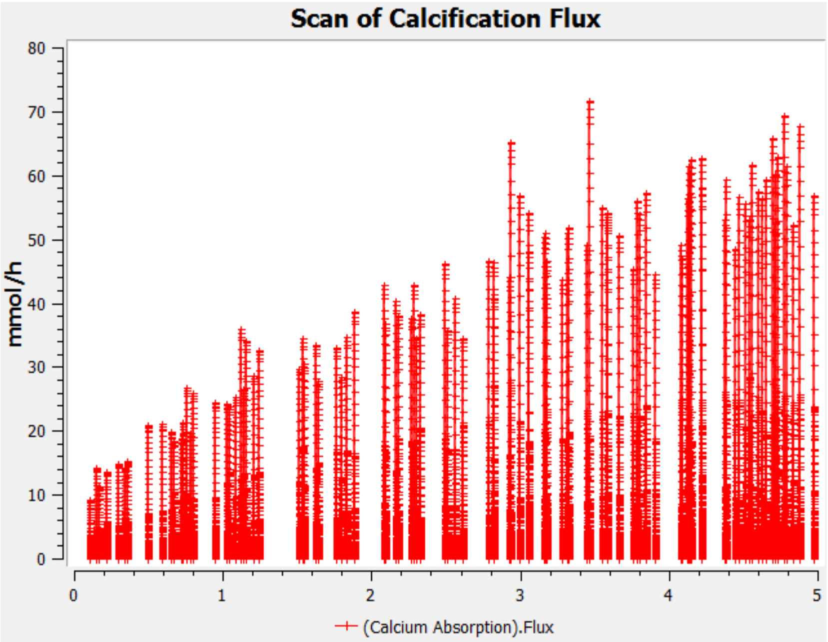

Figure 2. Parameter Scan analysis of absorption of calcium by mushroom biomass

Provided a possible rational intervention in mushroom cultivation leading to increment of calcium absorption in it, theoretical reflection of this was seen through increment of parameter Keq (calcium absorption) in the model. As evinced in the result, increment in parameter Keq from 0 to 5 would lead to progressive increment of rate of absorption of calcium in oyster mushroom. For example, for a parameter Keq value 3, calcium absorption value was 56 mmol/hr. This means, if rate constant Keq (calcium absorption) value in created model was 3, mushroom biomass would absorb approximately 2 grams of calcium in 1 hour.

3.3. Experimental design-based analysis of calcium absorption

Among experimental set of mushroom bags,

1. Spawned polythene bag with no egg shell and calcium hydroxide = Control

2. Spawned polythene bag with egg shell but no calcium hydroxide = ES

3. Spawned polythene bag with egg shell and 0.2% calcium hydroxide = ES + 0.2%Ca(OH)2

4. Spawned polythene bag with egg shell and 0.4% calcium hydroxide = ES + 0.4%Ca(OH)2

Growth analyzed after pinhead formation with an interval of 3 days, yielded calcium content in mushroom's fruiting body as follows:

Table 3. Calcium absorption at different condition.

Intervention (mg/100g)	3 Days FB	6 Days FB	9 Days FB
Control	32.23 ± 1.98	40.05 ± 3.17	41.15 ± 2.96
ES	127.72 ± 2.53	134.85 ± 5.22	146.5 ± 5.11
ES+0.2%$Ca(OH)_2$	140.4 ± 3.15	149.32 ± 2.75	156.72 ±6.62
ES+0.4%$Ca(OH)_2$	151.12 ± 3.01	157.42 ± 3.51	168.72 ±5.39

Note: Here, ES is egg shell and FB is fruiting body. Tabulated data represents mg of calcium in 100 grams of mushroom's fruiting body (mean ± margin of error).

The addition of calcium source from animal wastes into paddy straw substrate improved the calcium content of the cultivated P. sajor caju, which are different from those harvested from paddy straw alone. The animal wastes; ES supplemented into paddy straw improved the calcium content from 32.23 mg/100 g to 127.72 mg/100 g in 3 days old fruiting body, 40.05 mg/100 g to 134.85 mg/100 g in 6 days old fruiting body and 41.15 mg/100 g to 146.5 mg/100 g in 9 days fruiting body. P. sajor caju grown in paddy straw with egg shell and Ca(OH)2 in combination, increased absorbed calcium content significantly. The increment was more prominent with increasing percentage of Ca(OH)2. This was reflected in calcium content which improved from 127.72 mg/100 g in egg shell treated mushroom to 140.4 mg/100 g in mushroom grown in ES+0.2%Ca(OH)2 and to 151.12 mg/100 g in mushroom with a substrate of ES+0.4%Ca(OH)2. Allowing some growing time, increase in calcium content to 157.42 mg/100 g in 6 days fruiting body of mushroom and to 168.72 mg/100 g in 9 days fruiting body of mushroom was seen. The trend is further analyzed through Two factor analysis of variance in table below.

Table 4. Two factor ANOVA of calcium absorption with different treatment condition

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	103759.343[a]	11	9432.668	8477.144	.000
Intercept	5.964	1	5.964	5.360	.028
Group	6.165	3	2.055	1.847	.162
Three_Days_FB	6.121	1	6.121	5.501	.026
Six_Days_FB	4.427	1	4.427	3.978	.056
Group * Three_Days_FB	5.726	3	1.909	1.715	.187
Group * Six_Days_FB	4.122	3	1.374	1.235	.316
Error	31.156	28	1.113		
Total	761943.870	40			
Corrected Total	103790.500	39			

Dependent Variable: Nine_Days_FB

According to the analysis, P. sajor caju grown in paddy straw without any calcium source or with egg shell and Ca(OH)2 in combination don't absorb calcium on an increasing trend such that level of calcium in these treatment group are significantly different. However, there is significant difference in this treatment group when calcium level in three days fruiting body and nine days fruiting body are compared. Similar differences in calcium level don't hold when six days fruiting body and nine days fruiting body are contested. This is reflected through a P-value of more than 0.05 in interaction terms, (Group) and (Six_Days_FB) but a lesser P-value in (Three_Days_FB). Although reflective of a pattern, a more robust mathematical model is required to predict calcification rate based on its dependency on time and treatment condition for calcium enrichment. This is done through multiple linear regression model.

A continual increment of absorbed calcium in treatment group from 3 to 9 days has some implication for forecasting required time for calcium absorption to reach a limit. It is reflected in regression analysis data presented below:

Table 5. Table representing multiple linear regression model of calcium absorption in different treatment group

ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	103634.628	3	34544.876	7978.451	.000[b]
	Residual	155.872	36	4.330		
	Total	103790.500	39			

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.999[a]	.998	.998	2.08081	1.423

Coefficients

		Unstandardized Coefficients		Standardized Coefficients		
Model		B	Std. Error	Beta	t	Sig.
1	(Constant)	3.258	1.260		2.587	.014
	Group	-1.615	.603	-.035	-2.679	.011
	Three_Days_FB	.820	.101	.762	8.106	.000
	Six_Days_FB	.289	.100	.268	2.894	.006

Dependent Variable: Nine_Days_FB

According to the statistical model, significant P-values throughout with R square and adjusted R square value of 0.998 suggested that the model is a best fit with approx. 99% of variation explained by the model. The regression equation for the model was $y = -1.615x1 + 0.82x2 + 0.289x3 + 3.258$. This predicted that for a growth of 1 mg of calcium in three days fruiting body of mushroom, growth in calcium in nine days fruiting body is likely to be 1.82 mg. In the same way, for a growth of 1 mg of calcium in six days fruiting body of mushroom, growth in calcium in nine days fruiting body is likely to be 1.289 mg.

3.4. Parameter estimation and time course analysis of created model

In a completely different approach to least square regression model, similar data set of calcium absorbed in a growth condition for optimal calcium absorption, estimated parameter Keq (calcium absorption) from created model along with supportive data is shown in Table

Table 6. Result of Parameter Estimation

Time (h)	CalciumAb (Data) (mmol)	CalciumAb (Fit) (mmol)	Estimated Parameter (Calcium absorption) Keq	Lower Bound	Upper Bound	Standard Deviation	Coefficient of Variation (%)
72	3.77	1.68	0.000123196	0	2	1.84359e-05	14.39
96	3.74	2.29					
120	3.82	2.90					
144	3.92	3.53					
168	3.97	4.20					
192	3.93	4.90					
216	4.2	5.66					

In the above table, experimental data represented in a continual increment of absorbed calcium from 151.12 mg/100g to 168.72 mg/100g from 3 to 9 days were projected as time in hours and absorption in mmol as per the unit setting of the model. Based on parameter estimation tool of COPASI, calcium absorbed fit value and weighted error value were calculated through genetic algorithm of 200 iterations and population size 20. Following the calculation, estimated desired parameter Keq (calcium absorption) was found to be 0.000123196. For this calculation, lower bound was set at -100%=0 and upper bound was set at +100%=2 with model parameter value 1 set as start value. This produced an acceptable standard deviation and coefficient of variation value as 1.8435e-05 and 14.39 respectively. The extent of calcium absorption by replacing model parameter value with the estimated one is shown through time course analysis tool in COPASI as in Figure 3.

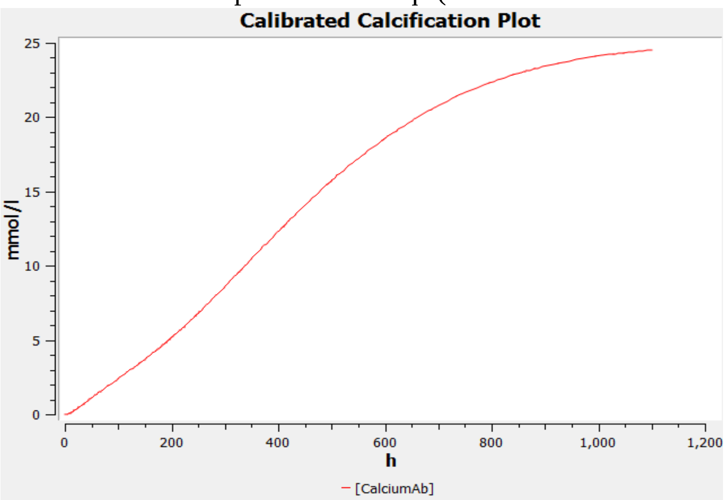

Figure 3: Time course analysis of absorption of calcium by mushroom biomass

Creating similar condition of duration, interval, interval size and method of determination as in time course analysis using model parameter 1, the graphical extension predicted that mushroom's biomass formed, could absorb 10.87 mmol or 434.8 milligrams of calcium in approximately 360 hours, i.e 15 days.

Yellow coloration in one of the spawn may be attributed to bacterium Pseudomonas sp. accumulation of excess water in spawn site (Rahman et al., 2019). While, a spawn bag lost to green colored velvety growth could be because of the fungus, *Trichoderma harzianum* that results from contamination due to improper boiling of the straw (sterilization) or due to contaminated spawn from growth in subculture (Rahman et al., 2019). Long period for spawn growth could be because of a mal-adjustment of humidity and temperature which is respectively preferred at 80-85% and 20-30^{o}C (Rahman et al., 2019). Among mushroom bags which succumbed to fungal contamination, 0.8% Ca(OH)2 treated bag was fully infested by brown plaster mold indicative of poor and old straw use with high moisture content in compost (Rahman et al., 2019). Basic pH of 0.8% Ca(OH)2 treated bag could have also attributed to excessive growth of brown plaster mold as they prefer basic pH for growth (Ghimire et al., 2021). While, 0.6% Ca(OH)2 treated mushroom bag infested with both green mold and ink caps couldn't recover for plausible reasons of basic pH triggering mold growth, excessive moisture, fungal contaminated environment of mushroom house and so on (Ghimire et al., 2021). However, 4 of the mushroom bags with mushroom growth were also affected by ink caps but were outlived by mushroom's mycelium over ethanol syringe treatment and fungicide treatment prior to mushroom pinhead formation. The reason for so many mushroom bags suffering from it was due to infection of Coprinus spp. (ink caps) favored by ammonia, present in composting straw during peak heating which gives an appearance of black inky liquid (Ghimire et al., 2021). It is reported that the optimal temperature for the mycelial growth of C. comatus was found at 23-26^{o}C while the pH range of 6-8 favored the mycelial growth of this fungus which also explains the likelihood (Ghimire et al., 2021).

Pertaining to the time course analysis plot, kinks formed in several locations reflecting absorption line was due to an event created in model which commands the repeat of the model calculation once half of the initial calcium content input in model was consumed. So, the model started on 50mmol of calcium starts again at 25mmol of calcium as initial input. This process carried on with time until 50mmol was completely absorbed. Since Langmuir isotherm was used for a particular reaction of calcium absorption in the model, event as such had to be created. A more accurate reaction set of freundlich adsorption isotherm, pseudo 1st order kinetics and pseudo 2nd order kinetics could have been selected for the purpose but the former was preferred over due to its relative ease during parameter estimation later. Note worthily, constancy of other reaction parameters were chosen based on the effect of product formation in redundant system using very few reaction set. This had to be done since there are only few research experiments attributing to parameter estimation of particular reaction in mushroom. For instance, parameter estimation of xylitol dehydrogenase in bi-substrate rate law formation in Pleurotus sp. was experimented but nothing else to follow it.

Parameter scan analysis of calcium absorption had its reflected values changed to an extent with each run of parameter scan in the model. This was due to number of iterations selected for the scan, but, with all set of conditions clicked similar, deterministic method used in the model would not give same value when calculated for multiple times. Mathematical intricacies may have a role to play in it. All the same, the trend of uphill in the graph remained.

Findings revealed that calcium content in egg shell is 632.29 ± 9.00 mg/g of substrate (Ogidi et al., 2020). This is the reason for a soar in calcium absorption in experimental mushroom grown. In support of this, P. ostreatus grown in coconut husk and egg shell produced a calcium absorption ratio of 3.4 (Ogidi et al., 2020). While with coconut husk +1.5% w/w Ca (OH)2, it produced a calcium absorption ratio of 2.4 (Ogidi et al., 2020). In comparison of the same mushroom species grown in palm kernel, calcium absorption ratios for egg shell and palm kernel +1.5% w/w Ca(OH)2 were 3.8 and 3.2 respectively (Ogidi et al., 2020). During this process improvement in calcium content was noted between 284.8 mg/100 g to 596.6 mg/100 g of mushroom (Ogidi et al., 2020). In the experimental finding, growth substrate with egg shell increased calcium content as in above studies. However, a relative difference in calcium uptake after introduction of 0.2%Ca(OH)2 and 0.4%Ca(OH)2 was seemingly due to calcium contents of Ca(OH)2 but its distinction from calcium uptake enhancing effect of increment in pH needs a more precise experimentation. For this experiment, however, calcium uptake had to be assumed to be attributed by both these effects. Though the relative insufficiency of calcium absorption, don't clearly justify combinatorial effect of these components, absorption reading of 596.6 mg/100 g was also a mindful reflection of variability in absorption ratio indicative of mineral absorption capability of the studied fungi, which may depend on different factors such as fungal species, calcium concentration or its source, type of substrate and mechanism of minerals assimilation by mycelium (Falandysz & Borovička, 2013).

Estimated parameter Keq (calcium absorption) value in the study was fairly less than model parameter value of 1 which gave more of an accurate prediction of absorption, although, calcium absorption was less than half of what was absorbed in the same time frame provided model parameter value was 1. So, tracing back to parameter scan result of the model, calcium absorption was negatively affected. However, since Keq (calcium absorption)= 1 was set as model parameter so that calcium absorption equation could produce adsorption approximating linear isotherm in surface adsorption resulting optimum absorption in the model, time course analysis of calcium absorption with corrected parameter could be compared to optimum calcification plot. This is because calcium absorption in the model was defined by Langmuir isotherm which suitably described a chemisorption process with ionic or covalent chemical bonds between calcium and functional groups in the cell surface of mushroom (Goldberg, 2013).

4. CONCLUSION

This study utilized animal wastes as supplements with paddy straw to produce calcium enriched-mushrooms. An animal waste in the form of egg shell of chicken along with lignocellulosic wastes (Paddy straw) supported the growth of mushrooms and thus, improved the calcium content. It has further been validated with a mathematical model of calcium absorption in COPASI. So, the study will be informative for mushroom farmers who wish to supplement their commonly used substrates (agro waste residues) with calcium-rich animal wastes to produce calcium-enrich mushrooms. Mushroom enrichment with calcium salt; Ca(OH)2 cultivated on calcium-rich wastes in this work is a surplus attribute for enhanced calcification in cultivated mushroom. This surely offers an alternative and reliable source of calcium foods for consumers since calcium-enriched mushrooms also have adequate potassium, protein, dietary fibers with low fat and sodium contents. Provided, lignocellulose waste alone cannot produce optimum yield of mushrooms but needed to be supplemented with other substrates, hence, the utilization of lignocellulosic biomass and animal wastes for mushroom cultivation is a standpoint towards a better prospect of food bio-fortification and nutrient availability.

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IMPACTS OF WTO AGREEMENTS ON THE EXCHANGE OF AGRICULTURAL COMMODITIES: A REVIEW

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ABSTRACT

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ABSTRACT

International trade agreements are often complicated to understand and bring them into action due to complicated writing with legal jargons and complex interdependent provisions with multitude of exceptions. This review article aims to demystify the intricate legal texts comprehensible for a broad audience to understand how these agreements affect world economies and exchange of goods and services. This exploration looks into the historical context and key insights on the principles of trading under the regulations of World Trade Organisation (WTO). Additionally, it highlights the various provisions of the international trade agreements with focus on those that regulate the exchange of agricultural goods, such as the Agreement on Agriculture (AoA), the Agreement on the Application of Sanitary and Phytosanitary (SPS) Measures, and the Agreement on Technical Barriers to Trade (TBT). In essence, these agreements are meant to play a crucial role in creating a fair and equitable trading environment for agricultural products. However, empirical studies suggest that benefits of WTO membership are not found to be distributed evenly between developed and developing countries. In Nepalese context, the domestic support is well below the de minimis level and therefore implies that Nepalese government can further increase the support.

1. INTRODUCTION

General Agreement on Trade and Tariff (GATT), the predecessor of World Trade Organisation (WTO) Agreements, was signed initially by 23 nations to address the economic consequences and trade disruptions caused by the World War II, along with promoting stability and prosperity through international trade. The Great Depression and World War II eras from the 1930s and 1940s served as crucial reminders of gloomy protectionism policies adopted by the nations. As a result of the Smoot-Hawley tariffs imposed by the United States and the international retaliation that followed, the global trade almost ceased in the 1930s (Barton et al., 2015).

Eventually after World War II, a multilateral agreement for reciprocal tariff reductions and minimizing trade barriers was negotiated by various economies. As a result of these discussions, 23 countries including USA, UK and other major economies signed the GATT on November 30, 1947. Effective from January 1, 1948, the treaty was intended to boost economic recovery through reconstructing and liberalizing global trade.

As an early step towards globalisation, the GATT became an international instrument which sets rules to guide the global exchange of business. The GATT secretariat at Geneva facilitated trade negotiations among the signing nations. However, it had not been a formal organisation yet (Ayenagbo et al., 2011).

Over the next forty-seven years, more countries signed on to the GATT and refined it through several rounds of trade negotiations. The main goal of these rounds of negotiations was to progressively reduce the tariffs that countries had levied on imports from other nations. Despite several achievements in opening up global trade, the GATT framework started to experience some issues in the 1980s. First of all, the GATT's dispute settlement process was not working as anticipated. Secondly, a number of commodities, most notably textiles and agricultural goods, were largely exempted from GATT regulations. Third, the GATT lacked any regulations governing the services sector, which was growing rapidly. Last but not least, it lacked the measures of protecting intellectual properties (Crowley, 2003).

In order to address these issues, a new round of trade negotiations – popularly called the Uruguay Round – started in 1986. It aimed to implement significant changes to the way the global trading system would operate. It eventually led to the creation of the WTO on 1st January 1995. The WTO not only absorbed the GATT but extended its scope to services industry and covered the intellectual properties as well. Initially signed by 123 nations, the WTO currently covers 98% of the world trade and has 164 member countries (WTO, 2023).

This review paper is aimed at drawing key insights on the principles of trading under the regulations of WTO. Additionally, it highlights the various provisions of the international trade agreements with focus on those that regulate the exchange of agricultural goods, such as the Agreement on Agriculture (AoA), the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), and the Agreement on Technical Barriers to Trade (TBT Agreement). This study also examines how the agreements have affected the exchange of agricultural products in Nepalese context. By offering this comprehensive overview, we intend to foster a better understanding on its implications and recommendation for protecting farmers within the framework of the WTO agreement.

2. MATERIALS AND METHODS

This review examines the effects of WTO agreements using comprehensive research approach. The methodology entails a thorough examination of the provisions of global trade agreements, such as the GATT and WTO focussing on AoA, SPS and TBT agreements. We seek to offer a comprehensive understanding of these agreement specifically affecting the international trade. The analysis uses the primary sources, such as the official WTO documents and the original texts of the agreements for analysing legal provisions of trading the agricultural goods. Additionally, we used secondary sources, including research papers, reports, and academic literature related to WTO agreements which regulate the global trades, particularly the exchange of agricultural goods. In reviewing, we also analysed its impacts on developing countries, including Nepal.

3. RESULTS AND DISCUSSION

3.1 Principles of WTO

WTO agreements cover a wide range of topics, including agriculture, textiles and apparel, finance, telecommunications, government procurements, industrial standards and product safety, laws governing food safety, intellectual property, and much more. However, all of these agreements follow a set of fundamental principles which forms the basic foundation of the multilateral trading system. The basic principles are as follows:

3.1.1 Most favoured nation

Countries are not allowed to discriminate their trading partners. Each member should view all other members equally as "most-favourable-nation". This principle is so important that it was mentioned in the very first Article of GATT and is still continuously mentioned in other agreements under the framework of WTO.

3.1.2 National treatment

This allows equal treatment to domestic products and imported commodities by member states in trade. This principle takes into effect as soon as a good, service, or a piece of intellectual property has entered to the domestic market.

3.1.3 Free trade

The principle of "free trade" lies at the heart of WTO agreement which emphasizes removing barriers and advancing trade between nations based on fairness, reciprocity, and mutual benefits with negotiation. It encourages nations to implement reforms gradually through "progressive liberalisation".

3.1.4 Predictability

The WTO emphasizes the member nations to create predictable, precise and consistent regulations for global trade. This principle ensures transparent and accessible trade laws, rules and procedures, providing firms and governments a stable framework for doing business.

3.1.5 Promoting fair competition

Although non-discrimination principles of WTO intend to secure fair conditions of trade, there are chances of unequal benefits and market distortions by the practices like subsides and dumping. The WTO allows the member nation to implement restrictions on dumping and subsidies to ensure a level playing field so that businesses can compete fairly on the merit basis.

3.1.6 Encouraging development and economic reform

WTO facilitates developing nations by offering specific provisions, technical support, and capacity-building initiatives that are relevant to their particular needs. It helps these countries in improving infrastructure, developing industries and create jobs by opening up markets and facilitating foreign direct investment.

3.2 Overview of WTO agreements

The WTO agreement encompasses an extensive compendium of guidelines and rules that governs the international trade, covering key topics of trading goods and services, and issues of intellectual property. The Marrakesh Agreement on establishing WTO serves as an umbrella of the WTO agreements which contains a list of Annexes on area specific agreements and understandings. The provisions of these agreements are summarised as follows:

3.2.1 GATT (for goods)

The updated GATT represents the goods related obligations under the WTO agreements. The additional agreements that govern the trade of goods are as follows:

a. AoA
b. Agreement on SPS Measures
c. Agreement on Textiles and Clothing
d. Agreement on TBT
e. Agreement on Trade-Related Investment Measures
f. Agreement on Implementation of Article VI of the GATT
g. Agreement on Implementation of Article VII of the GATT
h. Agreement on Pre-shipment Inspection
i. Agreement on Rules of Origin
j. Agreement on Import Licensing Procedures
k. Agreement on Subsidies and Countervailing Measures
l. Agreement on Safeguards

3.2.2 GATS (for services)

Global trade has experienced a fundamental transformation, moving from a mostly goods-oriented trading system to one that places emphasis on the exchange of services. This development reflects the larger shift from an industry-driven to a service-driven economy. The cross-border movement of services like finance, telecommunication, etc. has become a defining characteristic of the global economy. The General Agreement on Trade in Services (GATS) established a comprehensive framework to negotiate obligations and rules specific to following service sectors:

a. Movement of natural persons supplying services
b. Air transport services
c. Financial services

d. Maritime transport services (Shipping)
e. Telecommunications services

3.2.3 Agreements on TRIPS

The agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) was necessitated by the evolving technical innovations and growing recognition of intellectual property. While GATT and GATS were primarily concerned with trade in commodities and services, the development of intellectual property (IP) across industries demanded the conception of TRIPS agreement. It sets out the minimum standards concerning the availability, scope and use of the following intellectual properties:

a. Copyright and related rights
b. Trademarks
c. Geographical Indications
d. Industrial designs
e. Patents
f. Layout-designs (Topographies) of integrated circuits
g. Protection of undisclosed information (Trade secrets)
h. Control of anti-competitive practices

3.2.4 Dispute settlement understanding

Disputes are an inevitable aspect in international trade because they result from the intricate interplay of various economies and political interests. It arises when one nation adopts trade policy measures that one or more member nations believe violate the WTO agreements. The Dispute Settlement Understanding (DSU) was agreed upon during Uruguay Round of negotiation to offer a clear, legally binding, and efficient means of resolving disputes among the WTO members. It ensures that disputes are resolved through a standardized procedure that encourages accountability, predictability, and the preservation of the international trading system based on rules and principles of fairness and cooperation.

3.2.5 Trade policy review

WTO requires that the businesses involved in international trade should be aware of the changing trade policies adopted by the nations. Therefore, it is important that trade rules and regulations must be transparent to all. In the WTO, this is accomplished in two ways: first, governments are required to regularly send notifications to the WTO and fellow members on any amendment to the trade policies and laws; second, the WTO conducts periodic reviews of the trade policies of all the member nations.

3.2.6 Plurilateral trade agreements

Within the greater framework of the WTO, a small group of nations sharing common interests and objectives signed some agreements which are referred to as plurilateral trade agreements. Unlike the multilateral agreements that cover all WTO members, the plurilateral agreements are negotiated and joined by a certain group of nations only. These agreements allowed the like-minded nations to expand their collaboration in particular fields without anticipating for agreement among all WTO members.

3.3 Key WTO agreements affecting agricultural trade

The key agreements, under the WTO framework, which affects the trade of agricultural goods are briefly described as follows:

3.3.1 AoA

The AoA is a comprehensive framework for governing agricultural trade balancing the complicated interactions between trade liberalisation and the intricacies of agricultural practices. All the WTO agreements and understandings on trade of goods is applicable to the agricultural products as well, but in the event of conflict, it is agreed that the provision of AoA prevails.

The agreement defines "agricultural product" as basic agricultural items such as wheat, milk, and live animals, along with the products that are derived from them including bread, butter, oil and meat. Additionally, it includes processed agricultural products like chocolate, yoghurt, sausages, wines, beverages, tobacco products, fibres including cotton, wool, silk and raw animal skins used for making leather. However, fish along with their products and forestry goods like timber and rubber aren't covered by the AoA.

The AoA establishes guidelines that focus on the three major pillars: market access, domestic support and export competition.

Market access: The AoA aims to increase market access for agricultural products and addresses the barriers that prevent admission into the market, ensuring transparency in trade policies, and promoting fairness among all the WTO nations. The market access pillar primarily focuses on reducing tariffs, eliminating non-tariff barriers, and enhancing opportunities for member nations to access each other's agricultural markets.

Domestic support: Countries all over the world provide domestic support to the agricultural sectors in the form of subsidies and price support mechanism for improving food security and assisting the livelihoods of their farming communities. At the same time, the provision of domestic support has the potential to significantly distort trade and result in unfair advantages in global agricultural trade. Therefore, the agreement aims to control domestic support policies to prevent them from irrationally distorting international agricultural markets while also leaving room for governments to create their own support systems considering different unique circumstances prevailing in individual nations.

Export competition: Governments often offer export subsidies in the agricultural sector to aid domestic producers and encourage the selling of their products in foreign markets. Although the export subsidies are aimed at boosting nation's agricultural exports, it can significantly distort global trade. The agreement attempts to control and eventually remove these subsidies considering its impact on international trade. It aims to create a trading system where nations can compete on the basis of the calibre and viability of their products rather than the degree of their financial support for domestic producers.

3.3.2 SPS measures

Although WTO was established with the goal of liberalising international trade and removing trade barriers among member countries, certain exceptions of non-tariff barriers exist to address legitimate concerns regarding human, animal and plant health and safety. One such exception as a Non-tariff Measure (NTM) is the Agreement on the Application of Sanitary and Phytosanitary Measures, also known as the SPS Agreement. Here, 'Sanitary' refers to human and animal health, while 'Phytosanitary' indicates the plant health.

As consumers in the developed nations are more concerned about health and food safety, governments often employ several measures to make sure that the products are free from contaminants, toxins and any other substances that may impact human health. The SPS agreement allows for implementing regulatory measure to protect the health and safety of plants, animals and humans. However, these measures must be based on scientific evidence and must not unjustifiably discriminate against trading partners to protect local producers. In essence, the SPS Agreement aims to achieve a compromise between safeguarding public health and preventing it from turning into an arbitrary trade barrier.

3.3.3 TBT measures

Along with the SPS measures, another category of NTMs that can be applied to the trade of agricultural commodities falls under the TBT Agreement. While SPS measures are largely concerned with protecting human, animal, and plant health, the TBT measures relates to the technical regulations, standards and conformity assessment processes. As the global market expands and becomes increasingly interconnected, governments recognize the need to implement several regulations to safeguard consumers and to ensure the quality of goods and safety of users.

Although technical regulations, standards and conformity assessments are important, they differ from nation to nation which make it difficult for the manufacturers and exporters to comply. There are chances that the arbitrarily set standards and regulations could be used as an excuse to protect domestic companies and become a trade barrier. The TBT Agreement intends to ensure that technical regulations, standards and conformity assessment processes are non-discriminatory and do not create trade barrier unreasonably.

3.4 *Effects of WTO agreements in global agricultural trade*

Agriculture has remained a contentious issue since the establishment of GATT because of its multifaced nature and the complex interplay of economic, social, and political issues (Reena, 2008). Due to its importance in food security, rural livelihoods and economic stability, agriculture was considered a unique economic sector that couldn't be treated like other sectors. Due to the competing interests and the need to strike a balance between promoting trade and safeguarding domestic farming sectors, agricultural trade was given “special treatment” and was exempted from some important provisions of GATT (Merlinda and Johan, 2004).

According to Warley (1976) and Jackson (1969), there were two reasons for this exclusion. The two main economies, the United States and the European Community (now European Union), did not comply with GATT regulations while trading agricultural commodities. The United States were using quantitative restriction to protect its domestic agronomic market since 1950s, while the Common Agricultural Policy (CAP) set by European Community was also protecting their markets through complex import levy and financial support mechanisms, regardless of the global market conditions. As a result, trading of the agricultural commodities remained as an exception to the GATT rules.

Even after the creation of WTO, agreements were crafted to specifically handle the special difficulties and opportunities presented by agricultural products. The AoA is regarded as a significant development in the trade of farm products because for the first time the exchange of agricultural products on a global scale was governed by a comprehensive set of regulations that aims to reduce and eventually eliminate trade barriers (Smith, 2011). It outlined rules on market access, export subsidies and domestic support policies, restricting members to impose trade barriers arbitrarily. It allowed members to employ trade measures only to accomplish non-trade goals like development policy, environmental conservation and protecting the rural livelihood.

Moreover, the trade measures countries employed were through quotas and other non-tariff barriers. As quantitative restrictions were no more permitted exceeding the limits, the agreement allowed for converting them into custom duties through tariffication process. Furthermore, the members were given preparatory period to implement the agreement. Developed nations were required to reduce their average amount of tariff protection for agricultural products by 36% in the 6 years, while the developing nations were given 10 years to bring it down to 24% and the least developed countries were exempt from this provision (Anderson and Martin, 2005).

The early researches indicated that there were challenges in implementing the new rules. According to Anderson and Martin (2005), the developed nations on an average imposed 14% tariff on agricultural items while only 1% custom duties were charged for non-agricultural products. As a result, markets in developing countries were affected by the disparity in the levels of protection between agricultural and non-agricultural goods. In addition, the authors found that a large number of agricultural exporters were still supported through export subsidies and other support measures. This suggested that despite decline in the global prices, the agricultural producers were able to sustain their production because the government support program helped them generate high incomes. Although it sounds legitimate, the rules of the AoA prevents such assistance because of its potency to distort global prices.

However, the recent studies suggest considerable changes in the agricultural industry including the reduction in the tariff rates. Between 2006 and 2021, there has been some progress in cutting down tariffs although

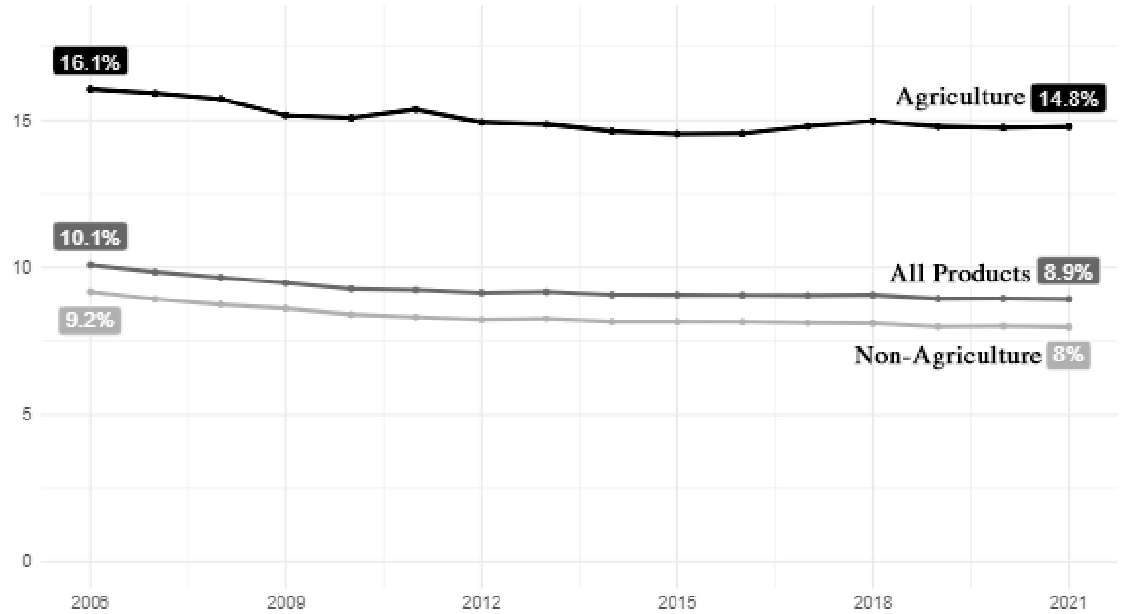

Figure 1. Average tariffs in percentage
Source: World Tariff Profiles (2023)

it has been relatively slow. The World Tariff Profile, 2023, a co publication of WTO, ITC and UNCTAD, showed that the total tariffs imposed by member states have decreased by 1.2 percentage since 2006, with average tariffs for agricultural and industrial products at 14.8% and 8% respectively in 2021 (Figure 1).

This aligns with the technical note issued by IICA (2019) which asserted that the AoA improved market access as signing parties fulfilled their commitments on decreasing the imposed tariff levels as per the agreement. These findings complement the conclusions of a study conducted by Baldwin and Evenett (2015) which claimed that the WTO agreements have improved trade performance by lowering trade barriers and raising trade policy transparency. Furthermore, the authors argued that the agreement has enhanced market access for developing nations and increased trade sizes.

The statistics of FAO (2022) on the trade of agricultural products also echoes the same trend. In nominal terms, the value of global agricultural exports (excluding fish) in 2020 was 3.7 times greater than it was in 2000, and the share of agriculture in the value of all merchandise commerce increased from 6.3 percent to 8.5 percent (Figure 2). However, Birovljev and Ćetković (2013) argued that the benefit of WTO membership might not be equally dispersed among various economies. While WTO membership has brought enormous opportunities for the export of agricultural commodities, developing countries appear to do just fine when it comes to the expansion of trade and agricultural production. At the same time, the WTO agreement is sometimes condemned for being a tool of the wealthy nation to institutionalise their economic dominance. In the same study, the authors also claimed that AoA has a negative impact on food security in developing nations as it exacerbated poverty and inequality by limiting government support program.

An empirical study conducted by Mujahid and Kalkuhl (2015) attempted to investigate the contribution of multilateral and regional trade agreement in supporting food security at the global level using Gravity model. The researchers found that WTO has a negative impact on food commerce when both trading nations are developed. However, when developing nations are involved in trade, the WTO has a positive impact on the food industry. They concluded that the global food security can be achieved if food can be moved efficiently from places of surplus to that of deficit. However, the regional and multilateral trade agreements have created disparities

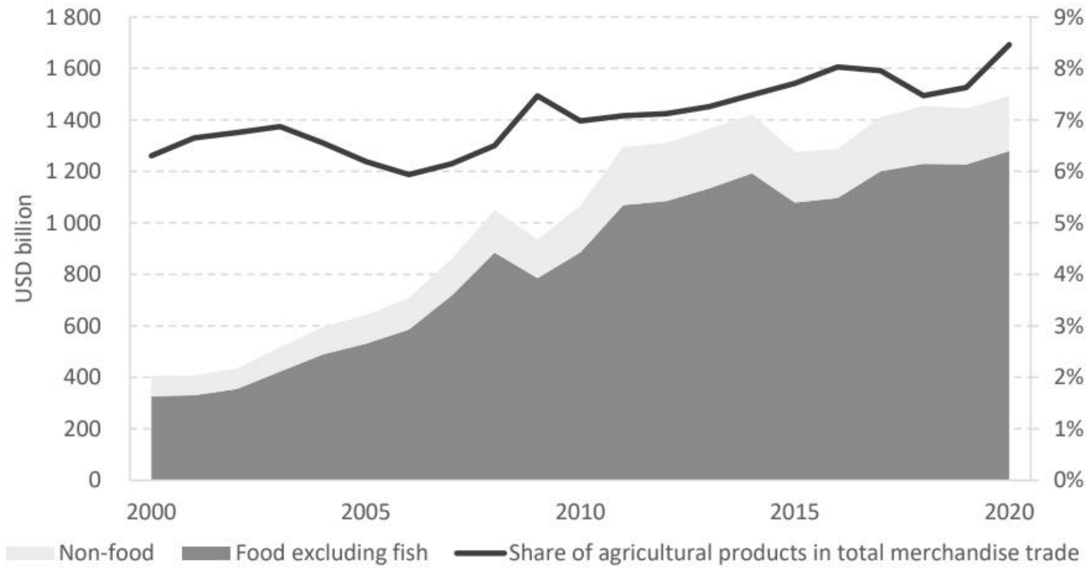

Figure 2 Value of world food and non-food exports and share in total merchandise trade
Source: FAO (2022)

particularly between developed and developing nations.

3.5 Implications in Nepalese Agriculture

Being the first Least Developed Country (LDC), Nepal joined WTO on 23rd April 2004 as the 147th member. Nepal agreed to a comprehensive set of reforms as stated in its accession package. The commitments affecting agriculture trade as briefly described below:

3.5.1 Domestic support

The domestic support measures are subject to gradual reduction as per the AoA commitment which seeks to eventually reduce these restrictions to minimize their negative effects on global agricultural trade. For Nepal, *the de minimis* threshold is at 10% which implies that Nepalese government can provide subsidies to its famers up to 10% of the value of total agricultural output (Awasthi & Adhikary, 2004). Nepal allocated NPR 58.98 billion in subsidies in the fiscal year 2023/24 which is 4% for the total agricultural production (Bhandari, 2023). Therefore, Nepal is within the threshold limit and can further increase the domestic support.

3.5.2 Market access

The market access pillar of AoA primarily focus on reducing tariffs and eliminating non-tariff barriers. Simple average of bound tariff of Nepal in agriculture at the date of accession was 51% which has been reduced to 41.1% by 2022 (Figure 3). Although it adheres to the provision of AoA, Nepalese farmers are having hard time competing with India as the domestic support level of India is comparatively higher. In such situation, Nepal can use Special Safeguard Measures (SSM) to protect the farmers by implementing quantitative restrictions or raise tariffs temporarily.

3.6 Way forward

It is worthwhile noting that The United Nations' 76th session adopted a resolution in February 2021 to graduate Nepal from the LDC to middle-income country with a preparatory period of five years. After 2026, Nepal will be considered a middle-income country and will no longer be enjoying the "duty-free and quota-free" exemption made for the LDCs. In this connection, Nepalese producers including those in the agricultural business have to be competitive in order to tap the export market and sustain the business in the domestic market. Instead of relying on temporary quantitative restrictions and tariff, government should prioritize long-term

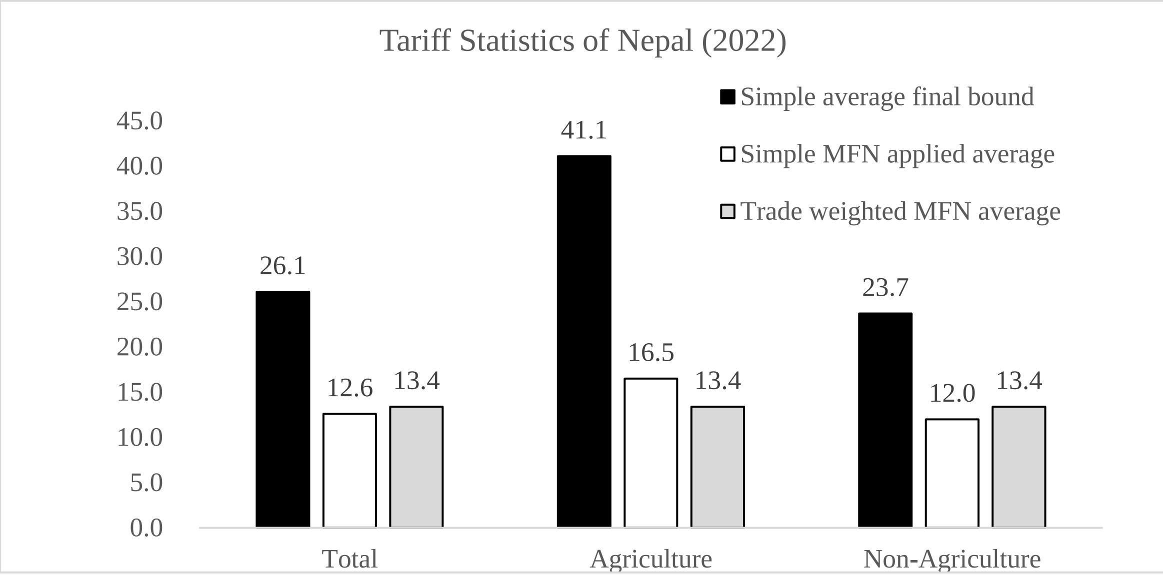

Figure 3 Tariff statistics of Nepal (2022)
Source: https://www.wto.org

strategies to enhance the competitiveness of Nepalese agricultural products. This includes investing in research and development to improve product quality, complying with international standards and certifications, and fostering sustainable agricultural practices.

4. CONCLUSION

To sum up, the advancement of WTO from GATT represented a significance evolution in the international trade. Initially signed by 23 nations to address the economic consequences and trade disruptions caused by the World War II, GATT helped lay the groundwork for a trade system based on rules by advancing ideas like transparency and non-discrimination. The creation of WTO in 1995 institutionalised these principles and extended their application to service industries as well, while also protecting the intellectual properties.

Numerous agreements under the WTO framework play an important role in shaping the global commerce. Especially in the agricultural trade, AoA stands as a key instrument in addressing issues pertaining to market access, domestic support and export competition. Moreover, the agreements of SPS and TBT establishes rules and regulations to ensure their quality, safety and conformity with international standards.

In essence, these agreements are meant to play a crucial role in creating a fair and equitable trading environment for agricultural products. They intend to balance the interest of both developed and developing countries by recognising their unique interest and challenges. However, empirical studies suggest that benefits of WTO membership are not found to be distributed evenly. There are concerns about how it affects food security, poverty, and inequality in developing nations despite improvement in trade and market access.

In Nepalese context, the domestic support is well below the *de minimis* level and therefore implies that Nepalese government can further increase the support. Moreover, it can use SSM to protect the farmers by implementing quantitative restrictions or raise tariffs temporarily.

As Nepal transitions to a middle-income country by 2026, careful attention to policy recommendations and proactive measures will be pivotal in ensuring the continued growth and sustainability of the agricultural sector.

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OPTIMIZATION OF GERMINATION TIME OF SORGHUM VARIETIES TO ENHANCE THE BIOACTIVE COMPOUNDS, ENZYMES, AND REDUCING SUGAR AND REDUCE THE ANTINUTRITIONAL FACTORS

Roman Karki*, Pravin Ojha, Sushma Maharjan, Utsah Manandhar, Sophi Maharjan, Ranjan Shrestha, Kabina Karki and Sanju Upadhyaya

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ABSTRACT

This study aimed to determine the effect of varietal difference and germination time and optimize germination time for sorghum (red and white varieties) to enhance its bioactive properties enzymes and reducing sugar concentrations while reducing anti-nutritional factors. Two-way ANOVA revealed a significant effect (p <0.0001) of varietal difference and germination time on most of the parameters with significant interaction terms (p <0.0001). A comprehensive Polynomial Regression analysis was conducted to investigate the relationship between germination time and the above-mentioned parameters critical for nutritional quality and functional properties. Significant coefficients and terms (p <0.05) associated with germination time across multiple parameters were identified, highlighting its pivotal role in shaping the biochemical composition and functional attributes of sorghum. The findings suggested an optimal germination time of approximately 3.50 days, which promises to yield sorghum with desirable levels of bioactive compounds, enzyme activity, and reducing sugar, and reduced anti-nutritional factors. Overall, this research underscores the potential of germination as a natural and sustainable approach for enhancing the nutritional quality and functional properties of sorghum. By optimizing germination time, this study paves the way for the development of healthier and more nutritious sorghum-based food products, aligning with contemporary dietary preferences and advancing the goal of sustainable food production.

1. INTRODUCTION

Sorghum, a member of the millet family, is a climate-resilient cereal with relatively low global consumption but high nutritional and functional value due to its bioactive content of polyphenols and flavonoids, known for their antioxidant properties and various health benefits such as reducing cardiovascular diseases, (Chaudhary et al., 2024; Mehany et al., 2023). The concentration of these compounds varies based on factors such as plant type, growing conditions, and processing methods (Shobana et al., 2013; Yousaf et al., 2021).

Germination, a common practice in many cultures across Asia and Africa to make various foods such as bread, porridge, and beer, can enhance the nutritional value of cereals by altering their biochemical composition, yet its effect on sorghum remains unclear (Aruna & Visarada, 2019). Thus, this study aims to elucidate the impact of germination time on the polyphenol, flavonoid, tannin, antioxidant activity, α-amylase, diastatic power, and reducing sugar content of sorghum, determining the optimal germination time for two sorghum varieties (red and white).

Polyphenols are natural compounds that have been shown to have antioxidant properties and can protect against various diseases (Hano & Tungmunnithum, 2020). Flavonoids have potent antioxidant and anti-inflammatory properties and have been associated with a reduced risk of several chronic diseases (Maleki et al., 2019). Germination has been reported to increase the polyphenol content of grains. Several studies have reported an increase in the polyphenol content of germinated barley, wheat, and rice (Lee et al., 2017; Tang et al., 2021). During germination, the concentration of polyphenols in cereals has been reported to increase due to the activation of enzymes, phenylalanine ammonia-lyase (PAL), and amino acid, phenylalanine, involved in their biosynthesis (Feduraev et al., 2020; Nkhata et al., 2018).
Similarly, flavonoids, polyphenolic secondary metabolites, known for their biological activities, increase during the germination of barley and rice through the activation of enzymes involved in flavonoid biosynthesis like flavonoid 3'-hydroxylase (F3'H) (Kumar et al., 2018; Liu et al., 2013). The enzyme, flavonoid 3'-hydroxylase (F3'H), is involved in the hydroxylation of flavonoids, leading to the formation of various flavonoid derivatives (Tohge et al., 2017). Tannins are water-soluble polyphenolic compounds that have been shown to have various biological activities, including antioxidant and anti-inflammatory properties (Sahakyan et al., 2020). Tannins, however, decrease during germination of cereals due to enzyme activation, such as tannase, which breaks them down into smaller, less astringent molecules (Garrido-Galand et al., 2021).

α- amylase is an important enzyme involved in the breakdown of starch during germination, leading to the production of smaller carbohydrates such as maltose and glucose (Damaris et al., 2019). The activity of α- amylase has been shown to increase during the germination of rice (Lee & Kim, 2018), wheat (Major et al., 2001), oats, and pearl millets (Lineback & Ponpipom, 1977). DP is another important indicator of the ability of grains to break down starch into simple sugars such as glucose and maltose (Olugbile et al., 2015). Fox et al. 2003 reported an increase in diastatic power during barley germination, which may be attributed to the activation of enzymes involved in starch breakdown.
The current study also aimed to determine the effect of germination time on Nepalese sorghum varieties and to find the optimum germination time for maximizing bioactive compounds, enzymes, and reducing sugar, and minimizing antinutritional factors to achieve the desired properties, and possible industrial applications. This information about Nepalese sorghum varieties was lacking.

2. MATERIALS AND METHODS

a. Varieties of sorghums analyzed

Two varieties of sorghum, *Junelo red (*red coloured seed) and *Junelo white* (white coloured seed) were collected from Benighat Rorang Rural Municipality, Dhading, Bagmati, Nepal (27° 49′ 0″ N, 84° 47′ 0″ E). Both varieties, in triplicate (300 g), were cleaned, and soaked in water for 24 h (18-20 ^{0}C). After that, the excess water was drained and placed in the germination paper (thickness of about 1 cm), covered by the germination paper, and left for germination for up to 5 days at room temperature (18-20 ^{0}C, relative humidity in the range of 31-37% that existed at the time the experiments were conducted). The total polyphenols, total flavonoids, total tannins, antioxidant activity, and antinutritional factor were measured in both varieties during germination from day 0 to day 5. For, determination of Diastatic Power, α-amylase activity, and reducing sugar (RS) concentration the germinated samples from day 1 to 5 were malted by killing for 22 h at progressively increasing temperatures at different time regimes; 45 ^{0}C- 6 h, 50 ^{0}C- 4 h, 55 ^{0}C- 8 h, 70 ^{0} C-1 h, and finally 84 ^{0}C- 3 h.

b. Extract preparation for bioactive compounds and antioxidant activity determination

The sorghum extract was prepared according to the method described by Sigdel et al. (2018) with some modifications. 1 g of grounded sorghum was shaken continuously for 20 minutes with absolute methanol (30 ml) and then filtered through Whattman No 42-filter paper. A similar process was repeated two times and the final volume was made up of 100 ml with methanol resulting concentration of 10 mg/ml.

c. Determination of total polyphenol concentration

The total polyphenol concentration (PC) was determined using the method described by Mahdavi et al. (2011) with some modifications, where the prepared extract was treated with Folin- Ciocalteau solution, and absorbance was measured at 750 nm in UV-Vis spectrophotometer (Cary 60 UV-Vis Spectrophotometer, Agilent Technologies, Inc. CA, USA). Phenols react with phosphomolybdic acid in the Folin-Ciocalteau reagent in an alkaline medium and produce the blue-coloured complex (molybdenum blue). Gallic acid (as a standard phenolic acid) solution at concentrations of 50-250 µg/ml was used to prepare a standard curve as a reference. The PC was expressed in mg Gallic Acid Equivalent (GAE)/100 g Dry matter (DM).

d. Determination of total flavonoid concentration

The determination of total flavonoid concentration (FC) was determined by using the Aluminum Chloride method as described by Walvekar and Kaimal (2014) and measuring the absorbance at 510 nm in a UV-Vis spectrophotometer (Cary 60 UV-Vis Spectrophotometer, Agilent Technologies, Inc. CA, USA). The principle of the aluminium chloride colorimetric method is that aluminum chloride forms acid-stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavanols. Gallic acid solutions at concentrations of 50-250 µg/ml using methanol as solvent were used for the preparation of a standard curve as reference. Total flavonoid was reported as mg Gallic Acid Equivalent (GAE)/100 g DM) of sorghums, *Juenlo red*, and *Junelo White*.

e. Determiantion of total tannin concentration

For total tannin concentration (TC) determination, Folin-Denish reagent was used for color development and absorbance was measured at 725 nm in a UV-Vis spectrophotometer (Cary 60 UV-Vis Spectrophotometer, Agilent Technologies, Inc. CA, USA) as described by Elgailani and Ishak (2014). The tannic acid solution was used to prepare a standard curve and the result was expressed in mg Tannic Acid Equivalent (TAE)/100 g DM.

f. Determination of antioxidant activity

The determination of antioxidant activity (AA) was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method as per Walvekar and Kaimal (2014) with some modifications. The principle of DPPH method is based on the reduction of DPPH in the presence of a hydrogen-donating antioxidant due to the formation of diphenylpicryl hydrazine. Briefly, 0.12 ml methanolic extract in a test tube was diluted with 2.88 ml of methanol and 1 ml of freshly prepared DPPH solution (0.004% w/v) was added followed by mixing (0.4 mg sorghum methanolic extract/ml of methanol). The solution was allowed to stand for 30 minutes at room temperature in the dark condition and the absorbance reading was measured at 517 nm using a UV-Vis spectrophotometer (Cary 60 UV-Vis Spectrophotometer, Agilent Technologies, Inc. CA, USA). Absolute methanol was used as a blank. The AA was expressed as the percentage of Radical Scavenging Activity (% RSA) using equation (1)

$$Radical\ Scavnging\ Activity = (A - B)/A \times 100 \qquad (1)$$

Where A is the absorbance of DPPH and B is the absorbance of DPPH and the extract combination.

The calculated value was given by 0.4 mg/ml of sorghum extract.

g. Determination of diastatic power

The diastatic power (DP) was determined by the Fehling solution modification method described by Horwitz & Latimer (2005). The malt infusion was prepared by grinding approximately 25.5 g sorghum malt (prepared by kilning the germinated sorghum at different times in the cabinet drier at temperatures) as per Association of Official Analytical Chemists (AOAC) Official Method No: 935.31. The DP of the above-digested starch solution was determined according to AOAC Official Method No: 935.31. The volume of digested starch solution required to reach the endpoint in this second titration was designated as ‘V’. The volume of digested starch solution using the blank was prepared used designated as ‘B’.

The DP as Degree DP (^{0}DP) using the equation (2)

$$Diastatic\ Power = (5000/V) \times (B/V) \quad (2)$$

h. Determination of α- Amylase Activity

The α- Amylase Activity was determined by the dextrinization method as described by Horwitz & Latimer (2005) according to the AOAC Official Method No: 955.22. The infusion was made from approx. 25.5 g grounded sorghum malt and the dextrinization of malt infusion was measured using dilute I_2 solution as an indicator and the time interval for dextrinization of starch was measured. An α-amylase unit is defined as an amount of α-amylase that will dextrinize soluble starch in the presence of excess β-amylase at the rate of 1 g/h at 20°C. The α-Amylase Activity concentration (Dextinizing units/g DM) was measured from the equation (3).

$$20^{0}DU(DB) = (24/(W \times T)) \times (100/(100 - M)) \quad (3)$$

where M = % moisture in the test portion, and 24 = weight starch used (0.4 g) multiplied by 1 h (60 min).

i. Determination of reducing sugar concentration

The determination of reducing sugar (RS) content was determined by using the 3,5-Dinitro-Salicylic Acid (DNS) method as per Ranganna (1986). In 3 ml clear supernatant, prepared from 1 g/ml sorghum malt, 3 ml of DNS solution was added and boiled for 5 minutes in a water bath. Then, 1 ml of 40 % Rochelle salt solution was added to the still-warm test tube and mixed thoroughly. Finally, 10ml distilled water was added and mixed again and the solution was cooled, and the absorbance was measured at 510 nm. The concentration of RS (% DM) was measured using a standard curve of absorbance vs glucose at concentrations of 50-250 µg/ml at 510 nm using a UV-Vis spectrophotometer (Cary 60 UV-Vis Spectrophotometer, Agilent Technologies, Inc. CA, USA).

j. Determination of trypsin inhibitor concentration

The trypsin inhibitor concentration (TI) was determined as per Bundy & Mehl (1958) with modifications. The suspension, prepared from a 0.2 g sample, was centrifuged at 5000 rpm for 5 minutes and filtered through the Whatman No. 42 filter paper. The volume of each was adjusted to 2 ml with phosphate buffer. Then, the test tube was placed in a water bath maintained at 37^{0}C and 6 ml of 5% Trichloroacetic acid (TCA) solution was added to one of the tubes to serve as a blank. 2 ml of Casein solution was added to all test tubes, which was previously kept at 37^{0}C and were incubated for 20 minutes. The reaction was stopped after 20 minutes by adding 6 ml TCA solution to the experimented tubes and shaken and left to react for 1 hour at room temperature after which it was filtered through Whatman No. 42 filter paper. The absorbance of filtrate from the sample and trypsin solution was read at 380 nm on a UV-Vis spectrophotometer (Cary 60 UV-Vis Spectrophotometer, Agilent Technologies, Inc. CA, USA) and the concentration of TI(TI) was calculated as mg/g DM using the equation (4):

$$TI = \frac{((Abs\ of\ Std - Abs\ of\ Sample) \times Dilution\ Factor)}{(19 \times Sapmle\ weight)} \quad (4)$$

k. Statistical analysis and optimization of germination time

The effect of varietal difference and germination time (0-5 days) on the concentration of PC, FC, TC, AA, DP, α-amylase, RS, and TI was analyzed by a two-way analysis of variance (ANOVA) with the full factorial generalized linear model using Minitab Version 21.2, Statistical Software (2017), State College, PA: Minitab, Inc., USA. (www.minitab.com). In addition, the post hoc Tukey test for multiple comparisons was performed at a 5% level of significance. The optimization of germination time for the response variable was carried out by fitting a Polynomial regression model (Equation 5) using Minitab Version 21.2 Statistical Software (2017), State College, PA: Minitab, Inc., USA. (www.minitab.com).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 {X_1}^2 + e \quad (5)$$

Where Y is the response variable, X_1 is germination time (0-5 days), β_0 is the coefficient or constant terms, β_1 is linear coefficients for X_1, β_2, is quadratic coefficients for X_1, e is the error term.

The model was simplified by backward elimination, with elimination criteria (α) significance set at 0.1 to remove insignificant terms in hierarchical order. The optimum germination time was calculated by response optimizer using a simplified model for maximizing PC, FC, TC, DP, AA, α-amylase activity, RS concentrations, and minimizing TI concentration.

3. RESULTS AND DISCUSSION

a. *Effect of varietal differences and germination time on total polyphenol concentration*

The total PC (mg GAE/ g DM) in two sorghum varieties (Junelo red and Junelo white) was evaluated over five different germination times (0-5 days). A two-way ANOVA revealed significant effects of sorghum variety ($p < 0.0001$) and germination time ($p < 0.0001$) on polyphenol concentration, with a significant interaction between the sample and germination time ($p < 0.0001$), with very high R-square values (Table 1). Moreover, PC decreased significantly ($p <0.05$) with increasing germination time in both sorghum varieties (Figure 1A). Several studies collectively suggested that both the variety of cereal and the duration of germination time have a significant impact on the polyphenol concentration, with a notable interaction between the sample and germination time in sorghum, corn, wheat, chickpea, hull-less spring cereals, and triticale. (Dicko et al., 2006; Gong et al., Khattak et al., 2007; Kim et al., 2018; Sokrab et al., 2012). Kathaak et al. (2007) observed that phytic acid was reduced and methanol extractable polyphenols were increased with an increase in germination time up to 48 hours significantly, but with extended germination beyond 48 hours, phytic acid was increased and polyphenols were decreased. However, an increase in PC with prolonged germination time was also observed, which was attributed to the formation of new polyphenols through the activation of polyphenol biosynthesis enzymes (Ling et al., 2018). Moreover, environmental conditions, such as plant type, age, stage of development, and stage of development, govern the polyphenol contents in plants, which can affect digestive enzymes, protein availability, mineral uptake, vitamin metabolism, and growth (Salunkhe et al., 1983). Notably, at all germination times, Junelo red maintained significantly higher ($p <0.05$) PC compared to Junelo white, suggesting its potential as a rich source of polyphenols for functional foods. These variations likely resulted from differences in polyphenol types, levels, biosynthesis, and degradation processes during germination (Shakune et al., 1983; Xu et al., 2020; Ti et al., 2014).

b. *Effect of varietal difference and germination time on total flavonoid concentration*

Similarly, total FC (mg GAE/g DM) in both sorghum varieties also varied significantly with both variety and germination time ($p < 0.0001$). FC decreased with increasing germination time for Junelo red (Figure 1B), consistent with previous studies on germinated seeds (Dicko et al., 2005: Dykes et al., 2009; Ghimire et al., 2021; Taleon et al., 2014). However, in the case of Junelo white, the FC did not differ between 2 and 3 days of germination as well as between 3 and 4 days of germination. The decrease in FC with an increase in germination time is probably due to the activation of enzymes that hydrolyze flavonoid glycosides, as well as possible oxidation reactions. During the germination process, enzymes such as β-glucosidase, other glycosidases, and esterase become activated and can hydrolyze flavonoid glycosides into aglycones (Zhu et al., 2005). The studies on soybean (Zhu et al., 2005), tartary buckwheat (Da-bing, 2013), brown rice (Ti et al., 2014), and Arabidopsis (Kubasek et al., 1992) provide evidence for the enzymatic degradation of flavonoids during the germination process.

Moreover, studies suggest that flavonoids are indeed used as nutrients during germination for various physiological processes, such as protecting the seed from oxidative damage and facilitating seedling growth (Pourcel et al., 2007; Shirley, 1998). However, the data does not provide direct evidence of other compounds competing with flavonoids for biosynthetic precursors during germination (Galland et al., 2014). Instead, the data suggests that flavonoids (flavanones, flavonols, flavones, and flavone C-glycosides) themselves are crucial for the proper development and protection of the germinating seed and growing plant (Doughty et al., 2014; Wang et al., 2020; Ylstra et al., 1994). Notably, like in the case of polyphenol, Junelo red exhibited the highest mean FC across all germination days, suggesting its potential as a source of flavonoids for functional foods.

The significant interaction between sorghum variety and germination time further highlights the variability in the effect of germination time on FC among different varieties. These differences may be attributed to genetic factors, specific flavonoid biosynthesis pathways, and environmental conditions during germination (Carbone et al., 2009). The observed decrease in FC with prolonged germination may result from enzymatic activity, nutrient utilization, or competition with other compounds for biosynthetic precursors. Furthermore, flavonoid biosynthesis and catalysis are known to be affected by environmental conditions, such as temperature, relative humidity, etc., during the germination process. water stress and light exposure, which can be induced by the germination process. This is supported by previous studies that have shown an increase in FC in response to water stress (Hodaei et al., 2018). Moreover, flavonoid biosynthesis is known to be inhibited by light (Zoratti et al., 2014).

c. Effect of varietal difference and germination time on total tannin concentration

Tannins, phenolic compounds abundant in plants, vary in concentration due to factors like species, variety, and growth conditions (Gebrehiwot et al., 2002). The current study observed a significant effect ($p < 0.0001$) of variety and germination time on TC (mg TAE/g DM) with a significant ($p < 0.0001$) interaction term (Table 1). *Junelo red* showed lower tannin levels on day 2 compared to day 1 ($p<0.05$), while *Junelo white* displayed consistent levels from day 0 to 2 and decreased significantly in both varieties after 5 days of germination (Figure 1C). Several studies conclude that the germination process in cereals affects tannin levels, with significant differences observed among varieties and germination times. Yan et al., (2009) found that germination reduced tannin content from 3.96% to negligible levels in a high-tannin sorghum cultivar. Chavan et al. (1981) also reported about 73% reduction of tannins during 120 hours of germination in high-tannin seeds of sorghum, while about 20% loss in low-tannin seeds. Additionally, germination under different environmental conditions was shown to reduce tannin content in lentils (Ayet et al., 1994). The decrease in TC with increasing germination time could be due to the degradation of tannins by endogenous enzymes like tannase into smaller phenolic compounds or their conversion into insoluble forms (Taylor & Duodu, 2015).

Table 1. Effect of variety and germination time differences depicted by Generalized Linear Model

Parameters	Variety *p-value*	Germination Time (Days) *p-value*	Var*Ger. Time *p-value*	R Square (%)	R Square (adjusted) (%)	R Square (predicted) (%)
Polyphenol (mg GAE / 100g DM)	<0.0001	<0.0001	<0.0001	100	100	100
Flavonoid (mg GAE / 100g DM)	<0.0001	<0.0001	<0.0001	99.99	99.98	99.97
Tannin (mg TAE / 100g DM)	<0.0001	<0.0001	<0.0001	99.97	99.96	99.94
AA(% RSA)	<0.0001	<0.0001	<0.0001	100	100	99.99
DP(^{0}DP)	<0.0001	<0.0001	<0.0001	99.98	99.82	99.72
RS(%DM)	<0.0001	<0.0001	0.003	97.64	96.58	94.69
α-Amylase (DU/ g DM)	<0.0001	<0.0001	<0.0001	99.62	99.45	99.15
TI(mg/g DM)	<0.0001	<0.0001	0.016	96.32	94.66	91.72

Note: GAE: Gallic Acid Equivalent, DM: Dry Matter, TAE: Tannic Acid Equivalent, RSA: Radical Scavenging Activity, ^{0}DP: Degree Diastatic Power, DU: Dextrinizing Unit

d. Effect of varietal difference and germination time on the antioxidant activity

The AA (% RSA) was observed to be significantly affected by both variety and germination time (p <0.0001) as well as significant interaction (p <0.0001). In both sorghum varieties, the antioxidant activity significantly (p<0.05) decreased with an increasing germination time (Table 1). *Junelo red* showed significantly higher values than *Junelo white* after day 1 of germination (Figure 1D). The concentration of polyphenols, flavonoids, and tannins is responsible for the AA of cereal. This is consistent with the findings of this study, where a significantly higher (p<0.05) concentration of these bioactive compounds has been observed in *Junelo red* than in *Junelo white* at all germination times.

Germination can significantly enhance the AA of seeds, although the extent of this increase and the optimal germination time can vary depending on the seed types (Abderrahim et al.2012; Aguilera et al., 2014; Dueñas et al., 2009 López-Amorós et al, 2006). The process generally leads to an increase in phenolic compounds and antioxidant vitamins, contributing to the improved antioxidant profile of germinated cereal and legume seeds. However, there are instances where certain antioxidants may decrease after prolonged germination (Fernandez-Orozco et al., 2006), indicating that there is a balance to be found in germination times to maximize the antioxidant benefits. Porokhovinova et al. (2022) have shown that variations in the phytochemical composition and antioxidant capacity of flaxseed as affected by seed coat color. The authors of that study found that the flaxseed with a brown seed coat had higher levels of phenolic compounds, flavonoids, and lignans compared to those with a yellow seed coat.

e. Effect of varietal difference and germination time on the diastatic power

Like other parameters, both the variety and germination time had a significant effect on the DP (p < 0.0001), as well as their interaction (p < 0.0001) (Table 1). The DP values for both varieties of sorghum increased significantly (p < 0.05) with an increase in germination time up to day 4, then did not change significantly (*p* >0.05) (Figure 1E). Several studies have reported that there are significant differences in DP values among different cereal varieties and germination times. The DP in cereal grains is influenced by both the variety of the grain and the conditions under which it is germinated, including time and temperature. Subramanian et al. (1992) reported that for sorghum cultivars, diastatic activity increased up to 96 hours of germination and then decreased at 144 hours, with significant variation among the cultivars themselves. Novellie (1962) supported this by stating that optimal DP in kaffir corn, a type of sorghum, requires specific conditions such as high temperatures and moisture content during germination. Furthermore, Niche & Okafor (1989) indicated that germination time and temperature significantly affect the DP of rice malt, with longer germination times and higher temperatures generally leading to increased diastatic power.

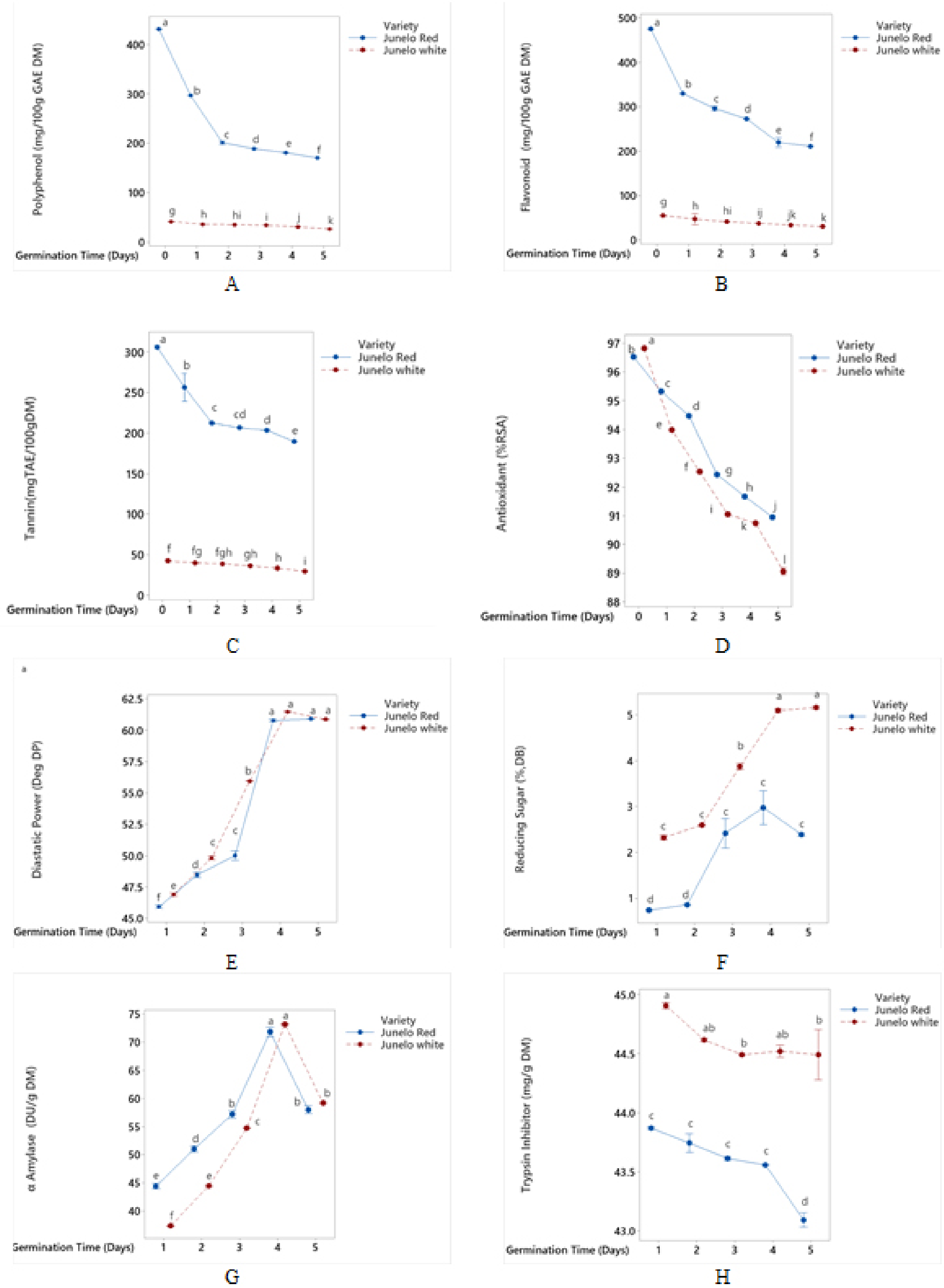

Figure 1. Effect of germination time on the concentrations of different parameters of two varieties of sorghum (*Junelo red* and *Junelo white*) A: total polyphenol (mg GAE/g DM), B: total flavonoid (mg GAE/g DM), C: total tannin (mg TAE/g DM), D: antioxidant activity (%RSA), E: DP(^{0}DP), F: α-amylase activity (DU/g DM), G: trypsin inhibitor (mg/g DM)

Moreover, Xia et al. (2019) and Xia et al. (2017) have reported that germination time and high hydrostatic pressure on other nutritional components of brown rice, which could indirectly influence diastatic activity.

Moreover, the DP was found to be significantly ($p < 0.05$) higher in *Junelo red* than *Junelo white* at each germination time up to day 4 suggesting it may have higher enzymatic activity and thus higher potential for use in brewing or other industrial applications such as brewing, distilling, or baking that require high DP grains (Faltermaier et al., 2014; Lalor & Goode, 2009). Further research is needed to fully understand the biochemical mechanisms underlying these differences in DP among cereal varieties and the effects of different germination times on enzymatic activity. Previous studies have shown that the rate of alpha-amylase activity can vary depending on the germination time, with the highest activity usually observed between 3 and 5 days (Farashah et al., 2011; Helland et al., 2002). It is important to note that the study only looked at DP values up to 5 days of germination, and further research may be needed to fully understand how DP changes beyond this time point. Additionally, other factors such as temperature, moisture concentration, and the presence of other enzymes could also affect DP and should be considered in future studies.

f. Effect of varietal difference and germination time on the reducing sugar concentration

Also, in the case of RS concentration (% DM), the p values for both the sample and germination time, as well as their interaction, were found to be highly significant ($p < 0.0001$) (Table 1). In *Junelo red,* the RS increased significantly ($p < 0.05$) after 3 days of germination than other times whereas in *Junelo white*, it did not differ significantly ($p > 0.05$) until 4 days of germination (Figure 1F). *Junelo red* had a significantly higher RS concentration than *Junelo white* at all germination times. Previous studies that have demonstrated the activation of endogenous enzymes during germination lead to an increase in RS concentration (Białecka & Kępczyński, 2007; Cheung & Suhadolnik, 1979; Jaya & Venkataraman, 1981; Ologhobo & Fetuga, 1986).

The differences in RS concentration among different cereal varieties and genotypes of the same cereal variety may be due to differences in the endogenous enzyme activity and starch concentration of these varieties, as shown by previous studies (Balcerek et al., 2016; Guzmán-Ortiz et al., 2019; Shaik et al., 2014; Sidari, et al., 2008; Singh et al., 2015). In addition to the increase in reduced sugar concentration, the DP of the cereal grains also increased with germination time. DP is a measure of the ability of the grain to break down starch into simpler sugars and is closely related to alpha-amylase activity (AOAC International, 2016). The increased availability of simple sugars may also have implications for the use of germinated cereal grains in the production of fermented foods and beverages. However, this study did not observe a significant correlation among RS concentration, α-amylase, and DP to make any conclusive statements. Further studies are needed to explore the relationship between alpha-amylase activity, diastatic power, and RS concentration in different cereal varieties and under different germination conditions.

g. Effect of varietal difference and germination time on α-amylase concentration

Significant variation in α-amylase concentration (DU/g DM) was found among varieties ($p < 0.0001$) and germination times ($p < 0.0001$) with a significant interaction ($p < 0.0001$) (Table 1). The α-amylase concentration for both varieties increased significantly ($p < 0.05$) with an increase in germination time from day 1 to 4 and then decreased significantly ($p < 0.05$) (Figure 1G). The increase in α-amylase activity is observed across various cereal grains, including rice, barley, maize, sorghum, and others, with the timing and extent of the increase varying among species, grain types, and germination conditions (Dicko et al., 2006; Gujjaiah & Kumari, 2013; Kikunaga et al., 1991; Lasekan, 1996; Lee et al., 2013; Palmiano & Juliano, 1972; Ranki & Sopanen, 1984; Sasahara & Ikarahi, 1989; Thévenot et al., 1992). These studies concluded that enzymatic activity is essential for the breakdown of starch into simpler sugars, which are necessary for the growth of the new plant. This enhanced enzyme activity during germination is a key factor in the transformation of stored nutrients within the grain, facilitating the early stages of plant development.

This finding agrees with the known physiology of seed germination, during which the stored starch in the endosperm is broken down by enzymes like α-amylase to provide energy for the developing seedling (Finch-Savage and Leubner-Metzger, 2006).

The α-amylase concentration was significantly higher for *Junelo red* at germination times up to 3 days; after that, the concentration did not differ. The differences in α-amylase concentration among the different varieties may be due to genetic differences in the enzymes responsible for α-amylase activity as well as environmental factors such as temperature, humidity, and soil conditions which could be utilized for specific industrial applications such as brewing, malting, and pediatric food development (Chelghoum et al., 2021; Hidalgo et al., 2013; Sundarram & Murthy, 2014). Further research is needed to better understand the mechanisms that contribute to the observed activity differences among different varieties and germination times and their potential impact on the nutritional and functional properties of the resulting products. These findings have important implications for the food and beverage industry, as well as for plant breeding programs aimed at improving the brewing quality of sorghum.

h. Effect of varietal difference and germination time on the trypsin inhibitor concentration

Similar to all observed parameters, the TI concentration (mg/g DM) was also significantly affected (p <0.0001) by both variables, sample and germination time, and their interaction (p <0.016) (Table 1). In *Junelo red*, only after 1 day of germination, *the* antinutritional factor was significantly higher (p <0.05); after that, there was no significant difference (p >0.05) with prolonged germination time (Figure 1H). Whereas in *Junelo white,* it was reduced significantly (p <0.05) only at 5 days of germination. On the contrary to bioactive compounds, *Junelo white* had significantly higher (p <0.05) antinutritional factor concentration than *Junelo red* at all germination times. This indicates that the bioactive compounds have a negative correlation to the antinutritional factor in the germinated sorghum. Several studies have reported that an increase in germination time decreased the TI concentration in cereals and legumes (El-Safy et al., 2013; Frias et al., 1995; Nkhata et al., 2018; Savelkoul et al., 1992; Wang et al., 2013).

i. Optimization of Germination Time

A polynomial regression analysis was done to optimize germination time and explore the intricate relationship between germination time and key attributes, including PC (mg/100g GAE DM), FC (mg/100g GAE DM), AA (% RSA), TC (mg TAE / 100g DM), DP (^{0}DP), RS (%DM), α-amylase (DU/g DM), and TI (mg/g DM). the objective was to find the optimal germination time that maximizes the concentrations of the bioactive components, enzymes, RS and minimize the concentration of anti-nutritional factor.

The regression analysis revealed that the mole was significant (p <0.05) with significant coefficients and terms associated with germination time across multiple parameters, as detailed in Table 2. PC also had a relatively lower significant (p <0.05) negative linear term, indicating a decrease in its value with the onset of germination (Table 2, Figure 2A). Furthermore, the variation of the leverage points was uniform, as suggested by a lower confidence level (Figure 2B), but the prediction ability is lower, due to large variability (Figure 2C), which is also demonstrated by lower level of significance of the linear prediction term ((p <0.05). Moreover, AA and α-amylase both had significant linear and quadratic terms (p <0.0001 and p <0.05, and p < 0.0001 and p <0.001, respectively). The AA has highly significant (p<0.0001) negative linear and positive quadratic terms for AA indicating that the rate of change in the concentration gradually decreased over time (Table 2, Figure 2J). The 95% confidence interval (CI) of the leverage points (Figure 2K) and prediction plots (Figure 2L) were very narrow indicating high prediction ability of the model. In addition, the positive linear terms related to germination time had significant effects (p <0.0001) on critical aspects such as DP and RS indicating that there is a linear increase in their value with an increase in germination time (Figure 3A, and Figure 3D, respectively). The DP has very low CI for both the leverage plot (Figure 3B) and prediction plot (Figure 3C) and RS moderate CI leverage plot (Figure 3E) and prediction plot (Figure 3F).

Table 2. Polynomial regression models with terms and coefficients for germination time and quality parameters.

Parameters	Polyphenol (mg GAE / 100g DM)	Flavonoid (mg GAE / 100g DM)	Antioxidant Activity (% RSA)	Tannin (mg TAE / 100g DM)	DP (^{0}DP)	Reducing Sugar (%DM)	α-Amylase (DU/ g DM)	TI(mg/ g DM)
Coefficient	202.60	236.50	96.596	154.064	41.852	0.801	20.52	44.489
Linear Term								
Germination Time (Days)	-25.1*	26.3	-1.884****	-11.916	4.084****	0.680****	20.32****	-0.133
Quadratic Term								
Ger. Time x Ger. Time			0.1172*	2.946			-2.369***	
p value	0.039	0.064	<0.0001	0.4556	<0.0001	<0.0001	0.001	0.074
R Square (%)	89	9.70	90.29	4.652	88.05	43.82	73.52	10.96
R Square (adjusted) (%)	9.30	7.04	89.70	1.126	87.62	41.82	71.56	217.78
R Square (predicted) (%)	0	0	88.45	0	86.80	34.43	68.39	0
Lack of fit (*p-value*)	0.875	0.973	0.527	0.0136	<0.0001	0.342	<0.0001	0.975

Note: the asterisks (*) indicate the level of significance of the coefficients of response variables: * p<005, ** p<001, and *** p<0001, **** p<00001

However, the positive linear and negative quadratic terms for α-amylase indicated that the concentration gradually decreased over time until it reached a maximum and started to decrease if we continued to germinate the sorghum (Figure 3G) with very low CI for leverage (Figure 3H) and prediction (Figure 3I) plots. The inclusion of quadratic terms for germination time highlighted the nuanced curvature present in the response surfaces of several parameters, providing deeper insights into their behavior over time. However, the FC and TI variables did not have any significant (*p* >0.005) terms indicating the observed variation in their value could not be explained by the used model (Figure 2D, 2E, and 2F, and Figure 3I, 3J, and 3K, respectively).

To ensure the reliability of our findings, we subjected the regression models to rigorous lack-of-fit tests. Encouragingly, the results indicated a non-significant lack of fit (p > 0.05) across most of the parameters, except TC, DP, α-amylase, and TI confirming the adequacy of our models in capturing the underlying trends. Moreover, the coefficients of determination (R^2) and adjusted R^2 demonstrated the models' effectiveness in explaining a significant proportion of the observed variability in the data, further strengthening our confidence in their predictive capability.

Leveraging the insights gleaned from the regression models, we identified the optimal germination time, estimated to be approximately 3.50 days. This optimized timeframe was projected to yield desirable levels of critical attributes such as PC (mg GAE/mg DM) (95% Confidence interval (CI): 67.4,161.8), FC (mg GAE/mg DM) (CI: 89.2, 199.8), AA (% RSA) (CI: 91.071, 91.793), DP (0DP) (CI: 55.292, 57.041), α-amylase activity (DU/gDM) (CI: 59.00, 65.61), RS (%DM) (CI: 2.738, 3.634), and TI (mg/ g DM) (CI: 43.803, 44.244). Notably, the composite desirability value associated with this optimal solution was calculated at 0.410, indicating its substantial merit. To offer stakeholders a comprehensive understanding of the implications of our optimization efforts, we provided predicted values and standard errors for each response variable at the optimal germination time. Additionally, we delineated the corresponding CI offering valuable insights into the range of variability and the precision of our optimization endeavors. Thus, our pursuit of optimizing germination time through rigorous regression analysis is poised to significantly enhance the nutritional quality and functional properties of our product. By elucidating the intricate dynamics governing the interplay between germination time and key parameters, we have paved the way for maximizing the nutritional value, bioactive components, antinutrtitional factors, and functional attributes of our final product, thereby recommending the optimal germination time for sorghum for possible industrial applications.

The implications of the study extend across various domains, including food processing, human nutrition, the brewing industry, and agricultural practices. Understanding the variations in TC and α-amylase activity among different cereal varieties and germination times provides opportunities for developing tailored food products with improved nutritional profiles. By manipulating germination time, it becomes possible to produce functional foods that cater to specific dietary needs. Additionally, variations in DP among cereal varieties suggest potential applications in brewing and other industrial processes, where identifying varieties with high DP can optimize efficiency and ensure consistent product quality. Furthermore, considering sorghum variety and germination time in agricultural practices informs breeding programs aimed at enhancing sorghum quality in addition to yield, and disease and pest resistance.

The optimization study on sorghum germination time holds significant implications for stakeholders in the food industry, agriculture sector, and academic research community alike. By identifying the optimal germination time, the study facilitates the production of sorghum with heightened levels of bioactive compounds such as polyphenols and flavonoids. These bioactive compounds are known for their antioxidant properties, which can contribute to improved health outcomes and disease prevention for consumers. Additionally, optimizing germination time results in elevated levels of α-amylase activity, crucial for starch hydrolysis and subsequent sugar production during food processing. This enhancement in enzymatic activity can lead to improved texture, flavor, and digestibility of sorghum-based food products.

Moreover, the study demonstrates that optimal germination time effectively reduces the levels of anti-nutritional factors such as trypsin inhibitors. This reduction enhances the bioavailability of nutrients present in sorghum, thereby improving its nutritional profile and making it a more wholesome dietary option. Additionally, the optimization of germination time influences functional properties such as DP and RS content, which are vital for food processing applications including fermentation and baking. By optimizing germination time to enhance bioactive properties and reduce anti-nutritional factors, sorghum-based products can gain a competitive edge in the market. This aligns with consumer preferences for healthier and more nutritious food options.

Furthermore, the study underscores the importance of germination as a natural and sustainable process for improving the nutritional quality of sorghum. By promoting the adoption of optimized germination practices, the agricultural sector can contribute to sustainable food production and address global challenges related to food security and nutrition. In essence, the implications of the optimization study extend beyond scientific research to practical applications in food processing, product development, and agricultural practices, offering tangible benefits to both producers and consumers.

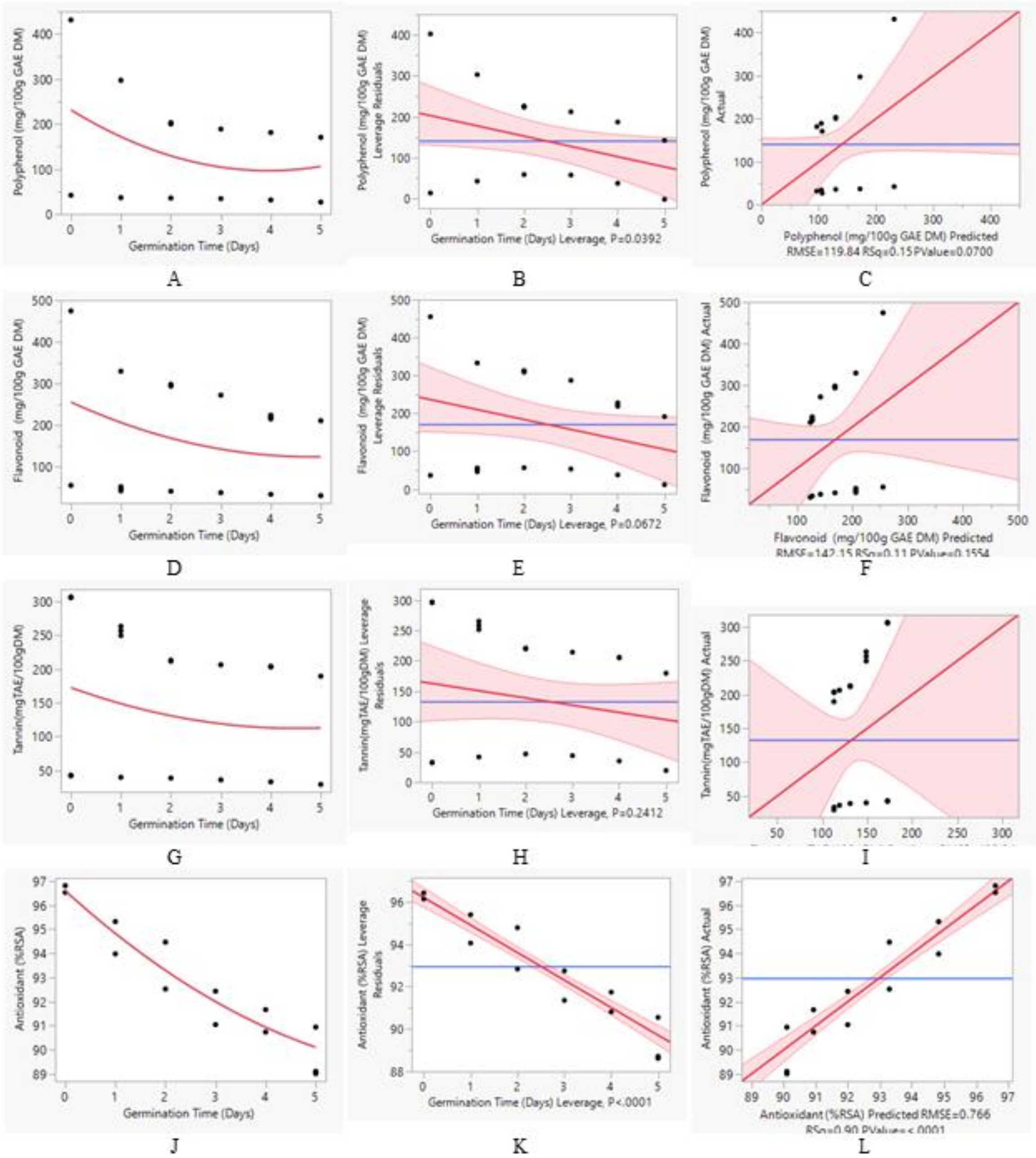

Figure 2. Various plots depict the effect of germination time on bioactive and antioxidant properties. A: regression plot for total polyphenol, B: leverage plot for total polyphenol, C: prediction plot for total polyphenol, D: regression plot for total flavonoid, E: leverage plot for total flavonoid, F: prediction plot for total flavonoid, G: regression plot for total tannin, H: leverage plot for total tannin, I: prediction plot for total tannin, J: regression plot for antioxidant activity, K: leverage plot for antioxidant activity, L: prediction plot for antioxidant activity

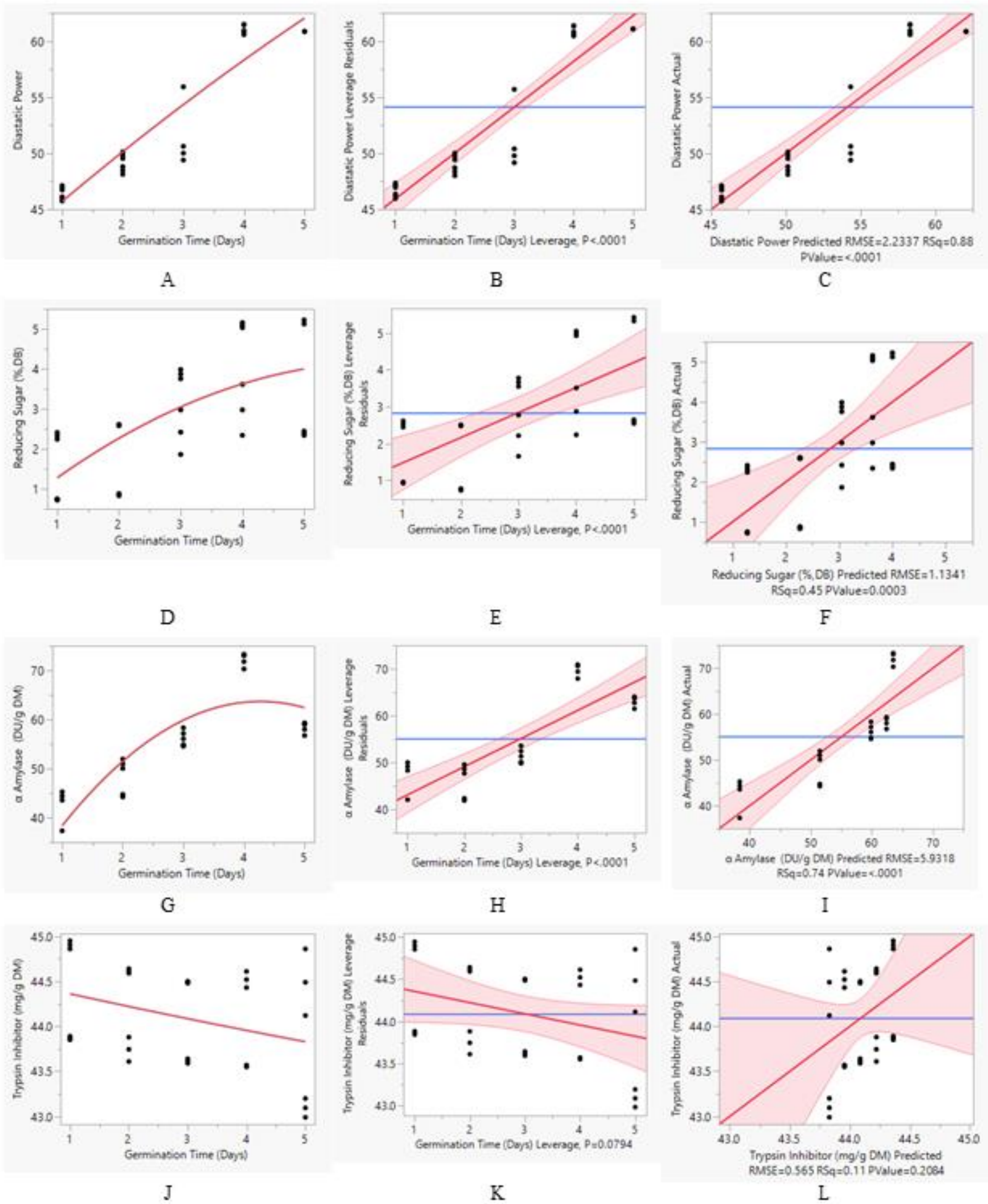

Figure 3. Various plots depicting the effect of germination time on enzymes, RS and antiantioxidant properties. A: regression plot for diastatic power, B: leverage plot for diastatic power, C: prediction plot for diastatic power, D: regression plot for total reducing sugar, E: leverage plot for reducing sugar, F: prediction plot for reducing sugar, G: regression plot for α-amylase, H: leverage plot for α-amylase, I: prediction plot for α-amylase, J: regression plot for trypsin inhibitor, K: leverage plot for trypsin inhibitor, L: prediction plot for trypsin inhibitor

4. CONCLUSION

In conclusion, our study provides valuable insights into the variation in PC, FC, TC, α-amylase activity, DP, RS, and TI concentrations among different cereal varieties and germination times. These findings underscore the complex interplay between genetic factors, germination conditions, and enzymatic activities, ultimately influencing the nutritional and functional properties of cereals. These insights have significant implications for food processing, human nutrition, the brewing industry, and agricultural practices. By understanding and leveraging these factors, researchers and practitioners can develop innovative strategies to enhance food quality, optimize industrial processes, and improve agricultural productivity.

However, the study acknowledges certain limitations, such as its focus on a limited number of cereal varieties and germination times, as well as its laboratory-based approach. Future research should explore additional factors influencing cereal properties under diverse environmental conditions to provide a more comprehensive understanding of cereal biology and its implications for various applications. Overall, the findings contribute to ongoing efforts to harness the potential of cereals for addressing food security, nutrition, and industrial needs. In essence, the optimization of the germination time of sorghum offers a promising avenue for enhancing its bioactive properties, enzymes, and RS content while simultaneously reducing anti-nutritional factors. Through a comprehensive polynomial regression analysis, we elucidated the intricate relationship between germination time and key parameters critical for nutritional quality and functional properties. Our findings reveal significant coefficients and terms associated with germination time across multiple parameters, indicating its pivotal role in shaping the biochemical composition and functional attributes of sorghum. By leveraging regression models and lack-of-fit tests, we established the optimal germination time, estimated to be approximately 3.50 days, promising to yield sorghum with desirable levels of bioactive compounds, enzyme activity, and reduced anti-nutritional factors. The implications of our optimization study extend to various stakeholders, including the food industry, agricultural sector, and the academic research community. Enhanced bioactive properties, increased enzyme activity, and improved functional properties contribute to the market competitiveness of sorghum-based products while promoting sustainable agricultural practices.

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EFFICACY OF FUNGICIDES FOR THE MANAGEMENT OF FOOT ROT DISEASE, *Sclerotium rolfsii* (SACCARDO) IN CHILLI

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ABSTRACT

Eight different fungicides were tested against Sclerotium rolfsii Sacc. in vitro and in vivo. In vitro evaluation was done by poisoned food technique with fungicides at three different concentrations of 10 ppm, 25 ppm and 100 ppm. In vivo efficacy of fungicides against foot rot of chilli was done under screen house condition in the pots. Among the fungicides, hexaconazole 5%, carboxin 37.5% + thiram 37.5%, and tebuconazole 50% + trifloxystrobin 25% showed 100 per cent growth inhibition of the pathogen even at 10 ppm; and difenconazole 5% and propiconazole 25% at 10 ppm inhibited the growth by 87.07% and 74.40%, respectively. In the pot experiment, hexaconazole 5% (1000 ppm), tebuconazole 50% + trifloxystrobin 25% (1000 ppm), and propiconazole 25% (1000 ppm) completely inhibited the disease by 100%. Carboxin 37.5% + thiram 37.5% (2000 ppm) and difenconazole 5% (1000 ppm) had 20% and 53.33% disease incidence, respectively. Carbendazim 50% (2000 ppm), carbendazim 12% + mancozeb 63% (2000 ppm), and azoxystrobin 23% (2000 ppm) did not reduce the disease in vivo. The fungicides seen effective in this study need to be tested in the field experiments for exploiting the use and benefits of fungicides against foot rot disease of chilli.

1. INTRODUCTION

Chilli pepper (*Capsicum frutescens* L.) is a spice crop of economic importance belonging to the family of Nightshade, Solanaceae (Muimba-Kankolongo, 2018). However, most of the chilli production in Nepal is challenged due to abiotic and biotic factors. Among biotic factors, soil borne diseases like Fusarium wilt (*Fusarium* spp.), Phytophthora wilt (*Phytophthora capsici*), foot rot (*Sclerotium rolfsii*), bacterial disease like bacterial wilt (*Ralstonia solanacearum*) are mostly limiting the higher production and yield of chilli pepper in Nepal (Baidya, 2018). Foot rot disease caused by *S. rolfsii* Sacc., is a serious threat to chilli causing huge economic losses at all stages of the crop especially at seedling stage of the crop when the crop is highly susceptible (Bhagat, 2014). The pathogen is mostly prevalent in the areas with warm climate, higher temperature and higher moisture. The management of the foot rot of chilli is quite challenging due to the prolific growth, wide host range of the pathogen and having the ability to produce large number of sclerotia that may persist in the soil for several years (Remesal *et al*., 2010). Being vigorous in nature, there are no substantial level of host plant resistance against the pathogen. However, the disease incidence and its severity can be minimized through integrated disease management strategy. Various measures like using healthy seedling, improving farming practices, use of resistant crop varieties, biological and botanical measures, chemicals and integrated disease management are strategically useful for the management of the diseases caused by *S. rolfsii* (Al-Askar *et al*., 2013). The present study was carried out to assess antifungal potential of fungicides against *S. rolfsii in vitro* and *in vivo* management of foot rot of chilli.

2. MATERIALS AND METHODS

2.1. Isolation of Sclerotium rolfsii from diseased samples

The pathogen was isolated from infected collar region of chilli crop collected from a field at Fulbari, Chitwan, Nepal. The collar region was washed and cut into small pieces. The infected samples were surface sterilized with one per cent sodium hypochlorite (NaOCl) for one minute followed by triple rinsing with distilled water. The samples were kept in the Petri plates with two layers of moist blotter paper. The plates were incubated at 25±1°C in an incubator until sclerotia were produced and the sclerotia produced were transferred to Potato dextrose agar (PDA) medium to obtain pure culture. The pure culture was maintained at 4°C.

2.2. In vitro evaluation of fungicides against S. rolfsii

The efficacy of five systemic fungicides (Carbendazim 50%, Hexaconazole 5%, Propiconazole 25%, Azoxystrobin 23% and Difenconazole 5%) and three combo fungicides (Carbendazim 12% + Mancozeb 63%, Carboxin 37.5% + Thiram 37.5% and Tebuconazole 50% + Trifloxystrobin 25%) were evaluated *in vitro* at different concentrations of 10 ppm, 25 ppm and 100 ppm on the mycelial growth of *S. rolfsii* on Potato dextrose agar (PDA) medium using poisoned food technique (Bhat *et al*., 2015). Fresh culture of the pathogen was grown on PDA medium five days before setting up the experiment. The stock solution of 10,000 ppm of different fungicides were prepared. The fresh PDA medium was prepared and melted. The required quantity of fungicide was added from the stock solution to the melted PDA medium to obtain desired concentration with the help of micropipette by using the formula:

V1=(C2*V2/C1) where

V1= Volume of stock solution to be added to the molten PDA
C2= Concentration of chemical fungicides to be prepared
V2= Final volume of PDA media (100 ml)
C1= Concentration of stock solution (10000 ppm)

Twenty milliliter of poisoned medium was poured in each sterilized plates and suitable check was maintained without addition of fungicides. A pinch of streptomycin sulphate was added to the medium at the time of pouring to avoid bacterial contamination. Mycelial disc (5 mm diameter) from the periphery of five days old culture of the pathogen was inoculated at the center of the Petri plates containing poisoned media with different concentrations of fungicides (10 ppm, 25 ppm and 100 ppm). The inoculated plates were incubated in BOD incubator at 25±1°C and five replications were maintained for each treatment. Colony diameter of the pathogen was measured from the underside of the plate in two directions in every 24 hours after inoculation until there was full growth of the pathogen in the control plate and the average was recorded. Final growth reading was recorded when the growth of the pathogen in control plate was full. The percent growth inhibition was calculated using the radial growth measurement of the test pathogen by using the formula given by Vincent (1947).

$$\mathrm{Pi}\ (\%) = (\mathrm{C} - \mathrm{T})/\mathrm{C}\ \times 100$$

Where, P_i = Percent growth inhibition
C = Radial growth of the test pathogen in control
T = Radial growth of the test pathogen in treatment

2.3. In vivo efficacy of fungicides against foot rot of chilli under screen house condition

The pot experiment was conducted under screen house condition in Completely

Randomized Design (CRD). For this, mass culture of the pathogen was prepared in the sorghum grain. Two hundred gram of the grains was autoclaved at 121°C at 15 psi for 20 minutes on two consecutive days and allowed to cool under aseptic condition. The substrate in polybag was inoculated with 5 mm diameter mycelial disc (2 discs per polybag) of 5 days old culture of *S. rolfsii* and incubated for 12 days at 25±1°C.

NS-1701 variety of chilli was selected for the *in vivo* experiment where seedlings were raised in plugmix media in seedling raising tray. The pot (10 cm diameter) with 1 kg capacity was filled with sterilized soil. The chilli seedlings having 2-3 true leaves were uprooted and dipped into the solution of different concentrations of eight fungicides. Fungicides solution were made based on their commercial formulation. Carbendazim 50%, carboxin 37.5% + thiram 37.5%, carbendazim 12% + mancozeb 63% and azoxystrobin 23% were tested at 2000 ppm (2 g or 2 ml per liter water) and respective solutions were prepared. Similarly, tebuconazole 50% + trifloxystrobin 25%, hexaconazole 5%, difenconazole 5%, and propiconazole 25% were tested at 1000 ppm (1 g or 1 ml per liter water) and respective solutions were prepared. Similarly, a control was kept where seedling was dipped in distilled water only. Single treated chilli seedling was transplanted in each pot and again drenched with 10 ml prepared solution of fungicides (Madhavi & Bhattiprolu, 2011). There were five plants per experimental unit. The pots were arranged in completely randomized design with five replications in the screen house. All seedlings were maintained in screen house at 27-30 °C with 60-70% relative humidity.

One week after transplanting, the seedlings already treated with fungicides were inoculated with two grams mass culture of *S. rolfsii* at the surface of soil and thoroughly mixed with upper two inches soil layer of the pot. The disease symptoms were observed and symptomatic plants were counted after 2 days of inoculation when white mycelial mat spread around the collar region of the chilli seedling where pathogen started to girdle the collar region by disintegrating the cell wall and exuding the cellular fluid outside the disintegrated collar part of the seedling (Madhavi & Bhattiprolu, 2011). Almost about 3 days after inoculation, diseased plant was completely wilted and complete girdling at the collar region of the chilli seedling was observed. Small, rounded white sclerotia started to form at 4-5 days after inoculation and it turned to brownish to blackish, small, round matured sclerotia in about 10-12 days after inoculation.

The disease incidence was calculated using the following formula given by Cooke (2006).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected seedlings}}{\text{Total number of seedlings observed}} \times 100$$

2.4. Statistical analysis

The data recorded under *in vitro* and *in vivo* tests were tabulated in Microsoft Excel 2007 data worksheets. Gen Stat 15th edition computer program was used for the analysis of variance (ANOVA) and for data with significant difference, mean comparison was done by Duncan's multiple range test (DMRT).

3. RESULTS AND DISCUSSION

3.1. In vitro evaluation of fungicides against S. rolfsii

Table 1 reveals that there was highly significant variation among eight fungicides at tested concentrations. Among the eight fungicides, hexaconazole 5% (Hexa Green), carboxin 37.5% + thiram 37.5% (Vitavax Power) and tebuconazole 50% + trifloxystrobin 25% (Nativo) were found to be highly significant that completely inhibited the colony growth of the pathogen even at lower concentration of 10 ppm (Figure 1). It was followed by difenconazole 5% (Purion) which recorded 87.07 per cent growth inhibition at 10 ppm concentration. At 25 ppm concentration, propiconazole 25% (Tilt) and difenconazole 5% (Purion) also completely inhibited the colony growth of the pathogen (Figure 2). Carbendazim 50% (Carbestin) and carbendazim 12% + mancozeb 63% (Saaf) were found to be most ineffective against the pathogen as carbendazim 50% recorded no inhibition against the colony growth of the pathogen and carbendazim 12% + mancozeb 63% recorded only 16.04 per cent growth inhibition against the pathogen even at higher concentration of 100 ppm (Figure 3). Chowdhury *et al*. (1998), Prabhu & Hiremath

(2003), and Das *et al*. (2014) reported that hexaconazole, tebuconazole, propiconazole, carboxin + thiram were found to have strong inhibitory effect on the growth of *S. rolfsii* even at lower concentration of 10 ppm while carbendazim and carbendazim + mancozeb were not so effective in inhibiting the mycelial growth of *S. rolfsii* at higher concentration of 100 ppm.

Table 1. *In vitro* efficacy of different concentrations of fungicides against *Sclerotium rolfsii*

Fungicide	Per cent growth inhibition at			Mean
	10 ppm	25 ppm	100 ppm	
Carbendazim 50%	0^{i}	0^{i}	0^{i}	0^{f}
Carbendazim12% + Mancozeb 63%	0^{i}	3.36^{h}	16.04^{g}	6.47^{e}
Hexaconazole 5%	100^{a}	100^{a}	100^{a}	100^{a}
Carboxin 37.5% + Thiram 37.5%	100^{a}	100^{a}	100^{a}	100^{a}
Propiconazole 25%	74.40^{c}	100^{a}	100^{a}	91.47^{c}
Azoxystrobin 23%	36.49^{f}	47.29^{e}	53.76^{d}	45.84^{d}
Tebuconazole 50% + Trifloxystrobin 25%	100^{a}	100^{a}	100^{a}	100^{a}
Difenconazole 5%	87.07^{b}	100^{a}	100^{a}	95.69^{b}
Mean	62.24	68.83	71.23	
	Fungicide	Concentration	Fungicide x Concentration	
F-test	***	***	***	
LSD (≤0.05)	1.88	1.15	3.26	
S.Em (±)	0.67	0.41	1.16	
CV (%)	3.8	3.8	3.8	

LSD is Least significant difference, S.E (±) is standard error of mean, CV is Coefficient of variation. Same letters in the treatments are not significantly different among each other based on DMRT at 0.05 level of significance

Figure 1. Poisoned food technique with fungicides at 10 ppm

Figure 2. Poisoned food technique with fungicides at 25 ppm

Figure 3. Poisoned food technique with fungicides at 100 ppm

Bhat *et al*. (2015) also found that tebuconazole + trifloxystrobin was highly inhibitory with ED_{50} value of 0.131 µg/ml, followed by carboxin + thiram (ED_{50} value of 0.27 µg/ml) and hexaconazole (ED_{50} value of 0.41µg/ml) which were respectively 75.41, 36.02 and 23.97 times inhibitory than mancozeb. Brenneman (1991) also discussed that tebuconazole, an ergosterol biosynthesis inhibitor, had a high level of activity against *S. rolfsii in vitro* with mean ED_{50} values for inhibition of mycelial growth of *S. rolfsii* was 0.08 µg/ml.

Rather *et al*. (2012) also found carbendazim and carbendazim + mancozeb as effective fungicides having high inhibitory effect against *Rhizoctonia solani* and less inhibitory effect against *S. rolfsii* both *in vitro* and *in vivo*. The study conducted by Sravani & Chandra (2021) found only 49.26 and 65.92 per cent mycelial growth inhibition of *S. rolfsii* by azoxystrobin 23% at 100 and 300 ppm concentration respectively.

Sharma & Dhruj (2018) found that among systemic fungicides, difenconazole and hexaconazole had completely inhibited growth of all the isolates of *S. rolfsii* while carbendazim was less effective in growth inhibition of *S. rolfsii* at all tested concentrations.

3.2. In vivo efficacy of fungicides against foot rot of chilli under screen house condition

Table 2 shows that the effect of different fungicides was highly significant in foot rot incidence in chilli seedlings. Tebuconazole 50% + trifloxystrobin 25% (Nativo), hexaconazole 5% (Hexa Power), and propiconazole 25% (Tilt) were found to be most effective as there was no disease incidence in chilli seedlings in these treatments. Similarly, it was followed by carboxin 37.5% + thiram 37.5% (Vitavax Power), and difenconazole 5% (Purion) as there was only 20 per cent and 53.33 per cent disease incidence of chilli seedlings respectively in these treatments. Carbendazim 50% (Carbestin) and carbendazim 12% + mancozeb 63% (Saaf) were found to be most ineffective against the pathogen as there was 100 per cent disease incidence in chilli seedlings treated with these fungicides similar to control.

Table 2. Effect of fungicides on the incidence of foot rot disease of chilli seedlings caused by *Sclerotium rolfsii* at Rampur, Chitwan, Nepal, 2020

Treatments	**Disease incidence (%)**
Carbendazim 50% (2000 ppm)	100
Carboxin 37.5% + Thiram 37.5% (2000 ppm)	20
Carbendazim 12% + Mancozeb 63% (2000 ppm)	100
Tebuconazole 50% + Trifloxystrobin 25% (1000 ppm)	0
Hexaconazole 5% (1000 ppm)	0
Difenconazole 5% (1000 ppm)	53.33
Propiconazole 25% (1000 ppm)	0
Azoxystrobin 23% (2000 ppm)	93.33
Control (water)	100

Shirsole et al. (2019) found that seed treatment of chickpea with hexaconazole 5% EC, propiconazole 25% EC, tebuconazole 50% + trifloxystrobin 25% and carboxin 37.5% + thiram 37.5% exhibited zero mortality and 100 per cent decrease in disease incidence over control. Tajane *et al*. (2002) found that these fungicides were absorbed by roots and translocated to shoot and leaf length. Similarly, maximum mortality (69.84%) was observed in treatment with carbendazim 50% WP and in contrary to our result only 38.42 per cent seedling mortality of chickpea was observed in treatment with carbendazim 12% + mancozeb 63% WP with 53.21 per cent reduction in disease over control (Shirsole et al., 2019). Madhavi & Bhattiprolu (2011) also recommended soil drenching of carbendazim

+ mancozeb as immediate control measure against collar rot of chilli caused by *S. rolfsii* which is contradictory to our finding. Sravani & Chandra (2021) found 100 per cent disease incidence of chickpea and disease severity of 42.88 and 34 per cent respectively at 100 and 300 ppm concentration of azoxystrobin in field condition. They reported azoxystrobin as least effective fungicide against the pathogen.

4. CONCLUSION

Fungicides hexaconazole 5%, tebuconazole 50% + trifloxystrobin 25%, propiconazole 25%, and carboxin 37.5% + thiram 37.5% were found very effective for controlling *S. rolfsii* both *in vitro* and *in vivo*. Difenconazole 5% was found to be very effective in inhibiting the mycelial growth of the pathogen *in vitro* but the disease incidence in chilli seedling treated with the same fungicide was found to be relatively high. Carbendazim 50% and carbendazim 12% + mancozeb 63% were found to be most ineffective fungicides against the pathogen both *in vitro* and *in vivo*. The effective fungicides found in the present study need to be tested in field conditions to verify their effectiveness against the pathogen.

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MANAGEMENT OF *Epilachna dodecatigma* (Wied.) (COLEOPTERA: COCCINELLIDAE) ON BITTER GOURD (*Momordica charantia* L.) IN CHITWAN, NEPAL

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ABSTRACT

Field experiment on management of epilachna beetle on bitter gourd (Variety: Palee) was conducted using randomized complete block design (RCBD) with four replications and six treatments, namely; (i) Beauveria bassiana (Bals.) @ 3gm/lit; (ii) Margosom @ 4 ml/lit; (iii) Derisom (Karanjin, 20000 ppm) @ 2 ml/lit; (iv) Chloropyriphos50% EC & Cypermethrin5%EC @ 2 ml/lit; (v) Spinosad @ 0.5 ml/lit (tracer 45% sc, microbial), and (vi) untreated control (water spray) at Rampur (Chitwan) during the spring season in 2015. The treatments were sprayed 3 times at 11 days intervals. The highest number of Epilachna beetle larvae was killed by chemical pesticides (Chloropyriphos 50% EC & Cypermethrin 5% EC) followed by Spinosad, botanicals (Derisom and Margosom), Microbials and the lowest in control, respectively. This study suggests that botanicals can be used as one of the economically viable and eco-friendly pest management measures in the Integrated Pest Management (IPM) of Epilachna beetles.

1. INTRODUCTION

The Epilachna beetle, *Epilachna dodecastigma* (Wied.), is a significant insect pest that poses a considerable threat to vegetable crops in South-East Asia, particularly in Nepal. Epilachna beetle attack especially the leaves and feed on the chlorophillous green portion of leaves and thereby preventing the synthesis of carbohydrates by the host plants due to lack of sufficient chlorophyll even though sunlight is present (Mishra, 2021) (Endo, Abe, Sekine and Matsuda, 2004). Grubs and adults both cause great damage to the host plants. Infestation primarily begins just after hatching of egg mass (Murata, Iwabuchi and Mitsuhashi, 1994). The nature of damage by the larvae are somewhat distinct from that of the adults (Pradhan, Jotwani and Prakash, 1990). Bitter gourd contains a variety of beneficial nutrients including thiamine, beta-carotene, folate, riboflavin, as well as essential minerals such as calcium, iron, phosphorus, manganese, potassium, magnesium, zinc, and dietary fiber. Consistently consuming bitter gourd juice enhances overall endurance and helps prevent the onset of chronic fatigue. The presence of beta-carotene in bitter gourd aids in managing eye-related disorders and improves vision. Bitter melon has Anti-Tumor, Anti-Inflammatory, Antioxidant, Ant diabetic, Hypo-lipidemic and Hypoglycaemic properties (Subha et al., 2018). Bitter gourd is highly regarded as one of the most nutritious types of gourds and holds significant commercial value as a vegetable. Bitter gourd possesses a multitude of medicinal properties and offers numerous health benefits. It contains momordicin (a chemical) which is effective in the treatment of diabetes mellitus (CABI, 2005). The production of bitter gourd faces various challenges, including issues such as infestation by insect pests, diseases, failure of fruit development, fruit dropping, and difficulties in post-harvest transportation. In a different part of the world, insect pests are reported major problems and the loss is estimated to be 50 to 100 percent in cucurbits (Singh, 2014). Given the high demand for vegetables and the relatively low productivity, there is a significant opportunity for expanding vegetable production in Nepal, including the cultivation of cucurbits.

Epilachna beetles are generalist feeders and have a wide range of host plants, primarily targeting vegetables such as bitter gourd, brinjal, potato, tomato, and others. The grubs feed on the epidermal layer of leaves and the adults feed irregularly upon the upper surface of leaves and its grubs also feed on the lower surface of leaves by scraping, causing net like appearance of the host plant leaves that turn brown in color, entirely dry up due to extensive infestation by the growing population and finally defoliate (Pradhan, Jotwani and Prakash, 1990). From economic stand point, the epilachna beetle bears a great significance because it cans alone damage up to 80% of the host plants (Rajagopal and Trivedi, 1989). The efficacy of the tested insecticides seven days after treatment was again lower than it was after three days (Milovanovic, Kljajic, Andric, Prazic-Golic and Popovic, 2013). Insecticides are usually rapid and curative in effect so that action can be taken quickly against a pest problem by the farmer (Fenemore and Prakash, 2009). His study aims to develop a sustainable and eco-friendly method for effectively controlling Epilachna beetle infestations on bitter gourd crops.

2. MATERIALS AND METHODS

The field trial was laid out at the experimental farm of Department of Entomology, AFU, Rampur, Chitwan (Bharatpur Sub metropolitan) during autumn season, 2015.The experimental station has subtropical and humid climate (NMRP, 2015) with cool winters (2-3 ^{0}C), hot summers (30^{0}C) and distinct rainy season with annual rainfall of about 1919.5 mm (NMRP, 2015). There were six treatments replicated four times, which were laid out in Randomized Complete Block Design (RCBD). The treatmens are (Spinosade 0.5ml /lit, Chloropyriphous & Cypermethrin 2ml/lit, Control (water spraying), Margosom 4ml/lit, Derisom 3ml/lit, *Beauveria bassiana* 3 gm/lit of water). The plot size was 4m×3m having 2-meter row to row and 1m plant to plant spacing. There were 2 rows in one plot and 3 plants in each row A Palee variety was selected to evaluate their pest infestation and Yield performance. Two sample plants were selected randomly in each plot and 3 branches were taken for the data collection from each, one from upper part, one from middle part and one from lower part of plant and yield was recorded from the whole plot. Change in population was calculated at 4th, 8th, 11th days. The production was recorded at each harvest from the whole plot and calculated into mt/ha. Populations of adult's beetle were recorded weekly throughout the crop growing period, data obtained were tabulated, analyzed and interpreted supporting with available literature. The percentage of population reduction over control was calculated by using the modified Abbots formula (Fleming and Retnakaran, 1985). The collected data in this study were arranged in MS Excel program and statistically analyzed with the help of R studio software packages. Means comparison was done by DMRT at 5% level of significance.

3. RESULTS AND DISCUSSION

During the first spray, the lowest number of larvae/branches was found in Chloropyriphous & Cypermethrin, Spinosad, Derisom, Margosom, *B. bassiana* and Water spray. During the second spray, the lowest number of adult epilachna beetle was found in the Spinosad, Chloropyriphous & Cypermethrin, Derisom Margosom, *B. bassiana* and Water spray. During the third spray, the lowest number of adult epilachna beetle was found in the Chloropyriphos and Cypermethrin (0.20±0.01), Spinosad (0.18±0.06), Derisom (0.24±0.06), Margosom (0.55±0.10), *B. bassiana* (2.30±0.26) and Water spray (9.62±0.40). According to Rajendran and

Gopalan (1999), direct spraying of *Beauveria bassiana* killed 58.1 percent first instar larvae and 35.2 percent of pre-pupal stage larvae. The adults were not susceptible to *B. bassiana*, though the maximum mortality being 10.3 percent in the case of newly emerged adults. The fungus caused 54.6 percent hatchability of one-day-old eggs of the spotted beetle. As the concentration of the test solutions increased, the rate of the larvicidal effect also showed an increase. Similarly, the research indicated that both concentrations of plant extract and spraying interval are equally important for effective pest management, indicating the possibility of using botanical plant materials in the development of organic insect pest management methods in vegetables (G.C. and Neupane, 2009).

After the second spray, the reduction of the larval population over control after the first spray was not calculated because the mean number of epilachna larvae was not significantly different ($p>0.05$) before spray and on the 4th day of the first spray. The treatments were found to be significantly different ($P<0.01$) in all the observations. On the 4th day after the second spray, larval population were not significantly different in the treatments, Chloropyriphous & Cypermethrin, Spinosad and Derisom had the lowest population followed by Spinosad. Similarly, the population of the larvae of Epilachna beetles was not significantly different in the Chloropyriphous & Cypermethrin and Spinosad on the 8thday of the second spray. Similarly, on the 11th day after spraying the lowest number of larvae was found in the Spinosd, Chloropyriphous & Cypermethrin followed by botanicals and the highest mortality was recorded in the Chloropyriphous & Cypermethrin (77.74%), Spinosad (70.27%) and botanicals; viz. Derisom (43.47%) and Margosom (40.69%), followed by microbials viz. *B. bassiana* (16.16%) respectively during the second spray after 4thday. After the 8thday of spraying the highest mortality rate was recorded in the same pattern Chloropyriphous & Cypermethrin (68.60%), Spinosad (62.78%) and botanicals; viz. Derisom (58.72%) and Margosom (47.39%), followed by microbial viz. *B. bassiana* (27.51%) respectively. But on the 11th day after the spray, the trend was found to be different. Botanicals had the highest effect on the reduction of the larval population of the beetles whereas microbials were found to be effective for the further reduction of the population due to the fact that microbials are slower in their action as they take a longer time to sporulate and develop mycelium for the penetration into the insect body. Spray of Derisom @ 1-2 ml/liter of water depending upon the intensity of the pest is recommended (Gyawali, 2009)So it takes 7 to 10 days to kill the insects in the favorable humid weather condition by that time affectivity of chemicals and botanicals had been lost due to rainfall and effectiveness of their course of action and larval population already built up after hatching the eggs laid by the adult female which can fly up to one and ahalfmiles. Chloropyriphous & Cypermethrin were found to be the quickest of all in their action and losing their action as well but in contrast, botanicals were quicker than microbial and more durable in effect than chemical Chloropyriphous & Cypermethrin.
Ali (2007). Cypermethrin 10 EC and Chlorpyriphos 20 EC reduced more than 90% of the epilachna beetle population in the brinjal field. Neem is effective against larvae of first and second instar only (GC, 2009). Microbial generally takes7-10 days affecting insect pests when the environment is favorable (GC, 2009).

After the third spray, the treatments were found significantly different in all the observations. On the 4th day after spray, the highest reduction of the larval population over control was recorded in the Chloropyriphous & Cypermethrin (77.18%), Spinosad (60.87%) and botanicals; viz. Derisom (44.98%) and Margosom (36.76%), followed by microbial viz. *B. bassiana* (8.07%), respectively. Chloropyriphous & Cypermethrin (77.18%) it was the quickest in their effect and losing their effectiveness followed by biopesticide (60.87%), botanicals and microbial, respectively. The highest mean larval population was recorded in the control. On the 8thday after spray, the highest reduction of the larval population over control was recorded in the Chloropyriphous & Cypermethrin (64.64%), Spinosad (50.50%) and botanicals; viz. Margosom (54.28%) and Derisom (44.75%) followed by microbial viz.

B. bassiana (18.58%) respectively but on the 11th day after spray, Botanicals had the highest effect on the reduction of the larval population of the beetles followed by chemical and microbial. The highest reduction of the larval population over control was found in the Margosom (62.44%), Chloropyriphous & Cypermethrin (47.19%), and Spinosad (42.97%) Derisom (40.59%) followed by *B. bassiana* (30.31%) respectively. The efficacy of the tested insecticides seven days after treatment was again lower than it was after three days (Milovanovic, Kljajic, Andric, Prazic-Golic and Popovic, 2013). Insecticides are usually rapid and curative in effect so that action can be taken quickly against a pest problem by the farmer (Fenemore and Prakash, 2009).

Table 1. Effect of different treatments on the reduction of *Epilachna* beetles' larvae, after the second spraying at Rampur, Chitwan, 2015

Treatments	Before spray	Second spray					
		4 days	PROC 1	8 days	PROC 2	11 days	PROC3
Beauveria@3gm/lit	2.83^{b}±0.12 (1.82)	2.66^{b}±0.03 (1.77)	16.16	2.45^{b}±0.11 (1.66)	27.51	2.66^{b}±0.30 (1.85)	35.86
Ch+Cy@2ml/lit	0.40^{e}±0.11 (0.90)	0.10^{d}±0.04 (0.77)	77.74	0.15^{d}±0.01 (0.80)	68.60	0.30^{c}±0.09 (0.87)	57.35
Control (water spray)	5.20^{a}±0.27 (2.37)	5.83^{a}±0.27 (2.50)	0	6.21^{a}±0.33 (2.57)	0	7.62^{a}±0.72 (2.78)	0
Derisom@3ml/lit	0.71^{cd}±0.32 (1.24)	0.45^{d}±0.18 (0.96)	43.47	0.35^{cd}±0.07 (0.91)	58.72	0.32^{c}±0.12 (0.90)	69.24
Margosom@4ml/lit	1.91^{bc}±0.16 (1.54)	1.27^{c}±0.35 (0.29)	40.69	1.20^{c}±0.28 (1.16)	47.39	1.16^{c}±0.24 (1.27)	58.55
Spinosade@0.5ml/lit	0.45^{de}±0.12 (0.97)	0.12^{d}±0.04 (0.78)	70.27	0.20^{d}±0.05 (0.83)	62.78	0.25^{c}±0.06 (0.85)	62.09
F-value	27.584**	46.597**		60.177**		17.724**	
CV%	14.38794	14.81275		13.40353		25.564	
LSD	0.3206	0.3012		0.26800		0.5485	

DAS: Days after spraying; PROC: Population Reduction percent over control; CV: Coefficient of variation; LSD: Least Significant Difference; values with the same letters in a column are not significantly different at 5% by DMRT; figures after± indicate standard error and figure in parentheses indicate √(x+0.5) transformation Ch+Cy mean Chloropyrifos 50% EC and Cypermethrin 5%EC.

Table 2. Effect of different treatments on the reduction of *Epilachna* beetles' larvae, after the third spraying at Rampur, during 2015

Treatments	Before spray	Third spray					
		4 days	Proc1	8 days	Proc2	11 days	Proc3
Beauveria@3gm/lit	2.66[b]±0.30 (1.85)	2.50[b]±0.32 (1.70)	8.07	2.45[b]±0.15 (1.85)	18.58	2.30[b]±0.26 (1.65)	30.31
ch+cy@2ml/lit	0.30[c]±0.09 (0.87)	0.07[d]±0.04 (0.75)	77.18	0.12[d]±0.05 (0.79)	64.64	0.20[c]±0.01 (0.82)	47.19
Control (water spray)	7.62[a]±0.72 (2.78)	7.79[a]±0.12 (2.75)	0	8.62[a]±0.08 (2.87)	0	9.62[a]±0.40 (3.16)	0
Derisom@3ml/lit	0.32[c]±0.12 (0.90)	0.18[d]±0.01 (0.82)	44.98	0.20[d]±0.04 (0.85)	44.75	0.24[c]±0.06 (0.85)	40.59
Margosom@4ml/lit	1.16[c]±0.24 (1.27)	0.75[c]±0.23 (1.09)	36.76	0.60[c]±0.09 (1.03)	54.28	0.55[c]±0.10 (1.03)	62.44
Spinosade@0.5ml/lit	0.25[c]±0.06 (0.85)	1.10[d]±0.02 (0.79)	60.87	0.14[d]±0.01 (1.80)	50.50	0.18[c]±0.06 (0.82)	42.97
F-value	17.724**	104.431**		354.70**		78.299**	
CV%	25.564	11.6496		6.5420		14.982	
LSD	0.5485	0.23205		0.1349		0.3145	

DAS: Days after spraying; PROC: Population Reduction over control; CV: Coefficient of variation; LSD: Least Significant Difference; values with the same letters in a column are not significantly different at 5% by DMRT; figures after± indicate standard error and figure in parentheses indicate √(x+0.5) transformation Ch+Cy mean chloropyrifos 50% EC and Cypermethrin 5%EC.

4. CONCLUSION

The Epilachna beetles are one of the most problematic insect pests of the bittergourd. Grubs and adults both cause great damage to the host plants. Infestation primarily begins just after the hatching of egg mass. The nature of damage by the larvae is somewhat distinct from that of the adults which attack especially the leaves and feed on the chlorophillous green portion of leaves. The lowest population of adult Epilachna beetles and highest reduction of the larval population over control was obtained in chemical chloropyriphous & cypermethrin followed by spinosad, botanical (Derisom and Margosom), and in the microbial (*Beauveria bassiana*), respectively and all treatments were found superior over control (water spray). This suggests that botanicals could be used as eco-friendly and sustainable pest management measures in the Integrated Pest Management (IPM) program for this pest. The strategy to reduce misuse of chemical pesticides in agriculture is very difficult to achieve until and unless strengthens the quality of pesticide retailers along with effective plant protection extension mechanisms and a strong emphasis on research for the production of eco-friendly plant protection measures.

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ECOLOGICAL STATUS, THREATS, AND HABITAT CHARACTERISTICS OF PANCHAULE (*Dactylorhiza hatagirea*) IN GHASA OF MUSTANG, DISTRICT.

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ABSTRACT

Dactylorhiza hatagirea, commonly known as "Panchaunle," is a critically endangered medicinal plant found in altitudes ranging from 2500 to 5000 meters, primarily in temperate to alpine regions. Although it naturally thrives in the Annapurna Conservation Area of Nepal at 3200-3600 meters, local communities lack the authority to harvest it. The plant's tubers and leaves possess significant medicinal properties, treating ailments like diabetes, dysentery, and fractures. However, various threats, including habitat destruction, illegal activities, and unregulated harvesting, imperil its survival. This study aimed to assess its ecological status, habitat characteristics, traditional uses, and threats. Methodologies included key informant surveys, focus group discussions, household surveys, and participatory resource mapping. Field sampling was conducted in 45 plots using semi-systematic methods, with soil samples analyzed for nitrogen, organic matter, and carbon content. Our study revealed a density of 533.33 individuals per hectare, with a frequency of 44.74 and an abundance of 1200 individuals per hectare. Despite its ecological importance, anthropogenic actions pose a severe risk, necessitating immediate conservation measures. The study identified suitable habitats for Dactylorhiza hatageria is characterized by low biotic disturbance, acidic soil, and silty loamy conditions, underlining the urgency to protect these areas.

1. INTRODUCTION

Nepal boasts an exceptional and vast array of flora and fauna, thanks to its diverse topography, altitude variations, and climate (Kohler et al., 2014). Within its relatively small geographical area, Nepal encompasses a remarkable range of plant and animal species (Aryal et al., 2012). Non-timber forest Products (NTFPs) play a significant role in forest ecosystem, encompassing various goods and services derived from forests, excluding timber and fuel wood (Shanley et al., 2015). NTFPs have gained recognition for their vital role in supporting the subsistence livelihood of local communities and their potential contribution to the economy of many developing countries (Shackleton et al., 2011). Nepal is home to over 700 species of NTFPs out of the 6,500 flowering plants found in the country (Maraseni et al.,2011). Among these, approximately 100 species are commonly traded (Shrestha et al., 2020). Annually, Nepal exports an average of 13,230 metric tons of medicinal and aromatic plant (MAP) products each year, generating USD 39.34 million in revenue and reaching more than 50 countries (Kalauni and Joshi, 2018).). Harvesting of non-timber forest products (NTFPs) is generally considered to have a lower impact on the forest ecosystem compared to timber harvesting. NTFPs, such as *Dactylorhiza* *hatagirea (*commonly known as "Panchaunle" in Nepali), belong to the Orchidaceae family and have been listed in Appendix II by the Convention on International Trade in Endangered Species (Shrestha et al., 2021). *Dactylorhiza hatagirea* is a medicinal orchid endemic to the Himalayas, specifically the Hindu Kush Himalaya range (Shrivastava and Jain, 2023). It can also be found in similar altitudinal ranges in India, Pakistan, Bhutan, and China. This orchid thrives in grassland slopes within the sub-alpine and alpine zones, typically between altitudes of 2800 m and 4200 m above sea level (IUCN, 2004). In the Annapurna Conservation Area of Nepal, *Dactylorhiza hatagirea* is reported to be distributed at altitudes ranging from 3200 to 3600 meters above sea level, with abundance observed on slopes with a 30° to 60° inclination on the north-east aspect (Ranpal, 2009). *Dactylorhiza hatagirea* is a terrestrial plant that grows up to 60 cm tall and has palmately split tuberoids. Its leaves are generally lanceolate, oblong-lingulate, or elliptic in shape. The flowers, which can be purplish-lilac, rose, or white, grow densely in a cylindrical inflorescence (Baral & kurmi, 2006). This perennial medicinal plant has an erect, hollow, and obtuse stem, with lanceolate and palmately lobed leaves that have sheathing leaf bases (Jodh et al., 2022). The flowers range in length from 1.7 to 1.9 cm and have a curved spur. The inflorescence consists of a compact raceme with 25 to 50 flowers that develop from axillary buds. *Dactylorhiza hatagirea* has tuberous roots that can store a large quantity of water, enabling it to survive in arid conditions (Chaurasia, 2007).

Propagation of *Dactylorhiza hatagirea* can be done through various methods, including green pod culture, shoot pod culture, tuber and leaf segment culture, and the use of vegetative parts. However, in-vitro seed germination using green pods is very poor, slow, tedious, and difficult (Giri & Tamta, 2012). This orchid is known to respond well to environmental quality (Kindlmann et al., 2002).

Dactylorhiza hatagirea a monocotyledonous, perennial, and terrestrial orchid, holds great value for its ornamental and medicinal purposes (Pandey et al., 2016). This species is known for its slow growth rate and specific habitat requirements. It faces challenges in regeneration due to its tiny, non-endospermous seeds with undifferentiated embryos. Germination of *Dactylorhiza hatagirea* relies on a mycorrhizal association, which becomes a significant concern for its long-term survival in natural conditions (Magar et al., 2020). Despite these difficulties, this remarkable plant can remain upright even under heavy snowfall. The Government of Nepal has recognized the endangered status of *Dactylorhiza hatagirea* and has given it high priority for research and conservation efforts. *Dactylorhiza hatagirea* is a plant species that has a flowering period from June to July, a fruiting period from August to September, and a harvesting time from September to November (Janečková et al., 2006). The medicinal properties of *Dactylorhiza hatagirea* are the main reason for its high value, with its geographical distribution playing a secondary role by affecting its availability and market price.

It is believed to be effective in treating diseases such as diabetes, dysentery, colic pain, seminal weakness, and diarrhea, and even has potential anti-cancer properties. The tubers and leaves of *Dactylorhiza hatagirea* are used for medicinal purposes, and its tubers yield a high-quality substance called "Salep," which is known for its aphrodisiac properties and use as a sexual stimulant in traditional medicine in the Himalayan region (Wani et al., 2020).

However, it is important to note that the collection, trade, and processing of the rhizome of *Dactylorhiza hatagirea* are prohibited under the Forest Act 1993 and Forest Regulation 1995 in Nepal. If authorized by the government, the collection of the plant requires payment of a royalty fee of NRs. 500 per piece, as stated in the Forest Regulation 1995 and its amendment in 2005 (Oslen, 1998). The Department of Plant Resources in Kathmandu has classified this plant as a national priority species for medicinal herb cultivation and conservation. Additionally, international organizations like the Convention on International Trade in Endangered Species (CITES) and the

Conservation Assessment and Management Plan (CMAP) have listed *Dactylorhiza hatagirea* as an endangered and vulnerable species, respectively.

Unfortunately, the abundance and distribution of orchid species, including *Dactylorhiza hatagirea* have significantly declined in recent decades (Damgaard, 2020). This decline is attributed to habitat loss and fragmentation, which have led to smaller population sizes and increased isolation of the species. Consequently, *Dactylorhiza hatagirea* is now confined to small, isolated populations, making it particularly vulnerable (Singh et al., 2021).

Considering these circumstances, a study has been conducted to gather baseline information on the characteristics, distribution, and status of the species, as well as its traditional uses and importance. The study also aims to address the challenges in conservation and management and propose measures for its conservation. Additionally, ecological data of the species has been sampled to better understand its current situation.

2. MATERIALS AND METHODS

Mustang district, located in the Gandaki province, covers an area of 3573 km2. Geographically, it is situated at 280 46' 59.99" N and 830 43' 29.99" E. The district experiences a diverse range of altitudes, ranging from 1372 to 8167 m, resulting in sub-tropical, temperate, and alpine climates. Despite its vast size, only 4.05% of the total area is covered by forests, amounting to 145.85 sq km. Grasslands make up a significant portion, with coverage of 1447.03 sq km (40.49%), followed by shrub-covered areas spanning 44.16 sq km (ACAP,2009). Within the Mustang district, Thasang Rural Municipality is chosen as the study area, occupying an area of 289 km2. Thasang Rural Municipality was established in 2017 (2073 BS) as a Local government of Nepal. The office of the Thasang Gaunpalika (Rural Municipality) is located in Kobang, Mustang which is located in Gandaki province of Nepal. Thasang Rural Municipality is divided into five wards (Tukuche, Kobang, Lete, Ghasa, and Kunjo). The climate in this region can be described as a cold desert, characterized by strong winds and high solar radiation. It falls under the sub-alpine category, with average minimum and maximum temperatures measuring 5.7 C and 12.5 C, respectively. Annual rainfall is relatively low, around 250 mm, and a majority of it occurs as snow during the winter months. The area is predominantly inhabited by traditional ethnic groups such as Thakali and Sherpas, who have resided here for generations. Other ethnic communities like Magar, Gurung, Pariyar, and Chhetris also call this region their home. Nibek, an open grassland, is located at a high elevation in the ward 4 name called Ghasa of Thasang Rural municipality. Nibek lies border of Ghasa side by side with kunjo. Nibek grassland ranges in altitude from 3200-4000 m.

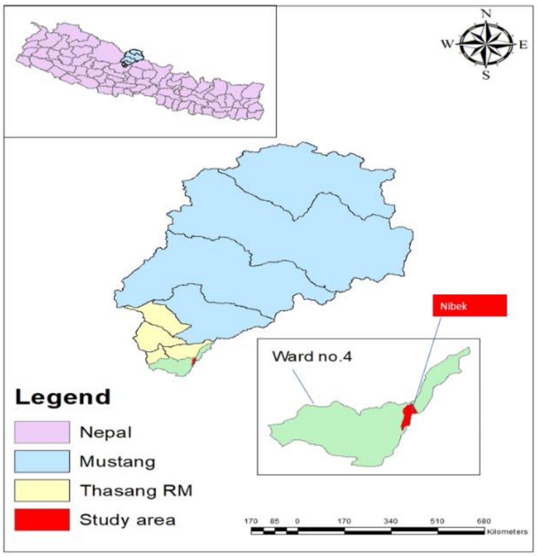

Figure 1. Map Showing Study Site

2.1 Data collection surveying

The research conducted with the help of the OSWD Project (2020-2022) focused on understanding the status, habitat characteristics, traditional use, and threats of Dactylorhiza hatageria in the ACA (Annapurna Conservation Area). The project was granted a research permit by the Annapurna Conservation Area Project (ACAP). To familiarize themselves with the research site, primary data gathering was carried out in the Thasang village community. Techniques such as key informant surveys, focus group discussions, household surveys, and participatory resource mapping were utilized to plan the sampling method within the research region and identify the numerous medicinal plants. Sampling was subsequently conducted in October at the designated research sites. This process allowed for the collection of valuable data on Dactylorhiza hatageria and other significant medicinal plants within the Annapurna conservation area (ACA).

2.2 Field sampling

Based on the information provided, a preliminary study was conducted in February 2022 to gather general information about the flora, specifically Dactylorhiza hatigeria the main visit took place in October 2022, during which all the necessary data was collected. To ensure comprehensive data collection, a semi-systematic sampling method was employed. This method covered various habitats and vegetation types associated with *Dactylorhiza hatigeria* within the elevation range of 3200 - 4000 m. A total of 45 plots, each measuring 5 × 5 m2, were sampled at a distance of approximately 15 m between each plot. The sampling was performed on the southern aspect of Sarri Khola, a river located in the study area. The major species observed in the sample plots were Delphinium and Acotinum species. In the Nibhek grassland area, sparse vegetation consisting of dwarf rhododendron shrubs and a few Juniper species were found, predominantly occupied by alpine meadows. During the data collection process, the longitude, latitude, and elevation of each sample plot were recorded using a Global Positioning System (GPS) device, specifically the eTrex Garmin model. Additionally, the slope and aspect of each plot were measured using a clinometer compass. Soil samples were collected from the four corners of each plot, mixed thoroughly, and stored in airtight plastic bags. These soil samples, numbering 20 per sampling site, were air-dried in the shade and kept in plastic bags for laboratory analysis. Within each plot, the number, height, and girth (measured 20 cm above ground surface) of individual Dactylorhiza hatigeria plants were noted. This preliminary study aimed to gather detailed information about *Dactylorhiza hatagirea* and its associated habitat and vegetation types in the study area. The data collected will likely contribute to further research and understanding of this plant species. Secondary data were collected by reviewing various literature sources such as journals, articles, theses, publications, maps, and websites. Additionally, specific libraries like the IOF library, DNPWC, ICIMOD, ACAP library, and online resources were utilized.

2.3 Laboratory test

First, the soil samples are passed through a fine screen with a mesh size of 0.5 mm. These samples have previously been air dried and their content of total nitrogen, organic matter, and soil organic carbon has been measured. To determine the total nitrogen content, the micro-Kjeldahl method, as described by Gupta in 2000, was used. The OC (organic carbon) and OM (organic matter) levels were determined using the Walkley and Black method.

3. RESULTS AND DISCUSSION

The height and collar diameter of a single *Dactylorhiza hatagirea* were measured in each plot in which *Dactylorhiza hatagirea* was found. Altogether 60 *Dactylorhiza hatagirea* were found in 20 plots out of which only 15 *Dactylorhiza hatagirea* were measured and the no. of plots in which *Dactylorhiza hatagirea* was absent was 15 (other herbs were present) excluding barren, forest, rocky, and stiff areas. Similarly, out of 45 plots, 4 plots include barren areas, and 6 plots include rocky and stiff areas in which inventory was not possible.

Table 1. pH, soil organic matter, Nitrogen, Phosphorous,potassium, clay,sand silt content of soil in the study site.

Plots	pH	SOM %	Nitrogen (%)	Phosphorous (mg/kg)	Potassium (mg/kg)	Clay%	Sand%	Silt%
1	5.11	11.25	0.42	25.13	1231.04	10	18.84	71.16
2	5.07	31.5	1.1088	23.89	1441.8	8	7.9	84.1
3	5.5	27.7	0.854	35.52	1328.4	6	33.1	60.9
4	6.15	9.62	0.3948	34.77	1728.1	6	43.76	50.24
5	6.13	12.62	0.5124	33.1	1629.2	6	26.62	67.38
6	6.52	9.49	0.3668	19.07	1515.6	8	36.35	55.65
7	5.51	8.66	0.28	15.17	1611.3	12	13.64	74.36
8	6.17	14.38	0.4816	21.21	1915.6	10	25.4	64.6
9	4.76	20.78	0.742	8.06	4119.6	6	19.54	74.46
10	4.73	12.06	0.4312	4.05	3412.5	6	17.36	76.64
11	4.98	9	0.3752	2.26	2813.5	8	14.2	77.8
12	5.62	14.1	0.7672	36.05	4723.6	8	34.04	57.96
13	4.3	7.63	0.42	30.2	3222.01	6	29.64	64.36
14	4.56	6.51	0.3528	9.67	2918.6	6	30.78	63.22
15	3.8	5.89	0.476	27.5	2360.02	10	34.88	55.12
16	3.93	11.52	0.2548	41.94	2711.04	8	32.13	59.87
17	4.42	7.12	0.2016	5.18	2111.3	12	22.98	65.02
18	3.45	15.13	0.6468	30.13	2640.3	10	21.8	68.2
19	5.12	6.41	0.3024	8.86	3017.6	10	29.44	60.56
20	4.15	21.29	0.9268	16.99	2830.1	8	29	63
Mean SD	±4.99±0.86	13.13±7.10	0.51 ±0.24	21.44±12.2	2464.06±955.2647	8.2±2.04	26.07±8.960	65.73±8.60

3.1 Habitat characteristics of Dactylorhiza hatagirea

In the study site, the *Dactylorhiza hatagirea* was observed from 3200 to 4000 m altitude. The species was more prevalent in open meadows with very low disturbances. i.e. in places with no trees. A previous study conducted in ACA found that the altitude range of distribution of D. hatageria was 3200 -3600. Ranapal (2009) but we found out it can be up to the range of 4000 meters. The soil was acidic in the sites with a pH ranging between 4.76 and 5.62. The organic matter ranged from 14.1-20.78 %, total nitrogen 0.42 – 0.85 %, available phosphorus 25– 35 mg/kg, and exchangeable potassium 1231- 1328 mg/kg. The soil type in the study area was silt loamy.

3.2 Mean height and mean diameter of Dactylorhiza hatagirea

The mean height of *Dactylorhiza hatagirea* in the study area was 54.62 cm which is less than the reported mean height of *Dactylorhiza hatagirea* in Nepal i.e. 60 cm (Dutta, 2007), was greater than 41.97 cm found by Ranapal (2009) and smaller than study conducted by Khadka et. al (2016) which was found to be 91.08 cm. It may be due to age factor, topographic factors, soil factors, and/or climatic factors. Likewise, the mean diameter of *Dactylorhiza hatagirea* at 5 cm above the ground was 0.42 cm.

3.3 Density, frequency and abundance

In terms of distribution, the population of *Dactylorhiza hatagirea* was found between 3200 - 4000 m of elevation. The species tends to grow in association with other species, with a preference for southern aspects. The slopes of the natural habitats ranged from 9˚ to 58˚, and the species exhibited a patchy or scattered distribution in small fragmented patches. The frequency, density, and abundance of *Dactylorhiza hatagirea* were recorded as 44.74%, 533.33 per hectare, and 1200 per hectare, respectively. The main associates in the study site were *Actonium* and *Delphnium* species. The frequency, density, and abundance of *Actonium spicatum* were 26%, 311 per hectare, and 700 per hectare, respectively, while the frequency, density, and abundance of *Delphnium denudatum* were 33%, 355 per hectare, and 800 per hectare, respectively. The frequency of *Dactylorhiza hatagirea* in the study area was lower compared to studies conducted in the Mansalu Conservation Area by Bhattarai et al. (81.81%), and in Lete, Mustang by Ranpal (72%) and Khadka et al. (71%). The lower frequency in your study area could be due to varying habitat suitability, human activity, and competition with other species. The density of *Dactylorhiza hatagirea* in the study area was 533.33 per hectare, which was higher compared to its associates. However, it was lower than the density indicated by Ranapal (1,671 per hectare). The difference in density could be attributed to the smaller area of the project site during the research period and different management practices, including grazing and trampling, which resulted in a lower density of the targeted species. Similarly, the abundance of *Dactylorhiza hatagirea* reported by Ranapal (2,367 per hectare) was double than that found in this study. The difference in abundance could be due to the smaller number of Himalayan orchid plants found in the study area.

3.4 Use patterns and threats

According to the responses of local households of Thasang village, Mustang several medicinal plants were used in the village. These medicinal plants were collected from vicinity forests and grasslands.

Before the ban on the collection of *Dactylorhiza hatageria*, people used to use D. hataregia to prevent numerous diseases and infections. A Key informants survey conducted in Thasang village revealed that it is used for cut parts, burns, all types of skin allergies, infectious wounds, and skin diseases for fast recovery, a power which can be used in drinking with milk as a tonic made from the dried rhizome is used in the wound.

Table 2. Traditional uses of Dactlorhiza hatageria

Ailments	Way to use in the local community
General weakness	The roots, fruits, and leaves undergo a process of crushing, resulting in a paste that is subsequently employed in the creation of pills.
Cough	Fresh tuber extract is taken.
Bone fracture	Boiled extract of tubers with milk is used orally
Diarrhea	Tuber paste is used orally.
Wound healing	Tubers are robbed to obtain a paste.
Stomach ache	Fresh root juice is used.
spermatorrhea	Refined tubers, sugar and milk are mixed.

The decline in the population distribution and density of *Dactylorhiza hatagirea* is attributed to both natural and anthropogenic activities, as mentioned in the study by Shrestha et al. (2021). The lack of awareness among villagers about the importance of *Dactylorhiza hatagirea,* inadequate ground-based research, and a lack of proper conservation and management plans from the government have all contributed to the species being at risk of extinction. Despite various conservation initiatives at the study site, the high medicinal value of *Dactylorhiza hatagirea* has led to its overexploitation. This, combined with the destruction of ecosystems, small and isolated populations, and unrestrained illegal activities, are the major driving factors behind the decline in its distribution range. These findings align with the studies conducted by Bachman et al. (2019) and Nic Luganda et al. (2020). Furthermore, increasing trampling and grazing activities have been identified as additional factors causing a decline in the number of locations where *Dactylorhiza hatagirea* habitats can be found, as well as negatively impacting its population. It is crucial to address these driving factors and implement effective conservation measures to safeguard the future of this species.

4. CONCLUSION

The study reveals continuous threats to *Dactylorhiza hatagirea* in protected areas, as it's naturally found in remote grasslands, making human labor unfeasible for conservation. Both in-situ and ex-situ conservation efforts are absent, leading to illegal collection and trade, and driving a significant population decline. Grazing indirectly impacts the species by promoting undesirable growth. Unprotected grasslands suffer from heavy livestock activity, disrupting the species' life cycle and damaging plants. The lack of a comprehensive MAP management plan worsens the situation. Insufficient awareness, research, government action, and increased disturbances contribute to potential extinction. Urgent awareness programs for in-situ and ex-situ conservation are vital to combat illegal activities and overgrazing, preventing the endangered *Dactylorhiza hatagirea* from extinction.

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CHARACTERIZATION OF FUNCTIONAL AND VISCOELASTIC PROPERTIES OF FLOUR FROM RELEASED WHEAT VARIETIES IN NEPAL

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ABSTRACT

The present study endeavors to assess the functional properties of flour, and the viscoelastic properties of dough derived from Nepalese wheat varieties, with the principal goal of enhancing processing methodologies and ensuring the adaptability of flour to diverse commercial applications. A total of eleven wheat varieties (ten registered cultivars and one local strain) were procured, milled, and subjected to comprehensive analysis. The findings revealed significant differences (p<0.05) in gluten content among the different wheat varieties. Moreover, significant variations (p<0.05) were observed in the functional properties, encompassing water holding capacity, and oil absorption capacity, underscoring the influence of the wheat genotype on these attributes. Additionally, the flowability parameters, including Carr's index and Hausner value, exhibited significant differences (p<0.05) across the wheat varieties. Furthermore, the textural properties of the dough, including elastic work, total work, degree of elasticity, relaxation time, and stress relaxation, were found to be significantly influenced (p<0.05) by the wheat genotype. The study illuminates the diverse potential applications of wheat varieties based on their functional properties and textural profile.

1. INTRODUCTION

Triticum aestivum L., or wheat is used as an industrial crop from noodles to bakery food, ethanol production, feed, and also in domestic consumption (Bhatta et al 2020, Kandel et al 2018). Nepal has released 43 wheat varieties until 2017, however only 30 varieties are under cultivation because of genetic redundancy, increasing vulnerability, and frequent replacement by improved varieties (Timsina *et al.*, 2018). A total of 52 types of wheat and 2 types of durum wheat have been released by Nepal since 1960 (Thapa et al 2019). Wheat is categorized based on end use, durum wheat is used for pasta production, hard wheat flour is used for bread production, and soft wheat flour is used for cookies and cake production (Turksoy et al 2021).

Functional properties are the fundamental physicochemical characteristics of food that represent the intricate relationships between the compositions, structures, and physicochemical characteristics of food components (Awuchi et al 2019). Viscoelastic properties of wheat dough have a high significance in the commercial production of pasta, bread, and biscuits, and are related to extensibility, resistance to stretching, and gas retention capacity (Hoseney and Rogers 1990, Lefebvre 2006, Hernández-Estrada et al 2017). Wheat kernels with a moisture content of less than 12% are brittle and elastic, while those with a moisture content of between 12 and 15% shift to elastic-plastic and display viscoelastic behavior, which is both plastic (able to deform without reverting to its original shape) and elastic (able to recover its shape after stress) (Devi and Khatkar 2016). Although there are some studies about the quality characteristics of wheat, there are limited studies concerning the characteristics of some widely popular wheat varieties grown in Nepal. This research aims to determine the functional characteristics of wheat flour and the viscoelastic properties of dough of some widely released wheat varieties grown in Nepal and also to evaluate their end-use. The research also aims to document the functional properties of flour and the viscoelastic properties of dough obtained from different varieties of Nepal. The output of this study can be used as a guide by food processors in selecting flour depending on the desired function or end product quality.

2. MATERIALS AND METHODS

A total of eleven, including ten registered varieties, were collected from National Plant Breeding and Genetics Research Centre, Khumaltar, and National Wheat Research Program, Bhiarawa, and one local variety *mudule* was collected from Lalitpur. The research was completely randomized design. To achieve homogenization, samples from the three plots were precisely combined to obtain a consistent blend. The methodology employs triplicate analyses, with each analysis performed three times for every sample. Results were expressed as the mean and standard error of the mean. The differences between treatments were conducted using the Student-Newman-Keuls test, executed through SPSS software version 26.

The percentage of gluten was measured by the method with some modifications described by AACC (2000). The obtained gluten was dried in a hot air oven till constant weight was obtained and the percentage was determined based on the weight of wheat flour. The gelatinization temperature was determined by the method described by Chavan et al *(*2010). The water holding capacity (WHC) and oil absorption capacity (OAC) were measured by the method described by Ojha et al (2020), but determined in percentage. The method employed by Ikpeme-Emmaanuel et al (2009) was used to measure loose bulk density and tapped bulk density. Hausner value is calculated as the ratio of tapped bulk density and loose bulk density, and Carr's index is calculated as the percentage of the difference between tapped bulk density and loose bulk density to tapped bulk density as explained by Bala et al (2020).

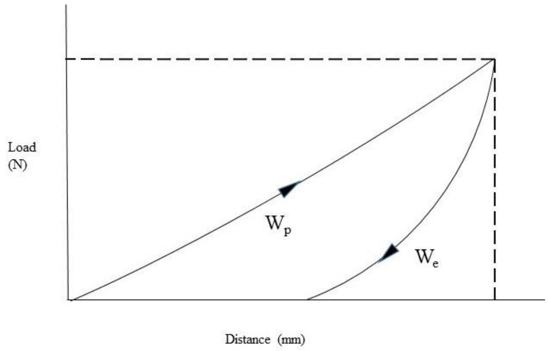


Figure 1. Degree of elasticity analysis graph from texture analyzer

The viscoelastic property and stress relaxation test were analyzed by TA.XTPlus texture analyzer (Stable Micro Systems, Great Britain). For the degree of elasticity, the dough was prepared from conditioned flour (14% moisture) and left for 30 minutes to rest (60-80% RH and 30 °C) in an incubator. A single compression test was carried out with a pre-test speed of 0.5 mm/s, test speed, post-test speed of 0.1 mm/s,3% strain, data acquisition rate of 500 pps, load cell 50 kg, trigger type auto, and force of 0.05 N. The degree of elasticity was calculated as described by Ponce-Garcia et al (2017) as the ratio of elastic work to the total work. The relation is demonstrated in Figure 1. The stress relaxation test was determined by the method with slight modification as described by Singh et al (2006). The water was added according to WHC in the conditioned flour (14% moisture content) to prepare the dough and allowed to rest for 5 minutes. analysis. A single compression test was carried out with a pre-test speed of 0.5 mm/s, 0.5 mm/s of test and post-test speed, 20 % strain, holding time of 300 s, data acquisition rate of 500 pps, load cell 50 kg, trigger type auto and force of 0.05 N. The graph of transformation of the force versus time measurements was achieved according to Ponce-Garcia et al (2017). The stress relaxation was determined at 35 seconds. The relaxation time was recorded as a time when the force reaches 36.8% of maximum force as documented by Singh et al (2006). The relation is demonstrated in Figure 2.

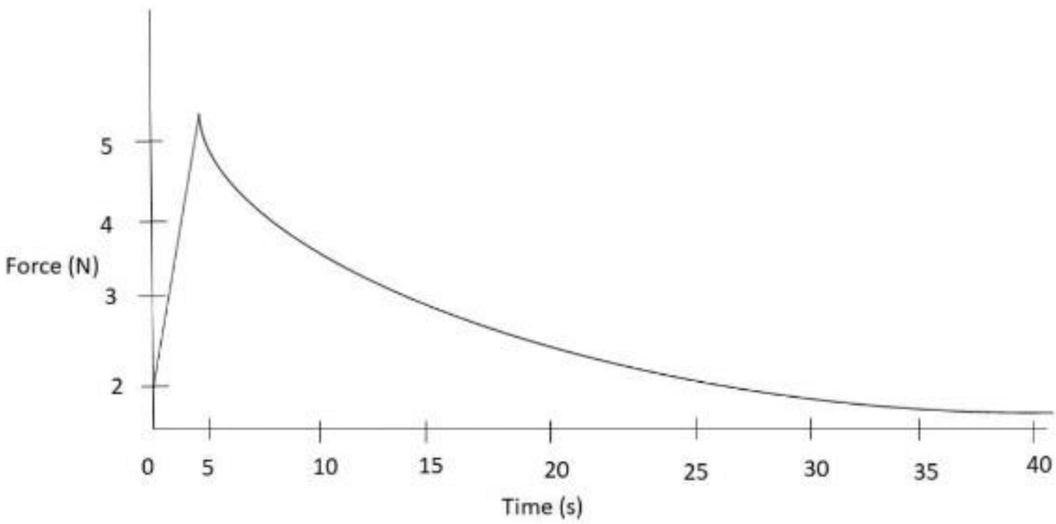
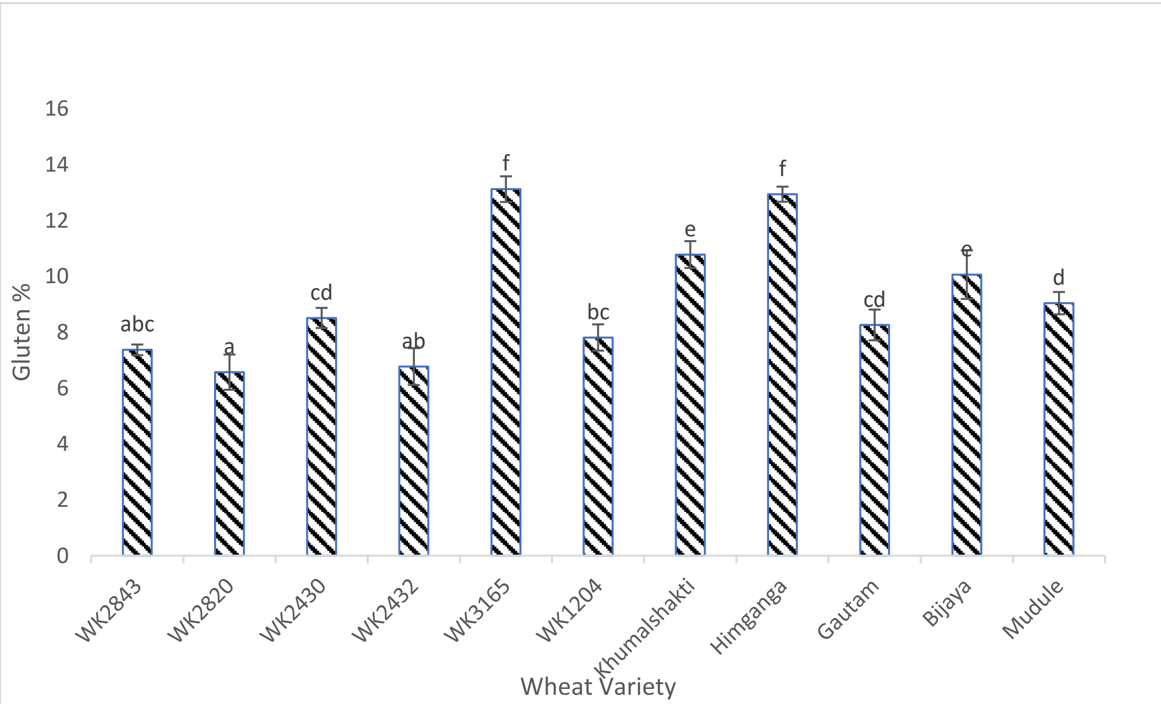


Figure 2. Stress relaxation profile graph from texture analyzer

3. RESULTS AND DISCUSSION

3.1 Gluten and functional properties of wheat flour



Note: The different letter in the bar indicates a significant difference ($p<0.05$)

Figure 3. Gluten% of different wheat varieties

The gluten content of eleven varieties of wheat is shown in Figure 3. The dry gluten content (%) of different heat varieties ranges from 6.57-13.12. The gluten content of WK 3165 and Himganga variety was significantly ($p<0.05$) higher among all wheat varieties. Varied dry gluten content was reported in wheat flour, which was 9.4-12.7% in 6 varieties, 8.28-10.35% in 4 varieties, 10.4-13.8% in 15 varieties, and 10.1-11.7% in 14 varieties (Ahmad et al 2016, Kaushik et al 2015, Khan et al 2012, and Supekar et al 2005). The quantity of gluten protein and its water-absorbing capacity in flour are exhibited by dry gluten (Kaushik et al 2015). High-dry gluten flour can be used for bread and noodles preparation, while low-gluten flour is used for biscuits and cake making (Khan *et al.*, 2012),

The functional quality of wheat flour is shown in Table 1. The water absorption capacity was found in the range of 67.52-82.77%. The highest water absorption capacity (WHC) of 82.77% was found in flour obtained from Khumalshakti ($p<0.05$). Siddiq et al (2009) reported a WHC of 85%, Azima et al (2013) reported 60-64% WHC in 5 varieties, while Mesias and Morales (2017) reported a WHC of up to 90.7%. The flour having WHC of 60-64% or more is suitable for bread making, WHC of 58-60% for cake, and WHC less than 58% is suitable for biscuit making (Mesias and Morales 2017). The variances in the flours' structural characteristics, protein content, and degree of water interaction could be the cause of the observed disparities between them (Chandra et al 2015). Wheat flours damaged by starch and higher in protein have a higher water-absorbing value (Erensteinet al 2022). A lower water-holding capacity is the result of reduced friction during the separation of bran and endosperm in the grinding process as opposed to the current milling technique, which accounts for less starch degradation (Bressianiet al, 2017).

Table 1. Functional quality of wheat flour

Wheat variety	WHC(%)	OAC(%)	GT (° C)	Hausner value	Carr's Index
WK 2843	79.31 ± 0.59^{e}	177.46 ± 1.08^{e}	70.67 ± 5.13^{a}	1.29 ± 0.02^{a}	22.58 ± 0.95^{a}
WK 2820	72.08 ± 0.43^{cd}	165.63 ± 0.57^{cd}	69.67 ± 2.08^{a}	1.32 ± 0.02^{a}	24.27 ± 0.88^{ab}
WK 2430	70.86 ± 0.32^{bc}	184.59 ± 0.89^{g}	64.5 ± 2.5^{a}	1.35 ± 0.01^{a}	25.7 ± 0.5^{ab}
WK 2432	70.79 ± 0.37^{bc}	159.66 ± 0.56^{a}	67.1 ± 3^{a}	1.34 ± 0.01^{a}	25.26 ± 0.61^{ab}
WK 3165	68.77 ± 0.60^{ab}	162 ± 0.94^{b}	71 ± 2^{a}	1.34 ± 0.01^{a}	25.19 ± 0.67^{ab}
WK 1204	73.15 ± 1.47^{cd}	181.54 ± 1.2^{f}	64.67 ± 2.08^{a}	1.36 ± 0.02^{a}	26.53 ± 1.34^{b}
Khumalshakti	82.77 ± 1.06^{f}	168.1 ± 0.39^{d}	71.5 ± 2.5^{a}	1.34 ± 0.02^{a}	25.22 ± 0.99^{ab}
Himganga	74.78 ± 0.99^{d}	166.76 ± 0.99^{cd}	63.5 ± 5.5^{a}	1.42 ± 0.02^{b}	29.74 ± 1.03^{c}
Gautam	71.82 ± 0.60^{cd}	163.95 ± 0.51^{bc}	62 ± 5.29^{a}	1.63 ± 0.01^{d}	38.72 ± 0.31^{e}
Bijaya	74.74 ± 0.95^{d}	163.9 ± 0.1^{bc}	66.67 ± 1.53^{a}	1.52 ± 0.03^{c}	34.33 ± 1.14^{d}
Mudule	67.52 ± 1.28^{a}	168.04 ± 0.04^{d}	67.33 ± 2.52^{a}	1.47 ± 0.01^{bc}	32.19 ± 0.24^{cd}

Note: Variations in the superscript in each column indicate significant differences ($p<0.05$) in each parameter between wheat cultivars.

The oil absorption capacity (OAC) of eleven varieties of wheat was found in the range of 159-.66-184.59. The highest OAC of 184.59 was observed in WK2436 ($p<0.05$). Different authors reported 163.5%, 169.97%, and 243,64% oil absorption capacities of wheat flour respectively (Njintanget al2008, Baljeetet al2010, Meherunnaharet al2018). The higher OAC is due to more hydrophobic binding sites in protein (Noor et al 2012).

The gelatinization temperature (GT) of wheat flour was found in the range of 62-71.5 °C and no varietal effect was observed ($p<0.05$) in gelatinization temperature. Azima et al (2013) reported GT of five wheat varieties in the range of 65-67 °C, while Chandra et al (2015) and Lo et al (2020) reported GT of 59.22 °C and 75 °C respectively. Ridwan et al (2020) suggest that high GT is preferable for noodles and pasta making, as high GT improves cohesion and reduces the amount of amylose leaching.

The Hausner value (HV) and Carr's index (CI) of eleven varieties of wheat flour range from 1.29 to 1.63 and 22.58 to 38.72% respectively. Flour obtained from Gautam has both the highest ($p<0.05$) HV and CI. According to Sonawane et al (2021), flowability with an HV of >1.6 and CI of >38 is extremely poor, while flour with an HV of <1.25 and CI of 1.26 to 1.34 has fair flowability. As per the data obtained, the flowability of flour obtained from wheat varieties Gautam, Bijaya, and Mudule is very poor, while other flowability ranges from passable to poor. Higher CI and HR values suggested that the flours were cohesive, which could lead to issues with bridging, incorrect flows, and discharge issues in hoppers (Bala et al 2020). As revealed by Mcsweeny et al (2020) reported hard wheat flour has poor flowability compared to soft wheat flour, as protein compress readily.

3.2 Viscoelastic properties of dough prepared from different varieties of wheat flour

The degree of elasticity, stress relaxation (%), and relaxation time (s) of 11 varieties of wheat dough were found in the range of 22.41-29.09, 23.41-29.93, and 6.2-12.72 respectively, and is shown in Table 2. The wheat variety demonstrates a significant effect ($p<0.05$) in the textural property. Ponci-Garcia et al (2016) observed a degree of elasticity in the range of 20-25% in 6 different wheat varieties. Singh et al (2006) revealed stress relaxation of 25 to 27% in dough prepared from 3 wheat varieties and a relaxation time of 4.98-5.52 s.

Table 2: Viscoelastic properties of dough prepared from different varieties of wheat flour

Wheat variety	DOE	SR(%)	RT(s)
WK 2843	22.44 ± 0.73^{a}	27.3 ± 1.47^{abcd}	8.8 ± 1.56^{ab}
WK 2820	22.88 ± 1.08^{a}	28.8 ± 0.82^{cd}	10.56 ± 0.9^{ab}
WK 2430	22.41 ± 1.45^{a}	24.33 ± 0.67^{ab}	6.2 ± 0.23^{a}
WK2432	24.83 ± 1.46^{ab}	27.49 ± 0.84^{abcd}	9.76 ± 0.99^{ab}
WK 3165	26.48 ± 0.81^{ab}	27.93 ± 1.22^{bcd}	10.24 ± 1.15^{ab}
WK 1204	27.17 ± 1.66^{ab}	26.64 ± 0.69^{abcd}	9.19 ± 0.81^{ab}
Khumalshakti	28.95 ± 1.21^{ab}	29.93 ± 0.72^{d}	12.72 ± 1.26^{b}
Himganga	29.09 ± 2.13^{b}	25.48 ± 0.57^{abc}	$9.16\ \pm0.19^{ab}$
Gautam	26.19 ± 1.19^{ab}	27.23 ± 0.85^{abcd}	9.77 ± 0.76^{ab}
Bijaya	23.43 ± 0.28^{a}	23.41 ± 0.42^{a}	6.92 ± 0.36^{a}
Mudule	22.94 ± 0.38^{a}	26.79 ± 0.8^{abcd}	8.76 ± 0.98^{ab}

Note: Variations in the superscript in each column indicate significant differences ($p<0.05$) in each parameter between wheat cultivars.

A low value of the degree of elasticity represents the dominancy of low molecular weight glutenin subunits, gliadins, lipids, pentosans, and starch, while a high value of the degree of elasticity is provided by high molecular weight glutenins (Ponci-Garcia et al 2016). Best et al (2023) revealed that glutenin is mainly responsible for dough resistance and elasticity, while gliadins contribute to the dough's viscosity and extensibility. A high value of the degree of elasticity means higher gluten strength (Maucher et al 2009). As reported by Hatcher et al (2008), the noodles prepared from durum wheat flour had a more elastic behavior and dissipated the stress energy more slowly. According to Sozer et al (2008), viscous materials have shorter relaxation times compared to elastic materials.

4. CONCLUSIONS

The research findings conclude that there is a significant impact of variety on both functional and viscoelastic properties. It's noteworthy that all varieties exhibited improved water-holding capacity and oil absorption capacity. However, the flowability of flour across all varieties ranged from poor to extremely poor. Among the varieties studied, WK 3165, WK 1204, Khumalshakti, Himganga and Gautam emerge as promising varieties for utilization in the noodle, pasta, and bread industries, owing to their favorable viscoelastic properties. The study suggests avenues for future exploration, particularly in understanding the interplay between proximate composition and the functional properties of flour, along with the viscoelastic behavior of dough. This could provide valuable insights for optimizing product quality and processing techniques in the food industry.

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EVALUATION OF RADISH (*Raphanus sativus* L.) VARIEITES IN DIFFERENT SOWING DATES FOR OFF-SEASON PRODUCTION AT DAILEKH

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ABSTRACT

Field experiments were conducted at Horticulture Research Station (HRS), Dailekh to identify the suitable radish varieties for the off-season production during two consecutive years (2019–2020). Six varieties of radish (40-Day, All Season, Mino Early, Tokinashi, Puthane Red and Lumle Red) were sown each at four dates; February 13, March 14, April 13 and May 14 in 2019 and 2020, and experiments were laid-out in factorial randomized complete block design with two replications. The combined analysis over the years revealed that varieties significantly affected on plant traits and root yield. Plant and yield traits were significantly influenced by sowing dates. Higher (63.2%) bolting was recorded in 40-Day, followed by Lumle Red (56.0%), Puthane Red (52.2%) and Mino Early (38.8%) than All Season (1.7%) and Tokinashi (0.0%). All Season and Tokinashi gave the highest yield of 30.4 mt/ha and 26.8 mt/ha, respectively and radish sown on May 14 produced the highest root yield (24.0 mt/ha). Therefore, All Season and Tokinashi are identified as the best varieties to grow from February to May for the off-season production at the agro-climatic condition of Dailekh.

1. INTRODUCTION

Radish (*Raphanus sativus* L.), a highly important annual vegetable crop worldwide belongs to Brassicaceae family. In temperate regions, the small-rooted radishes are mostly cultivated, while large-rooted cultivars are widely cultivated in East and Southeast Asia (Crisp, 1995). Generally, it is either eaten as raw as salads, fermented pickles or cooked as vegetables. Besides, radish sprouts and young leaves are also cooked, preserved by salting or pickling or eaten in salads (Kim et al., 2021). The consumption of tender root acts as a cooling effect, prevents constipation and increases appetite. The juice of fresh leaves has also diuretic and laxative value. Iso-thiocyanates are major volatile compound present in radish which gives pungent flavour (Bose et al., 2000). In Nepal, this is a very popular vegetable crop particularly in hills of Nepal owing its short season as well as wide adaptability. It can be grown at open field and protected structures for year-round supply (Mishra et al., 2010).

Radish is cultivated about 18,175 ha of land with total production of 2,89,558 mt and productivity of 15.93 mt/ha (MoALD, 2022), but in Dailekh, it is estimated to be cultivated 233 ha with the total productivity of 12.06 mt/ha. The altitude of Dailekh district ranges from 544 m (Tallo Dungeshwor) to 4, 168 m above sea level (Mahanbu mountain) which creates much climatic diversity to grow radish for normal season as well as off-season. Off-season vegetable production refers the cultivation of fresh vegetables before or after their normal season. Off-season production can be achieved by employing different climatic regions, adjusting planting times, selecting of varieties, creating artificial and controlled environment through use of tunnels, polythene houses, glass houses and hot bed (Joseph et al., 2021). Radish is a cool season crop and moderate day length, and cold temperatures favour its root development. Temperature ranges from 20 to 23°C is good for root development. Meanwhile, long day with hot weather results early bolting before the development of root and pungency develops before maturity (Selvakumar et al., 2019).

At Dailekh, radish can be cultivated twice a year i.e. August–September and January–February for main season production, but it depends on the genotypes and agro-climatic regions. The production of radish from February to May is considered as off-season at Dailekh which has also good price in the market. Varieties such as 40-Day, Mino Early, Tokinashi, Puthane Red and Lumle Red are developed and recommended for normal season production in Nepal (CPDD, 2014), but these varieties are not studied for their off-season potentiality particularly at the agro-climatic region of Dailekh. There is tremendous scope to increase off-season radish production through the proper selection of varieties in mid-hill regions. Tokinashi is a heat tolerant and late maturing variety and recommended for mid-hills of Nepal (Giri et al., 2017). Most of the researches on radish so far in Nepal are conducted in variety evaluation (Panday 1999, Gautam et al., 2017, Dahal et al., 2021, Gotame et al., 2021, Shrestha et al., 2021) and cultivation practices (Chapagain et al., 2010). However, the evaluation of varieties with different sowing dates from spring to summer season especially for off-season radish production at open field condition has not been conducted yet. The objectives of present study were to evaluate radish varieties at different sowing dates from spring to summer and to identify the suitable radish varieties for off-season production at open field condition of Dailekh district.

2. MATERIALS AND METHODS

2.1 Study Site and Climate

This study was conducted at HRS, Dailekh (28˚ 50' 49.8" N longitude and 81˚ 43' 19.4" E latitude with an elevation of 1, 255m above sea level) during two years (2019 and 2020) in Karnali Province. The research site is characterized by sub-tropical climate and average annual distribution ranged from 160 mm to 170 mm (HRS, 2020). The soil texture was loamy type with pH 5.5–6.0 (HRS, 2019). Average monthly maximum and minimum temperature was higher in 2019 than in 2020 during February to June. Rainfall was inconsistent in the both years, but as compared to 2019, the research station was received more amount of rainfall in 2020 (Figure 1).

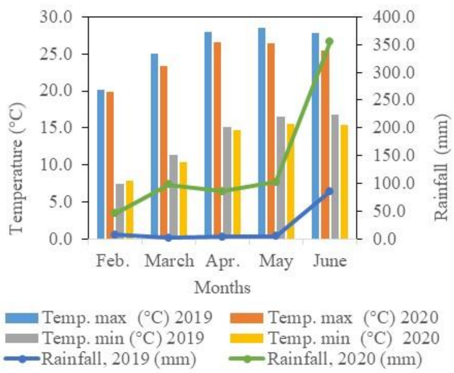

Figure 1. Monthly weather status of growing months (February–June) of radish cultivars in 2019 and 2020 at HRS, Dailekh, Nepal (Source: Department of Hydrology and Meteorology, Surkhet, 2019 and 2020).

2.2 Plant Materials and Cultivation

Seeds of six varieties (40-Day, All Season, Mino Early, Tokinashi, Puthane Red and Lumle Red) were used to evaluate for off-season production. Seeds of two varieties (Puthane Red and Lumle Red), two (All Season, Tokinashi) and the remaining two (40-Day and Mino Early) were received from the Directorate of Agricultural Research (DoAR), Lumle, Gandaki Province, National Horticulture Research Center (NHRC), Khumaltar and HRS, Dailekh, respectively. Manures and fertilizer were used at the rate of 20 mt farm yard manure (FYM) and 100:50:50 kg $NP_2O_5K_2O$/ha, respectively (Shrestha & Dhakal, 2018). All quantity of FYM, phosphorous, potassium and half quantity of nitrogen were applied as basal doses during land preparation and remaining half amount of nitrogen was top-dressed equally at 30 days after sowing (DAS). Urea (46% N), diammonium phosphate (18% N, 46% P_2O_5) and muriate of potash (60% K_2O) were used as the sources of fertilizers. The trial comprised of two factors; varieties (six levels) and sowing dates (four levels). Altogether, 24 treatments were designed at factorial randomized complete block design (RCBD) with two replications. Seeds of each six varieties were sown manually on February 13, March 14, April 13 and May 14 in 2019, and 2020. Initially, three seeds were sown at each hill at the spacing of 25 cm × 20 cm and after the crop emergence, thinning was done to maintain 10 plants at each row. Each plot contained five rows and the plot size was 2.5 m^2 with the total 50 plants. Irrigation, weeding and top-dressing were followed for radish as per the recommendation of Shrestha & Dhakal (2018).

2.3 Data Recording

Observations on bolting (%), leaf weight/plant (g), root length (cm), root diameter (mm), individual root weight (g), and fresh root yield (mt/ha) were taken. Bolting (%) was taken as number of bolted plant divided by total number of plant in a plot. Leaf weight (g) was measured on randomly selected five plants, weighted using digital balance (H-Honda™, India), and averaged it. Likewise, root length (cm) was measured using meter scale, whereas root diameter (mm) was measured using digital Vernier caliper (150mm, Model: DC-515). The 40-Day variety was harvested after 40 days after sowing (DAS), and other varieties were harvested at 60 DAS. Individual root weight (g) was measured on five plants using digital balance (H-Honda™, India) and root yield (mt/ha) was calculated based on total root weight/plot.

2.4 Statistical Analysis

Data were processed using MS Excel (version 16.0, Microsoft, Redmond, WA, USA) and then, analyzed using GenStat Release 10.3 DE Software (VSN, Rothamsted, UK) Discovery Edition. Phenotypic correlation coefficients between the traits were analysed using SPSS (SPSS Inc., Chicago, Ill., USA).

3. RESULTS AND DISCUSSION

3.1 Effect on Plant Characteristics and Root Length

Varieties showed a highly significantly ($p<0.01$) difference on bolting (%) (Table 1). The combined mean showed that 40-Day showed the highest (63.2%) bolting, followed by Lumle Red and Puthane Red. But the average bolting was higher in 2020 (51.3%) than 2021 (20.3%). This might be due to the occurrence of higher temperature and low rainfall in 2019 than 2020 (Figure 1). Temperature higher than 25°C has reported to be early bolting in radish (Selvakumar et al., 2019). Pandey et al. (2017) reported that long day conditions and high temperature influence in bolting of radish. Varieties affected significantly ($p<0.05$) on leaf weight and it was recorded the highest (66.2 g/plant) in All Season, but it was statistically similar to Mino Early, Tokinashi, Lumle Red and Puthane Red. The differences in leaf weight may be due to genetic differences and similar results have reported in radish genotypes (Mapari et al., 2009, Shrestha et al., 2021). Nieuwhof (1976) have also mentioned the differences in leaf growth in radish cultivars. Similarly, highly significant ($p<0.01$) differences were observed in root length among the cultivars. All Season produced the longest root (23.4 cm), followed by Tokinashi (22.5 cm), but the shortest root length (11.6 cm) was measured in 40-Day. The differences in root length among the cultivars were due to genetic trait and similar report has mentioned by previous researchers (Deotale et al., 1994, Shrestha et al., 2021, Gotame et al., 2021, Luitel et al., 2022). Furthermore, root length in radish genotypes is also affected by environmental factors and soil conditions.

Sowing date revealed a highly significant ($p<0.01$) difference in bolting percentage (Table 1). Radish sown on March 14 showed the maximum (43.0%) bolting, but it was statistically at par with the radish sown on Feb. 13 and April 13. The minimum (17.2%) bolting was recorded in radish sown on May 14. The interaction effect among varieties, sowing dates and year was significant (Table 1). The sowing date also significantly ($p<0.05$) affected on leaf weight and the significant effect of growing season on leaf weight has also reported (Akoumianakis et al., 2011). The highest leaf weight (66.6 g/plant) was produced on May 14 sown radish. Year had a highly significant (p<0.01) effect on leaf weight, but the interaction effect of different treatments was non-significant (p<0.05). The effect of sowing dates on root length was highly significant (p<0.01). Radish sown on May 14 produced the longest (20.6 cm) root, but the shortest (12.4 cm) root was measured on Feb. 13 sown radish. The varieties, sowing date and year, and their interaction on root length was highly significant (p<0.01). Seeds sown on May 14 showed less bolting, higher leaf weight and higher root length and this might be due to more rainfall which could also lower the soil temperature. Further, the soil temperature is also critical for root development in radish and McGill (2018) has reported the reduced root growth of radish at 26°C compared to 20°C. The significant effect of sowing time on plant and root traits has reported in the main season radish (Alam et al., 2010, Khan et al., 2022). Khan et al. (2022) have mentioned that early sowing (October 19) of radish cultivar Mino in normal season of Pakistan resulted the highest leaf and root length. In this study, late sowing (May 14) coincided with rainfall that might have favoured for developing less bolting, higher leaf weight and root length than those of radishes sowing in earlier dates.

Table 1. Plant characteristics and root length of radish varieties established at different sowing dates

Treatments	Bolting (%)			Leaf wt. (g/plant)			Root length (cm)		
Varieties (V)	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
40-Day	72.0	54.5	63.2	10.0	70.5	40.3	3.8	19.3	11.6
All Season	3.5	0.0	1.7	59.8	72.6	66.2	24.4	22.5	23.4
Mino Early	63.8	13.8	38.8	47.0	78.5	62.8	10.3	21.4	15.8
Tokinashi	0.0	0.0	0.0	48.5	74.5	61.5	22.5	22.6	22.5
Puthane Red	86.5	24.0	55.2	13.6	74.5	44.0	5.1	20.8	12.9
Lumle Red	82.2	29.7	56.0	18.9	74.0	46.4	5.8	20.3	13.1
F-Test			**			*			**
LSD (0.05)			9.85			20.92			2.14
Sowing date (SD)									
Feb. 13	64.8	19.0	41.9	17.0	69.3	43.2	8.9	15.7	12.4
March, 14	53.0	33.0	43.0	22.8	85.7	54.2	12.6	21.1	16.8
April, 13	65.3	17.2	41.3	35.8	58.7	47.2	7.3	25.6	16.5
May, 14	22.2	12.2	17.2	52.5	82.8	67.7	19.2	22.1	20.6
F-Test			**			*			**
LSD (0.05)			8.04			19.53			1.75
Mean	51.30	20.30	35.8	32.90	74.10	52.70	11.99	21.15	**
Year (Y)			**			**			1.23
LSD (0.05)			5.69			13.8			16.57
V × SD			**			NS			**
LSD (0.05)			19.70			47.84			4.29
V × Y			**			NS			**
LSD (0.05)			13.93			33.83			3.03
SD × Y			**			NS			**
LSD (0.05)			11.37			27.62			2.47
V × SD× Y			*			NS			**
LSD (0.05)			27.86			67.65			6.07
CV (%)			38.7			63.8			18.2

NS, non-significant at p >0.05. *Significant at p <0.05; **Highly significant at p <0.01.

3.2 Effect on Root Diameter, Root Weight and Root Yield

The combined analysis showed that varieties had a highly significant (p<0.01) effect on root diameter, root weight and root yield (Table 2). The average root diameter was higher (26.30 mm) in 2020 than that of 2019 (16.84 mm). The average root diameter was measured the highest (26.9 mm) in Tokinashi and All Season (26.8 mm), but the lowest (15.9 mm) root diameter was measured in 40-Day. Likewise, individual root weight was also higher in 2020 than that of 2019. The average root weight was the highest (175.0 g) in Tokinashi and it was statistically similar to All season (162.6 g) and the lowest (93.6 g) was recorded in Puthane Red. The highest root yield (30.4 mt/ha) was recorded in all season, followed by Tokinashi (26.8 mt/ha) and the lowest (10.6 mt/ha) was recorded in Puthane Red. In this study, yield differences found among the varieties might be due to genetic trait. The significant yield differences among commercial radish cultivars in Dhankuta have reported by Chapagain et al. (2010). Besides, Poudel et al. (2018) have reported the highest root yield (59.5 mt/ha) in normal season radish genotype HRDRAD002, but we reported the highest (30.4 mt/ha) in off-season radish cultivar All Season. In a study by Gotame et al. (2021), have reported the maximum root yield (29.04 mt/ha) in genotype HRDRAD004 in the main season of terai. The production of radish also depends on genotype and season. Panday (1999) reported the highest (30.8 mt/ha) root yield in Mino Early in the main season (August–September), but our study showed yield of 13.5 mt/ha in off-season in Mino Early.

The effect of sowing date on root diameter, root weight and total root yield was highly significant (P<0.01) (Table 2). Root diameter was measured the highest (29.8 mm) on May, 14 sown radishes and the lowest (17.5 mm) was found on April, 13 sown radishes. The interaction effect of varieties, sowing data and year was highly significant ($p<0.01$). Similarly, radish sown on May 14 gave the highest root weight (182.8 g) and root yield (24.0 mt/ha). Year had a highly significant ($p<0.01$) effect both on root weight and root yield. The interaction effect between varieties and sowing dates, and sowing dates and years was highly significant ($p<0.01$). Dahal et al. (2021) have reported the highest root yield (30.87 mt/ha) in Ivory White F_1 cultivar at February third week sown for off-season radish under shade net at Terai. In our study, varieties showed low production in mid-hills during February to April and this might be due to high temperature. But seeds sown on May 14 coincided with high temperature, but plants received more rainfall which tended to lower the temperature effects, and thereby produced high yield in June. Radish is a winter vegetable and many researchers (Cheema et al., 2004, Dahal et al., 2021) have recommended to grow under shade net house during summer to get high off-season yield with quality roots.

Table 2. Root diameter, root weight and yield of radish varieties established at different sowing dates

Treatments	Root diameter (mm)			Root wt. (g)			Root yield (mt/ha)		
Varieties (V)	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
40-Days	9.0	22.8	15.9	40.8	183.1	111.9	10.0	16.8	13.4
All Season	26.9	26.8	26.8	138.5	186.6	162.6	32.2	28.7	30.4
Mino Early	20.2	26.1	23.1	87.2	144.4	115.8	11.7	15.2	13.5
Tokinashi	25.4	28.5	26.9	155.6	194.4	175.0	24.9	18.7	26.8
Puthane Red	7.9	27.2	17.6	48.2	138.9	93.6	2.2	19.0	10.6
Lumle Red	11.7	26.3	18.9	46.5	162.2	104.4	6.3	19.8	13.1
F-Test			**			**			**
LSD (0.05)			4.74			32.19			4.75
Sowing date (SD)									
Feb. 13	8.7	29.7	19.2	0.0	118.1	59.1	13.9	19.7	16.8
March, 14	16.8	22.8	19.8	109.4	120.8	115.1	14.2	16.4	15.3
April, 13	11.4	23.5	17.5	53.9	249.6	151.8	6.5	24.7	15.6
May, 14	30.3	29.2	29.8	181.2	184.5	182.8	23.5	24.7	24.0
F-Test			**			**			**
LSD (0.05)			3.87			26.28			3.87
Mean	16.84	26.30	21.57	86.1	168.3	127.2	14.52	21.37	17.96
Year			**			**			**
LSD (0.05)			2.74			18.58			1.36
V × SD			**			**			**
LSD (0.05)			9.49			64.37			9.49
V × Y			**			*			**
LSD (0.05)			6.71			45.52			6.71

SD × Y	**	**	**
LSD (0.05)	5.48	37.17	5.48
V ×SD ×Y	**	**	NS
LSD (0.05)	13.42	91.04	13.43
CV (%)	30.9	35.6	37.2

[NS], non-significant at $p > 0.05$. *Significant at $p<0.05$; **Highly significant at $p < 0.01$.

The interaction effect between varieties and sowing dates on root yield is given in Figure 2. The 40-Day variety sown on May 14 gave the highest yield (38.5 mt/ha), but it did not perform well on Feb. 13, March 14 and April 13. However, All Season provided the highest (45.2 mt/ha) root yield on Feb. 13 sowing, but it provided consistent yield in March 14, April 13 and May 14 sown radish. Compared to Mino Early, Puthane Red and Lumle Red, All Season, followed by Tokinashi gave the higher yield in all sowing dates. The 40-Day, Mino Early, Puthane Red and Lumle Red sown on May 14 showed increased root yield compared to others sowing dates, and this might be due to more rainfall during May–June which might be lowered the soil temperature and improved the yield. Sowing date is also important to reduce cold and heat and the significant effect of sowing dates on root yield of radish cultivars has reported in previous studies (Shamil et al., 2019). In the main season of radish, late sowing decreases plant traits and root yield. Shamil et al. (2019) stated that early sowing (October 20) increases leaf weight and root yield in radish in the normal season. The interaction effect of radish varieties and sowing dates on root traits and yield has also reported (Ebrahimi et al., 2013). In this study, early sowing (Feb. 13) of radish varieties coincided with high temperature that resulted high bolting and low root yield in 40-Day, Mino Early, Puthane Red and Lumle Red. Radish is sensitive to temperature requirement, thus manipulation of sowing time is important to achieve the highest yield (Alam et al., 2010). Our study revealed that All Season and Tokinashi seemed appropriate varieties for off-season production since these were well-adapted to the growing environments of Dailekh region. The combination of superior varieties and appropriate sowing date are the most important factor to achieve the highest yield (Alinaghizadeh et al., 2009).

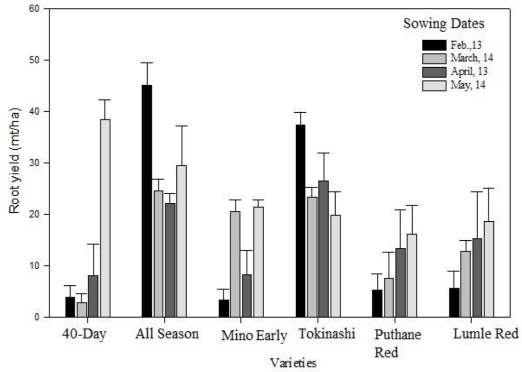

Figure 2. Effect of varieties and sowing dates on root yield (mt/ha) in radish at HRS, Dailekh, Nepal. Vertical bar represents mean ±SE (n = 4).

3.3 Phenotypic Correlation Among the Traits

The phenotypic correlation coefficients of the measured traits in radish varieties are presented in Table 3. Bolting exhibited a highly significant ($p<0.01$) and negative correlation with leaf weight (r = −0.48), root length (r = −0.81), root diameter (r = −0.74), root weight (r = −0.59) and root yield (r = -0.77). Bolting affected all the measured traits. The energy reserves are diverted for the production of flowering stems instead of roots and leaves which reduces leaf weight, root weight, and total root yield (Kamenetsky & Rabinowitch, 2001) which also confirms the result of our study. The leaf weight was positively and highly significantly ($p<0.01$) correlated with root length (r = 0.56), root diameter (r = 0.49), root weight (r = 0.41) and root yield (r = 0.48). Likewise, root length had a highly significantly ($p < 0.01$) and positively correlated with root diameter (r = 0.78), root weight (r = 0.72), and root yield (r = 0.77). The significant and positive correlations of root length with root weight and root yield have also reported (Luitel et al., 2022). Furthermore, Ullah et al. (2010) reported that root length and root diameter showed the significant, and positive correlation with root yield. Root diameter revealed a highly significant ($p<0.01$) and positive association with root weight (r =

0.64) and root yield (r = 0.67). A highly significant and positive correlation (r = 0.59) was found between root weight and root yield. This type of association is important and necessary for the selection and breeding program of radish (Kaur et al., 2017).

Table 3. Phenotypic correlation coefficients of phenotypic traits of radish varieties

Traits	Bolting	Leaf weight	Root length	Root diam.	Root wt.	Root yield
Bolting	1.0	−0.48**	−0.81**	−0.74**	−0.59**	−0.77**
Leaf wt.		1.0	0.56**	0.49**	0.41**	0.48**
Root length			1.0	0.78**	0.72**	0.77**
Root dia.				1.0	0.64**	0.67**
Root wt.					1.0	0.59**
Root yield						1.0

**Highly significant at p<0.01.

4. CONCLUSION

This research evaluated six important radish cultivars at different sowing dates i.e. February 13, March 14, April 13 and May 14 in 2019 and 2020 for off-season production in mid-hill condition of Nepal. Varieties and sowing dates both significantly affected in bolting, leaf weight, root length, root diameter, root weight and root yield. Bolting was more pronounced in 40-Day, followed by Lumle Red, Puthane Red and Mino Early particularly seed sown on Feb. 13, April 14, and March 13, but Tokinashi had no bolting, and low (1.7%) bolting was observed in All Season. All Season and Tokinashi produced the highest yield of 30.4 mt/ha and 26.8 mt/ha, respectively. Likewise, seeds sown on Feb. 13, March 14 and April 13 did not exhibit statistical differences for root yield, but seeds sown on May 14 produced the highest yield (24.0 mt/ha). All Season and Tokinashi sown on four different sowing dates (Feb. 13, March 14, April 13 and May 14) produced higher yield than others. High bolting is negatively associated with leaf weight, root length, root diameter, root weight and root yield and bolting is the predominate trait of radish cultivars for off-season production. Therefore, All Season and Tokinashi can be recommended to grow from February to May to get higher production in off-season radish at the similar agro-climatic conditions of Dailekh.

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PERFORMANCE OF TOMATO GENOTYPES UNDER OPEN FIELD CONDITIONS IN MID HILLS OF NEPAL

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ABSTRACT

Nepalese tomato growers are seeking new high-yielding open-pollinated varieties for open field conditions. An experiment was conducted at National Horticulture Research Center, Khumaltar, Lalitpur, Nepal to identify the best open pollinated genotypes among recently introduced genotypes from The World Vegetable Center, Taiwan. Eleven genotypes were evaluated in Randomized Completely Block Design with three replications. The result showed the shortest days to 50% flowering in genotype AVTO0922 (28 days after transplanting) while Pusa Ruby was reported to be the earliest for harvesting (66 DAT). Though the number of fruits per cluster (7) and the number of fruits per plants (80) were recorded the highest in genotype AVTO1350 but the fruit weight (26.8 g) and yield (23.4 t/ha) was reported the lowest, which make it un-preferable for commercial grower. Similarly, the yield per plant (3.4 kg) and yield per hectare (59.1 t/ha) was reported the highest in genotype AVTO 9802 and AVTO1429 respectively. Despite having the highest fruit yield, the genotype AVTO1429 was unsuitable for commercial cultivation due to its relatively large fruit weight (135.1 g). Based on fruit size and yield, genotypes AVTO9802 and AVTO1219 were found promising for commercial cultivation under open field.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L., 2n=24) is a very popular and most widely cultivated vegetable commodity around the world that is important both economically and nutritionally (Singh *et al.*, 2014). Globally, it is an important economic vegetable after potato with a cultivated area of 505 million hectares, production of 169.4 million ton, and productivity of 33.54 t/ha (FAOSTAT, 2020). Tomato is not only consumed as a fresh vegetable, as a processed product, and dried product, but also used as an important ingredient for many recipes (Labate *et al.*, 2007; Subramanian, 2016). Nutritionally tomato contains different phytochemicals, phenolic compounds, antioxidants, and vitamins (Hedges and Lister, 2005). Tomato is one of the most popular vegetable crops which is commercially grown both in the hills and plains of Nepal (Pandey *et al.*, 2006). Tomato has been accepted as a remunerative crop by the farmers of Nepal due to high market demand and high income from its cultivation therefore the cultivation of tomatoes is expanding rapidly in Nepal (Ghimire *et al.*, 2018). It is the third largest vegetable crop of the country in terms of area and production after cauliflower and cabbage whereas it is in second place in terms of productivity after watermelon. The total area, and production of tomato in Nepal is 22,911 ha and 4,22,703 ton, respectively (MoALD, 2023). The productivity of tomato in Nepal (18.45 t/ha) is lower as compared to India (24 t/ha), China (57 t/ha), and the world average (36 t/ha) (FAOSTAT, 2020). Lack of appropriate varieties resistant to disease and pests, improper management, lack of quality seed, and lack of awareness about improved production technologies are the main reasons for the low productivity of tomato in Nepal (Gotame *et al.*, 2021). Similarly, in the mid hills of Nepal, tomato is cultivated under open field conditions during the summer and rainy seasons with very low production because of flower drops and poor fruit set. A total of 38 varieties of tomato are registered under the National Seed Board (NSB) of Nepal for commercial cultivation, among which four are released open-pollinated (OP) varieties (Pusa Ruby, Monprecos, Roma, and NCL-1), three are hybrids developed within the country (Srijana, Khumal Tomato Hybrid-2, and Khumal Tomato Hybrid-3) and remaining thirty-one are exotic hybrids. Released OP varieties are not preferred by growers as they are old, not properly maintained, deteriorated, and susceptible to diseases and pests. On the other hand, exotic hybrids may not give stable performance over the season, their seeds are not easily available in the market and they are expensive too (Gotame *et al*., 2021). Nepalese tomato growers are seeking new high-yielding open-pollinated varieties for open field conditions of mid hills of Nepal (Devkota *et al.,* 2018). Hence, this experiment was carried out to identify high-yielding open-pollinated varieties of tomato that can be adapted for commercial cultivation in the mid-hills of Nepal.

2. MATERIALS AND METHODS

2.1 Experimental site

The experiment was conducted at the experimental field of National Horticulture Research Center, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal from March to July 2022 and 2023 (for two consecutive years). Geographically Khumaltar (27º67'N, 85º31'E, 1365 masl) is situated in the hilly region of Nepal with a subtropical climate. The average temperature was 21.7°C and the average relative humidity was 76.6% and the total rainfall was 825.7 mm during the entire research period. The soil of the experimental site has 2.8% organic matter, 0.12% total nitrogen, 181.7 vmg/kg available phosphorus, and 92.8 vmg/kg potassium with a pH 5.95.

2.2 Experiment details

The experiment was laid out in a randomized completely block design (RCBD) with eleven treatments and three replications. Among eleven genotypes ten improved lines of tomato were collected from World Vegetable Centre, Taiwan while Pusa Ruby, a released variety (used as a check) was collected from National Horticulture Research Centre, Khumaltar. The seeds were sown in a plug tray on March 20, 2022, and 2023. The size of the experimental plot was 3.24 m^2 (1.8 m length and 1.8 m breadth), and the space between experimental plots and within replication was 0.5 m each. Organic manure (FYM) was applied at the rate of 25 ton/ha whereas chemical fertilizers were applied at the rate of 200:150:120 kg $N:P_2O_5:K_2O$ per hectare. All FYM, full doses of P_2O_5, K_2O, and half of N required for the individual plot was calculated and applied equally into nine pits as basal dose while remaining half dose of N was top dressed in two splits at 30 days after transplanting (DAT) and 60 DAT. About 28 days old seedlings were transplanted in the open field on 18th April 2022 and 2023. Planting was done at a spacing of 60 cm × 60 cm distance accommodating nine plants per plot. Various intercultural operations like removal of sucker, weeding, irrigation, and plant protection measures were applied as per recommendation. After fifteen days of transplanting, staking was given with a bamboo pole. Three branches per plant were maintained and each branch was trained upward by using the jute rope.

2.3 Observations and data recording

Observations were recorded on various parameters like days to 50% flowering, number of flowers per cluster, number of fruits per cluster, days to the first harvest, fruit length, fruit diameter, fruit weight, number of fruits per plant, yield per plant and yield per hectare. Maturity character i.e. days to 50% flowering was recorded when 50% of plants had set flowers in each plot and expressed in days after transplanting (DAT) by counting the number of days after transplanting to 50% flowering. Number of flowers per cluster and number of fruits per cluster was counted from five selected cluster from each plant and the average was computed. The number of days to first harvesting was counted from days of transplanting and expressed as DAT. Similarly, fruit weight, fruit length, and fruit diameter of 20 sample fruits from each experimental plot were recorded by using a digital weighing balance (Phoenix PH12002NE) and vernier caliper (INSIZE 1139-150), respectively. Likewise, yield and yield attributing parameters like, the number of fruits per plant and fruit yield per plant was recorded from each sample plant from each harvest, and the average number of fruits and yield per plant was computed. The yield of the net plot was calculated by adding the yield of each sample plant. The total fruit yield per hectare was obtained by converting the yield of the net plot area into a hectare.

2.4 Data analysis

The collected data were compiled by using the Microsoft-excel program (Version 10). Analysis of variance for all parameters was carried out as per the procedures given in GenStat Teaching and Learning (Version 18) statistical computer package for RCBD. Duncan's Multiple Range Test (DMRT) for mean separations was done from the reference of Gomez and Gomez (1984).

3. RESULTS AND DISCUSSION

3.1 Days to 50% flowering

This parameter was observed to evaluate the earliness of different tomato genotypes. The difference among the genotypes on days to 50% flowering from transplanting was highly significant (Table 1). AVTO0922 was the earliest genotype to 50% flowering from transplanting (27 DAT in 2022 and 29 DAT in 2023) followed by AVTO1418 (31 DAT in 2022 and 30 DAT in 2023). AVTO1422 took the longest period for flowering (46 DAT in 2022 and 44 DAT in 2023) followed by AVTO0102 (45 DAT in both the years). The mean days to 50% flowering was the shortest for AVTO0922 (28 DAT) followed by AVTO1418 (31 DAT) and AVTO1219 (32 DAT) whereas it was the longest for AVTO1422 (45 DAT). The earliness in flowering is a genotypic characteristics and somewhat governed by environmental conditions like growing areas (Singh *et al.,* 2014). Gotame *et al.,* (2021) also reported similar results with shortest days to 50% flowering in genotype Sindhupalchowk Local (26 DAT) and the maximum days to 50% flowering in AVTO1420 (44 DAT). Tujuba and Ayana (2020) stated that both genotypic and environmental factors influence tomato plants to flower early or delay in flowering.

3.2 Number of flowers per cluster

This parameter was observed to evaluate the flowering characters of different tomato genotypes and relate the number of flowers per cluster with the yield. The difference among the genotypes on number of flowers per cluster was highly significant. The highest number of flowers per cluster was found in AVTO1350 (9 flowers per cluster) followed by AVTO1219, AVTO1429, AVTO9802 (8 flowers per cluster) in 2022 whereas AVTO1350, AVTO1429 and AVTO9802 had the highest number of flowers per cluster (9 flowers per cluster) in 2023. The lowest number of flowers per cluster was produced by AVTO1422 (5 flowers per cluster) in 2022 whereas it was produced by AVTO1418 (5 flowers per cluster) in 2023. The mean value of number of flowers per cluster was highest for AVTO1350, AVTO1424 and AVTO9802 (9 flowers per cluster) followed by AVTO1219 (8 flower per cluster) whereas it was lowest for AVTO1418, AVTO1422, AVTO1424 and Pusa Ruby (6 flowers per cluster). The remaining genotypes had the mean value of 7 flowers per cluster. The number of flower per cluster might be varietal characteristics which may be sometimes influenced by environmental conditions like temperature, light etc. Sureshkumara *et al.*, (2017) also reported similar results on number of flower per cluster of tomato genotypes with the highest flower in genotype IIHR-2200 (6 flower/cluster) and the lowest in Arka Ahuti (4 fruits/cluster).

Table 1. Performance of tomato genotypes on days to 50% flowering and number of flowers per cluster at Khumaltar, Lalitpur during 2022 and 2023.

Genotypes	Days to 50% flowering			Number of flowers per cluster		
	2022	2023	Mean	2022	2023	Mean
AVTO0102	43^{e}	45^{f}	44^{f}	7^{c}	8^{d}	7^{b}
AVTO0922	27^{a}	29^{a}	28^{a}	7^{c}	6^{b}	7^{b}
AVTO1219	31^{b}	33^{c}	32^{b}	8^{d}	8^{d}	8^{c}
AVTO1314	39^{d}	38^{e}	38^{e}	7^{c}	7^{c}	7^{b}
AVTO1350	36^{d}	35^{cde}	36^{d}	9^{e}	9^{e}	9^{d}
AVTO1418	31^{b}	30^{ab}	31^{b}	6^{b}	5^{a}	6^{a}
AVTO1422	46^{e}	44^{f}	45^{f}	5^{a}	6^{b}	6^{a}
AVTO1424	35^{cd}	34^{cd}	35^{cd}	6^{b}	7^{c}	6^{a}
AVTO1429	32^{bc}	33^{bc}	33^{bc}	8^{d}	9^{e}	9^{d}
AVTO9802	36^{d}	35^{cde}	35^{d}	8^{d}	9^{e}	9^{d}
Pusa Ruby (check)	36^{d}	37^{de}	37^{de}	6^{b}	6^{b}	6^{a}
Mean	**36**	**36**	**36**	**7**	**7**	**7**
F test	**	**	**	**	**	**
LSD	**3.47**	**3.28**	**2.27**	**0.41**	**0.95**	**0.56**
CV	**5.7**	**5.4**	**5.5**	**3.4**	**7.7**	**6.7**

*Note: LSD = Least significant difference, CV = Coefficient of variance, *, ** and *** Significant at 0.05, 0.01 and <0.01 levels, respectively. In column figures with same small letter(s) do not differ significantly by DMRT at 0.05 level*

3.3 Number of fruits per cluster

This parameter was studied to evaluate the fruiting potential of different tomato genotypes. The difference among the genotypes for the number of fruits per cluster was highly significant (Table 2). The highest number of fruits per cluster was produced by AVTO1350 (7 fruits per cluster) followed by AVTO0102, AVTO1219, AVTO1314 and AVTO9802 (6 fruits per cluster) in 2022. In 2023, The highest number of fruits per cluster was produced by AVTO1350, AVTO1429 and AVTO9802 (7 fruits per cluster). The lowest number of fruits per cluster was produced by AVTO1418, AVTO1422, AVTO1424 and Pusa Ruby (4 fruits per cluster) in 2022 whereas in 2023 it was produced by AVTO1418, AVTO1422 and Pusa Ruby (4 fruits per cluster). The mean value of number of fruits per cluster was highest for AVTO1350 (7 fruits per cluster) followed by AVTO0102, AVTO1219, AVTO1429 and AVTO9802 (6 fruits per cluster) whereas it was lowest for AVTO1418, AVTO1422, AVTO1424 and Pusa Ruby (4 fruits per cluster). Other than these genotypes, the number of fruits per cluster was 5. Sureshkumara *et al.,* (2017) also reported similar results on number of fruits per cluster among different genotypes of tomato.

3.4 Days to first harvest

This parameter was observed to study the earliness of the different tomato genotypes. The difference among the genotypes for days to first harvest was highly significant (Table 2). The days to first harvest was shortest for Pusa Ruby (64 days in 2022 and 68 days in 2023) followed by AVTO0102 and AVTO1350 (67 days in 2022 and 71 days in 2023). AVTO1422 took longest days to first harvest (81 days in 2022 and 85 days in 2023).

Days to first harvest determine the demand and market price because early harvesting genotypes can help to fetch comparatively higher prices in the market hence farmers prefer early maturing varieties. The mean days to first harvest was the shortest for Pusa Ruby (66 days) followed by AVTO0102 and AVTO1314 (69 days) whereas it was the longest for AVTO1422 (83 days).

Table 2. Performance of tomato genotypes on number of fruits per cluster and days to first harvest at Khumaltar, Lalitpur during 2022 and 2023.

Genotypes	Number of fruit per cluster			Days to first harvest		
	2022	**2023**	**Mean**	**2022**	**2023**	**Mean**
AVTO0102	6[c]	5[b]	6[c]	67[b]	71[b]	69[ab]
AVTO0922	5[b]	5[b]	5[b]	68[b]	73[bc]	71[bc]
AVTO1219	6[c]	6[c]	6[c]	71[c]	75[cd]	73[c]
AVTO1314	6[c]	5[b]	5[b]	71[c]	75[cd]	73[c]
AVTO1350	7[d]	7[d]	7[d]	67[b]	71[b]	69[ab]
AVTO1418	4[a]	4[a]	4[a]	68[b]	72[b]	70[bc]
AVTO1422	4[a]	4[a]	4[a]	81[d]	85[e]	83[d]
AVTO1424	4[a]	5[b]	4[a]	70[c]	75[cd]	73[c]
AVTO1429	5[b]	7[d]	6[c]	71[c]	75[d]	73[c]
AVTO9802	6[c]	7[d]	6[c]	71[c]	76[d]	74[c]
Pusa Ruby (check)	4[a]	4[a]	4[a]	64[a]	68[a]	66[a]
Mean	**5**	**5**	**5**	**70**	**74**	**72**
F test	**	**	**	**	**	**
LSD	**0.41**	**0.81**	**0.56**	**1.55**	**2.05**	**3.06**
CV	**4.7**	**9.0**	**9.4**	**1.3**	**1.6**	**3.7**

*Note: LSD = Least significant difference, CV = Coefficient of variance, *,** and *** Significant at 0.05, 0.01 and <0.01 levels, respectively. In column figures with same small letter(s) do not differ significantly by DMRT at 0.05 level*

3.5 Fruit length (mm)

This parameter was observed to evaluate the relation of fruit size to total yield of different tomato genotypes. The difference among the genotypes on fruit length (mm) was highly significant (Table 3). The fruit length was the longest for AVTO9802 (55.8 mm in 2022 and 55.3 mm in 2023) followed by AVTO1422 (54.0 mm) in 2022 and AVTO1424 (52.1 mm) in 2023, respectively. The shortest fruit length was observed in Pusa Ruby (37.9 mm in 2022 and 36.9 mm in 2023). The mean fruit length was the longest for AVTO9802 (55.6) followed by AVTO1429 (52.9 mm) and AVTO1422 (52.7 mm) whereas it was the shortest for Pusa Ruby (37.4 mm). The variation on fruit length of different genotypes might be associated with the genetic make-up of the genotypes (Jogi *et al.*, 2023).

3.6 Fruit diameter (mm)

This parameter was observed to evaluate the relation of fruit size to total yield of different tomato genotypes. The difference among the genotypes on fruit diameter (mm) was highly significant (Table 3). AVTO1429 had the longest diameter (66.7 mm in 2022 and 66.8 mm in 2023) followed by AVTO1314 (63.9 mm during both years). AVTO1350 had the shortest diameter (34.4 mm in 2022 and 34.5 mm in 2023). The mean value of fruit diameter was longest for AVTO1429 (66.8 mm) followed by AVTO1314 (63.9 mm) and AVTO1422 (53.3 mm) whereas it was the shortest for AVTO1350 (34.5 mm). The differences expressed among tomato genotypes with respect to fruit diameter might be as a consequence of a combination of factors including plant health, fruit shape (spherical, elongated, flat or pear-like) and ability of plant to absorb and utilize existing moisture (water) and nutrients (Kanneh *et al.*, 2017).

Table 3. Performance of tomato genotypes on fruit length and fruit diameter at Khumaltar, Lalitpur during 2022 and 2023.

Genotypes	Fruit length (mm)			Fruit diameter (mm)		
	2022	**2023**	**Mean**	**2022**	**2023**	**Mean**
AVTO0102	41.3[b]	40.7[c]	41.0[c]	45.1[b]	45.2[b]	45.2[b]
AVTO0922	46.0[c]	44.7[d]	45.4[d]	51.4[de]	51.5[de]	51.5[ef]
AVTO1219	51.5[d]	50.7[e]	51.1[e]	48.9[bcd]	48.9[bcd]	48.9[cd]
AVTO1314	52.7[de]	52.0[f]	52.3[ef]	63.9[f]	63.9[f]	63.9[g]
AVTO1350	40.2[b]	39.0[b]	39.6[b]	34.4[a]	34.5[a]	34.5[a]
AVTO1418	47.4[c]	44.7[d]	46.0[d]	51.2[de]	51.2[de]	51.2[ef]
AVTO1422	54.0[ef]	51.3[ef]	52.7[f]	53.3[e]	53.3[e]	53.3[f]
AVTO1424	51.6[d]	52.1[f]	51.9[ef]	50.6[cde]	50.7[cde]	50.7[de]
AVTO1429	53.8[e]	51.9[f]	52.9[f]	66.7[f]	66.8[f]	66.8[h]
AVTO9802	55.8[f]	55.3[g]	55.6[g]	46.9[bc]	46.9[bc]	46.9[bc]
Pusa Ruby (check)	37.9[a]	36.9[a]	37.4[a]	46.7[bc]	46.8[bc]	46.8[bc]
Mean	**48.4**	**47.2**	**47.8**	**50.9**	**50.9**	**50.9**
F test	**	**	**	**	**	**
LSD	**1.80**	**0.89**	**1.29**	**3.75**	**3.75**	**2.22**
CV	**2.2**	**1.1**	**2.3**	**4.3**	**4.3**	**3.8**

*Note: LSD = Least significant difference, CV = Coefficient of variance, *, ** and *** Significant at 0.05, 0.01 and <0.01 levels, respectively. In column figures with same small letter(s) do not differ significantly by DMRT at 0.05 level*

3.7 Fruit weight (g)

Fruit weight is one of the most important characteristics which determine the farmer's preference in tomato varieties. The difference among the genotypes on fruit weight (g) was highly significant (Table 4). The fruit weight was highest for AVTO1429 (137.3 g in 2022 and 132.9 g in 2023) followed by AVTO1314 (116.1 g in 2022 and 112.9 g in 2023). It was lowest for AVTO1350 (26.6 g in 2022 and 27.0 g in 2023). The mean value of fruit weight was the highest for AVTO1429 (135.1 g) followed by AVTO1314 (114.5 g) and AVTO1422 (74.3 g) whereas it was lowest for AVTO1350 (26.8 g). The variation among genotypes for individual fruit weight might be associated with heritability of the trait for the genotype as well as number of locule inside the fruit. According to Kanneh *et al.,* (2017) fruits with fewer locule numbers have smaller fruit size and those having more locules gave more fruit weight.

3.8 Number of fruits per plant

The difference among the genotypes on number of fruits per plant was highly significant (Table 4). The number of fruits per plant was the highest in AVTO1350 (79 in 2022 and 81 in 2023) followed by AVTO0102 (71 in 2022 and 73 in 2023). The number of fruits per cluster was lowest for AVTO1429 (30 in 2022 and 32 in 2023). The mean value of number of fruits per plant was the highest for AVTO1350 (80) followed by AVTO0102 (72) and Pusa Ruby (63) whereas it was the lowest for AVTO1429 (31). Number of fruits per plant is one of the most important yield attributing characteristics. The differences of tomato genotypes on number of fruits per plant could be ascribed to number of flower sets that developed into marketable fruits and retained by plants (Shrestha and Sah, 2014).

Table 4. Performance of tomato genotypes on fruit weight and number of fruits per plant at Khumaltar, Lalitpur during 2022 and 2023.

Genotypes	Fruit weight (g)			Number of fruits per plant		
	2022	2023	Mean	2022	2023	Mean
AVTO0102	46.3^{b}	47.2^{b}	46.8^{b}	71^{e}	73^{e}	72^{e}
AVTO0922	66.9^{c}	68.3^{c}	67.6^{c}	44^{c}	45^{c}	44^{c}
AVTO1219	73.9^{d}	75.5^{e}	74.7^{e}	60^{d}	61^{d}	61^{d}
AVTO1314	116.1^{f}	112.9^{f}	114.5^{f}	37^{b}	36^{b}	37^{b}
AVTO1350	26.6^{a}	27.0^{a}	26.8^{a}	79^{f}	81^{f}	80^{f}
AVTO1418	69.6^{cd}	71.0^{cd}	70.3^{cd}	45^{c}	47^{c}	46^{c}
AVTO1422	75.8^{d}	72.8^{de}	74.3^{e}	46^{c}	46^{c}	46^{c}
AVTO1424	74.4^{d}	72.0^{de}	73.2^{de}	37^{b}	37^{b}	37^{b}
AVTO1429	137.3^{f}	132.9^{g}	135.1^{g}	30^{a}	32^{a}	31^{a}
AVTO9802	70.7^{cd}	69.3^{cd}	70.0^{cd}	59^{d}	61^{d}	60^{d}
Pusa Ruby (check)	48.8^{b}	47.4^{b}	48.1^{b}	62^{d}	64^{d}	63^{d}
Mean	**73.3**	**72.3**	**72.9**	**52**	**53**	**52**
F test	**	**	**	**	**	**
LSD	**5.97**	**3.45**	**3.24**	**5.76**	**3.42**	**2.975**
CV	**4.8**	**2.8**	**3.8**	**6.5**	**3.8**	**4.9**

*Note: LSD = Least significant difference, CV = Coefficient of variance, *, ** and *** Significant at 0.05, 0.01 and <0.01 levels, respectively. In column figures with same small letter(s) do not differ significantly by DMRT at 0.05 level*

3.9 Fruit yield per plant (kg)

Yield is the major character that any genotypes are selected for as it determines the income generating capacity and the scale of commercialization. The difference among the genotypes on fruit yield per pant was highly significant (Table 5). The highest fruit yield per plant was obtained from AVTO1219 and AVTO9802 (3.4 kg) followed by AVTO1314 (3.1 kg) in 2022 whereas from AVTO9802 (3.4 kg) followed by AVTO1219 and AVTO1314 (3.2 kg) in 2023. The lowest yield per plant was obtained from AVTO1350 (1.5 kg in 2022 and 1.6 kg in 2023). The mean fruit yield per plant was the highest for AVTO9802 (3.4 g) followed by AVTO1219 (3.3 kg) and AVTO1314 (3.2 kg) whereas it was the lowest for AVTO1350 (1.6 kg). According to Bhattarai *et al.*, (2005) and HRD (2019), the fruit yield of tomato might be varying due to the varietal diversity as well as growing condition. Higher yield per plant in genotype AVTO9802 is due to higher number of fruits per plant and higher individual fruit weight (Table 5).

3.10 Fruit yield (t/ha)

The difference among the genotypes on fruit yield (t/ha) was highly significant (Table 5). The highest fruit yield was obtained from AVTO1429 (66.2 t/ha) followed by AVTO9802 (50.6 t/ha) in 2022 whereas from AVTO9802 (57.0 t/ha) followed by AVTO1219 (53.3 t/ha) in 2023. The lowest yield was obtained from AVTO1350 (26.5 t/ha in 2022 and 23.4 t/ha in 2023). The mean fruit yield was the highest for AVTO1429 (59.1 t/ha) followed by AVTO9802 (53.8 t/ha) and AVTO1219 (51.3 t/ha) whereas it was the lowest for AVTO1350 (23.4 t/ha). Yield is a complex trait governed by a number of factors like genotype, environment and crop management. The significantly highest yield of AVTO1429 was the results of cumulative effect of yield attributing characters especially fruit size (Gotame *et al.*, 2021).

Table 5. Performance of tomato genotypes on yield per plant and fruit yield at Khumaltar, Lalitpur during 2022 and 2023.

Genotypes	Fruit yield per plant (kg)			Fruit yield (t/ha)		
	2022	**2023**	**Mean**	**2022**	**2023**	**Mean**
AVTO0102	2.3^{b}	2.4^{cd}	2.4^{cd}	46.2^{b}	40.3^{cd}	43.2^{bcd}
AVTO0922	2.2^{b}	2.3^{bc}	2.3^{bc}	44.5^{b}	38.0^{bc}	41.3^{bc}
AVTO1219	3.4^{c}	3.2^{e}	3.3^{fg}	49.3^{bc}	53.3^{e}	51.3^{cde}
AVTO1314	3.1^{c}	3.2^{e}	3.2^{ef}	34.6^{ab}	52.8^{e}	43.7^{bcd}
AVTO1350	1.5^{a}	1.6^{a}	1.6^{a}	20.3^{a}	26.5^{a}	23.4^{a}
AVTO1418	2.3^{b}	2.5^{d}	2.4^{cd}	42.0^{b}	41.1^{d}	41.6^{bc}
AVTO1422	2.5^{b}	2.6^{d}	2.5^{d}	40.0^{ab}	42.8^{d}	41.4^{bc}
AVTO1424	2.0^{b}	2.1^{b}	2.1^{b}	34.3^{ab}	35.2^{b}	34.7^{b}
AVTO1429	3.0^{c}	3.1^{e}	3.1^{e}	66.2^{c}	52.0^{e}	59.1^{e}
AVTO9802	3.4^{c}	3.4^{f}	3.4^{g}	50.6^{bc}	57.0^{f}	53.8^{de}
Pusa Ruby (check)	2.1^{b}	2.3^{bc}	2.2^{bc}	35.8^{ab}	37.8^{bc}	36.8^{b}
Mean	**2.6**	**2.6**	**2.6**	**42.2**	**43.3**	**42.8**
F test	**	**	**	**	**	**
LSD	**0.42**	**0.18**	**0.10**	**18.32**	**2.99**	**9.90**
CV	**9.5**	**4.0**	**6.7**	**13.4**	**4.0**	**17.9**

*Note: LSD = Least significant difference, CV = Coefficient of variance, *, ** and *** Significant at 0.05, 0.01 and <0.01 levels, respectively. In column figures with same small letter(s) do not differ significantly by DMRT at 0.05 level*

4. CONCLUSION

In the present study the genotype AVTO9802 and AVTO1219 were found superior in terms of overall quality traits and yield. However, further verification in multi-location and farmer's field is necessary before commercial recommendation.

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A REVIEW ON YAK AND ITS HYBRIDS FARMING PRACTICE IN NEPAL

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ABSTRACT

The study covers the present situation of yak farming practice in Nepal as well as its future scope and opportunities. Yak / Chauri is generally reared in an altitude range of 2000-6000 meters above sea level, mostly in the Himalayas region as it requires little food and is highly insensitive to cold temperatures. Yak is seasonal breeders; the breeding season is especially between July to September with a gestation period between 257-270 days and a single calf born between May and June. Yak farming is not commercialized, and its population has been decreasing in recent decades. But there is great potential for yak farming due to the good market price of yak milk, and byproducts. In addition, yak can support directly in the transportation and tourism sectors. To improve the productivity and commercialization of yak farming in Nepal, the government should establish pasture banks, research centers, veterinary services, and industries for milk and byproducts, and economic support to the farmers of the Himalayan region.

1. INTRODUCTION

Yak, *Bos grunnieus* (Artiodactyla: Bovidae) is the bovine large, robust, and hardy ruminant animal that can thrive in an extremely cold climate (-50°C) and at an altitude ranging from 2000 to 6000 meters above sea level(MoALD, Stastical Information on Nepalese Agriculture, 2022). Yak is commonly found in high hills of Himalayan districts of Nepal such as Solukhambu, Dolakha, Mugu, Taplejung, Rasuwa, Manang, Mustang, and Dolpa, etc. Yak serves as a multipurpose and semi-domesticated animal in the Himalayas(CBS, 2004). They are vital sources of milk, hide, hair, energy, and transportation while playing a major role in maintaining the high rangeland ecosystem and conserving agro-biodiversity(Degen, et al., 2007). Moreover, yak is integral to cultural traditions, livelihood strategies, and all aspects of socioeconomic development in the high mountain areas(Joshi, et al., 2020).

The origin and evolution of yak are believed to be traced back to cattle from one to five million years ago, with recent studies suggesting a closer relation to bison than another genus according to apparent close fossil relatives of the yak(Pande, 1996). Initially named *Bos grannies* by Linnaeus in 1766, domestic yak is now referred to as *Bos grannies*, while wild yak is designated as *Bos mutus*(MoALD, 2017).

Yak and nak, their domestic counterparts, are large livestock adapted to higher altitudes, characterized by sturdy legs, bulky frames, rounded, cloven hooves, and dense, long fur that hangs lower than the belly. While wild yak typically exhibits dark coloration, ranging from black to brown, domestic yak displays a wide range of colors, often with patches of rusty brown and cream. Yak possesses distinct features such as small ears, wide foreheads, and smooth horns, with males sporting longer, forward-curving horns compared to females.

Both sexes have a short neck with a pronounced shoulder hump, more prominent in males. Yak weighs approximately 350 to 585 kg, while nak weighs about 225 to 255 kg, with wild males reaching weights of up to 1,000 kilograms. Domestic yak males stand at 1.11–1.38 meters at the withers, while females are 1.05–1.17 meters tall (Neupane *et al.*, 2001).

To protect against the cold, yak have long, shaggy hair with a dense wooly undercoat, particularly over the chest, thighs, and flanks. Male yak may have hair forming a long skirt reaching the ground. The tail resembles that of a horse, and due to genetic variation and crossbreeding, domestic yak exhibits a variety of coat colors. Notably, both male and female yak have long hairs on their udders and scrotums for cold protection. Yak produces lowing sounds similar to cattle, both domestic and wild. This distinctive behavior inspired the scientific name of the domestic yak variant, Bos grunniens (grunting bull)(Pandeya & Niroula, 2019).

2. METHODOLOGY

For this review, information was collected from surveys and various secondary sources including social media, newspapers, journals, reports, and internet sources.

DISTRIBUTION OF YAK IN NEPAL

Globally recognized as essential for habitation in high altitudes, yak is a common sight in Nepal, particularly at elevations around 2000 meters, where they thrive due to their minimal food requirements and remarkable tolerance to cold temperatures.(Alirol, 1976). Yak population in different districts of Nepal is shown in Table 1.

Table 1. Yak and hybrids population according to districts in Nepal

District	Yak/Chauri(No.)
Bhojpur	452
Ilam	30
Khotang	175
Okhaldhunga	705
Panchthar	1050
Sankhuwasabha	2758
Solukhumbu	8624
Taplejung	3802
Dhading	309
Dolakha	5350
Nuwakot	20
Ramechhap	1215
Rasuwa	3605
Sindhupalchowk	3908
Gorkha	3272
Kasko	386
Lamjung	100
Manang	2319
Mustang	3825
Myagdi	315
Dolpa	7579
Humla	8205
Jumla	951
Kalokot	75
Mugu	2607
Bajura	99
Bajhang	175
Darchula	650
Total (National)	62,561

Source; MoALD, Stastical Information on Nepalese Agriculture, 2022

BODY CHARACTERISTICS

Yak, with their larger lungs and hearts compared to cattle, possess an advanced capacity to transport oxygen throughout their bodies, aided by the presence of fetal hemoglobin throughout their lives. However, at lower altitudes, yak struggle and are prone to heat exhaustion above 15 degrees Celsius due to their thick subcutaneous fat layer and the absence of functional sweat glands (Long & Long, 1992). Despite consuming only 1% of their body weight in feed, yak efficiently converts low-quality food into nutrients through fermentation in their large rumen. They are seasonal breeders, with a breeding season typically from July to September. During this time, bulls exhibit aggressive behavior to establish dominance and mate.

YAK AND ITS CROSSES' FARMING IN NEPAL

In Nepal, yak and nak (female yak) are referred to as pure Himalayan cattle breeds and are predominantly reared above 3000m in 28 Himalayan districts. While the number of yak/nak and chauri has increased to more than 62000 in the past few years, the number of pure breeds is decreasing. Chauris, produced by crossing yak and local hill cows, are more productive and adaptive to lower altitudes, serving as a significant source of household income in the Himalayan regions (Sherchand, 1996). These hybrids thrive in the altitude range of 2000-5000 meters, bridging the gap between cattle and yak (Joshi, 1982; Robinson, 1992; Miller, 1993). The Chauri are more productive than female yak more adaptive to lower altitudes and are reared at the intermediate zone between cattle and yak. Chauri farming is a main source of households' income in the Himalayan regions (Sherchand, 1996).

The female Chauri are more productive than Nak. The hybrids are more adaptive to lower temperatures and are reared at the intermediate zone between cattle and Yak at an altitude between 2000-5000 meters (Joshi, 1982; Robinson 1992; Miller 1993). The population of the pure Yak/Nak is rapidly declining. It is estimated that there are more than 62000 yaks and Chauri in Nepal (MoALD, Stastical Information on Nepalese Agriculture, 2022).
Table 2 shows that the chauri exhibits greater milk production and gives more benefits in comparison to nak. Additionally, chauris reach maturity in a shorter time frame, and the interval between calving periods is also shorter compared to nak (Degan et al., 2001).

Table 2. Performance comparison of Nak and Chauri

S.N.	Characters	Nak	Chauri
1.	First calving age	48 months	36 months
2.	Milk yield	470 liters/ lactation	960 liters/ lactation
3.	Calving interval	687 days	425 days
4.	Lactation length	174 days	254-400 days

Dzo, which is a hybrid of yak and cattle, is similar to a mule in that the male hybrid is infertile, while the female hybrid, known as dzomo, is fertile. This fertility allows for the breeding of three-quarter mixes. A dzo is larger and stronger than both yak and cattle in the region, making them excellent pack animals for transporting equipment to the base of Mount Everest (Kharel, 2000).

The crossbreed of yak and hill cow, known as Urang chauri, and the crossbreed of nak (female yak) with a hill or Tibetan bull, called Dimjo chauri, are common. Male chauris, known as Jhopkyos, are sterile. Female yak and chauris are utilized for milk production, while yak and male Jhopkyos serve as pack animals (Neopane S. P., 2001).

Table 3 revealed 6,235 yak holdings with a total of 48,865 yak heads (MoALD, 2017). Provinces Madesh and Lumbini reported zero numbers of yak due to geographical and climatic conditions unsuitable for yak farming. This underscores the significance of favorable geographical and climatic conditions for yak farming, both in Nepal and worldwide

Table 3. Number of heads reporting Yak/Nak/Chauri by Provinces of Nepal (MoALD, 2021/22)

S.N.	Province	Yak/Nak/Chauri
1.	Koshi	17,596
2.	Madesh	0
3.	Bagmati	14,407
4.	Gandaki	10,212
5.	Lumbini	0
6.	Karnali	19,417
7.	Sudurpaschim	924
	Total	62,561

Table 4. shows that there is an increase in several yaks between 1999 A.D. to 2002 A.D. in Nepal. The upward trend persisted until 2002 A.D., after which there was a significant decline in the total yak population in Nepal.

Table 4. Total heads of yak/nak/chauri between 1999-2021

S.N.	Year	Total heads of yak/nak/chauri
1	1999	56488
2	2002	95447
3	2010	68097
4	2013	65661
5	2017	48865
6	2021	62561

HOUSING AND GRAZING PRACTICES IN NEPAL

Yak farming in Nepal is predominantly non-commercial, characterized by poor and unmanaged housing systems. Individuals engage in yak farming primarily as a supplementary source of income to meet both direct and indirect family needs. Hand milking is the traditional method employed. However, in districts like Dolpa and Rasuwa, there is a growing trend towards chauri farming due to increased demand for products like chhurpi and cheese, accompanied by the resumption of cheese factories by DDC. This gradual commercialization has led to an increase in household per animal ratio, encouraging the construction of better farmhouses and the adoption of modern farming tools and technologies. Conversely, in other districts, yak farming is on the decline due to outmigration and decreasing interest among the youth in agriculture (Paudel, 2016).

In Nepal, transhumance migration is widely practiced, with yak/chauri moved to lower altitudes (up to 2000m) for grazing during the winter and higher altitudes (up to 5000m) in the summer. Temporary sheds are assembled to accommodate the animals at different stages of migration (Shrestha B. S., 1996).

Common forage and fodder for yak include buki, alpine grass, ryegrass, white clover, oak, gojeng, kamlya, nigalo, pasi, tiure, bhena, bekhar, Rambo, khar, dhadebuki, singkshe, benakhi, and others. While in Manang and Mustang, free grazing is available throughout the year, in other districts, yak and chauris are grazed for 8-11 hours a day and then taken to sheds for rest, feeding, and water. Milking animals are provided with additional feeding, including concentrated feed supplemented with iodine salt, minerals, and vitamins (Shrestha, 1994).

BREEDING STRATEGIES

Yak undergoes a reproductive cycle known as the estrus cycle, consisting of proestrus, estrus, metestrus, and diestrus stages, completing the cycle in 21 days (Sen, 1992). Gestation ranges between 257 and 270 days, with calves typically born between May and June (Dong, 2009). Calves can walk within minutes of birth and are weaned at one year old. Females generally give birth for the first time at three or four years of age and reach peak reproductive fitness around six years (Miller, 1987).

Since ancient times, Nepal has engaged in the crossbreeding of purebred yak with cattle, resulting in enhanced performance in meat and milk production, as well as greater resilience to high altitudes. Typically, smaller humpless cattle, known as Tibetan cattle (Bos taurus), are preferred for crossbreeding at higher altitudes, while larger hump cattle, referred to as Zebu cattle (Bos indicus), are favored at middle altitudes. When pure yak bulls are crossed with female Zebu cattle, hybrids known as urang are produced, while hybrids called dimjo are the result of crossing pure nak with male Tibetan cattle. Although female hybrids retain reproductive abilities, male hybrids are sterile. The practice of backcrossing is not widely understood and is primarily carried out at the local level (FAO/RAPA, 1994).

Table 5. Comparison of Yak, cattle, dimjo, and rang (FAO/RAPA, 1994)

Items	Yak	Cattle	Dimjo	Urang
Altitude range(m)	>3000	<3000	2300-4900	2000-4300
Male weight(kg)	300	300	365	360
Female weight(kg)	225	210	235	230
Life span(yrs)	15	15	20	20
First heat(yrs)	3-4	2-3	2.5-3	3-4
First calving(months)	44-56	34-40	39-45	44-56
Gestation period (days)	260	300	270	270
Lactation length(months)	6	6-8	7-8	7-8
Annual milk yield(kg)	720	500-1000	1700	1300
Birth weight (kg)	12-15	18-20	12-17	15-19
Calving interval(months)	15-18	12-14	12-15	12-15

3. DISCUSSION

Yak is primarily concentrated in the high hilly and Himalayan regions of Nepal, where the climatic conditions are conducive to farming. They serve as a significant source of income in these mountainous areas, primarily utilized for milk and meat production as well as transportation purposes. Their farming, despite its economic importance, remains largely non-commercialized in Nepal, with traditional farming methods still dominant while livestock farming in the Terai regions, such as poultry, goats, cattle, and buffaloes, is undergoing commercialization, yak farming in the Himalayan areas still lacks the adoption of modern skills, tools, and technologies. The market prices for yak milk, meat, and byproducts are relatively high, yet the total population of yak and chauris (female yak) is declining in Nepal due to reduced interest among youths. Migration from the Himalayan region to the Terai region is contributing to this trend, shifting people's occupation away from yak farming to other businesses. Transhumance grazing is practiced, involving seasonal movements of yak to different zones based on environmental conditions and feed availability. However, there is a lack of routine and proper feed supply, particularly for lactating yak, which may hinder productivity. Disease prevalence poses a significant challenge in yak farming, exacerbated by limited knowledge among farmers regarding disease prevention and treatment, inadequate access to veterinarians, and a shortage of effective medicines. Moreover, the free-grazing nature of yak increases the risk of disease transmission during grazing and migration. Efforts to improve yak farming productivity are limited, with minimal research conducted at the government level. While some projects, like HIMALI, have been initiated, more extensive research, particularly at the genetic and breeding levels, is needed to enhance productivity.

4. CONCLUSION

The recent government survey in Nepal reveals a concerning decline in the total yak population over the past two decades. Yak farming in Nepal remains primarily traditional, with limited commercialization, often serving as a secondary income source or fulfilling basic family needs in high Himalayan areas. Challenges such as water scarcity, inadequate veterinary services, waning interest among youth, farmer awareness issues, wildlife predation, and heavy snowfall pose significant obstacles to yak/chauri farming in Nepal.

Despite these challenges, various ethnic groups and communities in high Himalayan areas have long depended on yak rearing, breeding, and farming for daily economic activities, livelihoods, and even tourism. Male yak is particularly crucial as transportation means in high-altitude regions where other alternatives are scarce. Therefore, there is an urgent need to enhance yak/chauri production, conserve genetic diversity, maintain traditional breed selection systems, and improve the livelihoods of yak herders.

In Nepal, farmer awareness about proper farm management practices is essential for successful yak farming. Access to veterinary services for regular health check-ups is crucial for productivity and disease control. The establishment of a pasture bank, the production of nutritious fodder and grasses, and ensuring regular water supply by local governmental bodies are vital for supporting yak farming. Furthermore, the establishment of milk chilling centres and byproduct production industries in various areas can modernize traditional yak farming practices and facilitate commercialization. Regular deworming, vaccination, and the provision of nutritious diets, vitamins, and mineral supplements are necessary for improving productivity.

Introducing superior and high-productivity breeds and implementing artificial insemination technology using sperm from superior breeds can significantly enhance genetic improvement in local yak breeds and increase profits from yak farming. Establishing yak research centres under the scope of central government is imperative for conducting new research and adopting modern skills, tools, and technologies to support yak farming.

Implementing insurance policies and services accessible in Himalayan areas can mitigate business risks associated with diseases, natural disasters, and predation. Therefore, direct and indirect support from government agencies, relevant authorities, certified veterinarians, and livestock experts is crucial for the development of yak farming in Nepal.

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CONSUMPTION OF MILLETS BY THE INDIGENOUS PEOPLE (IP): A CASE STUDY IN CHINGMEI VILLAGE, TUENSANG NAGALAND, NORTHEAST INDIA

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ABSTRACT

Millets are a type of grain with high levels of micronutrients that have the potential to become an important source of nutrition and food security. These crops are becoming increasingly popular as a healthy food option and are seen as valuable genetic resources in the face of climate change. A survey was conducted in the village of Chingmei in Tuensang Nagaland, Northeast India to determine the frequency of millet consumption by indigenous people and to document the types of millet found in this area. A systematic sampling method was used, and Indigenous people from 76 households were interviewed using an interview schedule. Descriptive analysis was used to examine the results. The survey revealed that millet has always been a part of the indigenous people's diet, which they consume daily. The main reasons for daily consumption were the availability, easy accessibility, and the perception that consuming millet is associated with good health. The survey also highlighted the indigenous people's interest in learning new methods for the preparation and development of products from millets. Sorghum, Coix lacryma-jobi (job's tear), Setaria italica (foxtail millet), Panicum sumatrense (little millet), and Eleusine coracana (finger millet) were cultivated and found in the village. Given its importance, it is necessary for indigenous people to develop millet products and test their utilization and consumption. This could also be a source of livelihood for them if they can market and sell millet products.

1. INTRODUCTION

Millets are the 'Miracle Grains', they grow tall and vertically and come in a variety of shapes and sizes. The seeds have colorful hulls that are frequently difficult to digest. Their growing season is only 3 to 4 months long and requires less human intervention because of their tremendous genetic variety and self–self-fertilization (Michaelraj & Shanmugam, 2013).

They are a staple food of most people around the world because they can grow and survive in unfavorable environments and produce sustainable yields (Sharma & Ortiz, 2000).

They can be kept for over three years in storage and are regarded as a famine crop since they can be kept readily for a longer amount of time than most cereals. They are now making a strong comeback because of their superior nutrition and innate climate tolerance. Due to their benefits for human health, low agricultural input requirements, lack of irrigation infrastructure, capacity to slow down climate change, and ability to provide secure food and nutrition, they have gained popularity on a global scale (Anbukkani et al., 2018, Ravikesavan et al., 2022)

They are a staple crop in civilization and are one of the main food crops, grown in more than 100 nations worldwide, with most of their production taking place in semiarid tropical regions of developing nations like Asia and particularly in India. India is the largest producer of millets, which makes up more than 80% of global production (Prashanthi et al., 2022)

To achieve nutritional security and combat developing climatic vagaries and life-threatening diseases, it is imperative to diversify food resources by including them. Small millets are extremely beneficial to health. When compared to rice, they are superior sources of minerals, energy, dietary fiber, and protein. The tiny "grain" is free of gluten and rich in vitamins and minerals. The body needs nutrients like calcium, magnesium, manganese, phosphorus, iron, and antioxidants, which are abundant in little millet. They are valued for their inherent antioxidant properties and becoming more significant as sources of all the essential nutrients. As a result of their high nutrient content, essential fatty acid, dietary fiber, vitamin B, and minerals including potassium, magnesium, manganese, iron, and calcium, millets are referred to as "Nutri – Cereals" (Sadhu & Naidu, 2021, (*NUTRI CEREALS-IYOM 2023*, n.d.). They are inferior to rice and wheat in every way, including health benefits, they are gluten-free and non-allergenic and aid in improving the height, weight, and hemoglobin level of children (Durairaj et al., 2019).

Millets are healthy and play a significant role in people's diets. They can be cooked, milled to form porridge flour, or used to make cakes, and it is nutrient-dense, easily digestible, and versatile. They are more nutritious than other cereals, although their consumption as food is still largely limited. The high protein content of millet compensates for the lack of calories in a vegetarian diet. The only crop that will handle pressing issues like food scarcity, fuel shortage, malnutrition, health problems, and climate change in the future is millet. They are suited to a wide range of ecological circumstances, requiring less water, and inputs, and are receiving increasing interest from food scientists, technologists, and nutritionists because of their contribution to food security and potential health advantages. The United Nations (UN) General Assembly in the 75th session acknowledged the potential of millet and designated 2023 as the International Year of Millets to encourage millet production and consumption all around the world (Kohlo, 2023).

Millets are also regarded as smart food which is excellent for consumers, producers, and the environment. Since millet has so many health advantages and can grow in a range of climates, production of millet is expected to increase. They are considered smart food and promoting them will help upcoming farming generations. They can contribute to the Sustainable Development Goals (SDGs), which are among the top priorities for attaining food and nutrition security globally (Nayana & Udaykumar, 2021).

SDG 2 is 'attaining zero hunger', this goal aims to '*end hunger, achieve food security, enhance nutrition, and support sustainable agriculture'*. Consuming millet could also help achieve SDG 3 which is to *'Ensure healthy lives and promote and well-being for all at all ages'*. SDG 12 is *'Ensuring sustainable consumption and production patterns'*. Millet growing and consumption are anticipated to promote sustainable agriculture with no harm to the environment because agriculture production can result in serious ecological damage due to the indiscriminate use of fertilizers and pesticides. They can also aid in the achievement of SDG 13, which is to '*Take urgent action to combat climate change and its impact'*. There are two ways in which they help SDG 13, by reducing the adverse effects of climate change and global warming and helping boost food production. In another way, millet-based agriculture lessens the production of synthetic fertilizers and pesticides and lessens their adverse effects on the environment.

They are essential for supplying food demand and enhancing food security in the future. Similarly, increasing their production's value-added processing and cultivating them may help rural areas generate income. Foods made from them are advised for a healthy and balanced diet and to treat a variety of ailments.

Changing the food system and promoting local foods like millet are essential for achieving many SDG goals (Maharajan, 2021).

In recent years, there has been a change in eating patterns and food choices are notably a result of globalization (Patgiri, 2022). According to the World Health Organisation (WHO), poor diet and improper eating habits have been associated with unfavorable outcomes like mortality and morbidity as well as the prevalence of chronic diseases like diabetes, cardiovascular disease, and hypertension Additionally, micronutrient deficits like vitamin D, iron, and folate are common in India (Venkatesh et al., 2021).

The Indian Green Revolution in the 1960s led to a decline in millet production and consumption. The cultivation of cereals, pulses, and commercial cash crops has decreased the area and production of minor millets as well (Singh & Benbi, 2016; Nelson et al., 2019). Millets have consequently lost favor in the community and have become a poor man's diet.

This study aimed to determine the frequency of millet consumption per year by Indigenous people and document the types of millet found in Chingmei Village, Tuensang Nagaland, Northeast India

2. MATERIALS AND METHODS

Area of Study

The survey was conducted in Old Chingmei Village, Tuensang district in Nagaland, Northeast India. The village is divided into old and new Chingmei. The people who live in the village are the Indigenous people called the Chang people. According to the United Nations (UN), Indigenous people have a distinct social, economic, and social system. They have a strong relationship with their land and have distinct knowledge and beliefs, culture and knowledge. The UN declaration of the rights of the Indigenous people has not come up with a definition but people can self-identify as Indigenous people. This was their fundamental right (United Nations, n.d.). The Chang people have a distinct language, culture, knowledge, and belief system. In India, the Chang are tribal people of Northeast India.

There are 370 households in old Chingmei village as reported by the headman and the population size is 4143 as per the 2022-2023 record. This village was purposely selected because it is well known for consuming and cultivating varieties of millets. The October of every year, the people of Chingmei village celebrate the millet festival where they display varieties of millets.

Research Design

The research design used for the present study was descriptive. Descriptive research involves surveys and fact-finding inquiries that reflect the current situation (Kothari & Gaurav, 2019).

2.1. Sampling method

The sampling method was a systematic random sampling method, where every fifth house was selected for the interview. Systematic random sampling is a practical method where every i^{th} item is selected on a list (Kothari & Garg, 2019).

2.2. Sample unit and sample size

The sample unit was the Chang Indigenous people who prepared food for their households. There were 370 households in the villages and every fifth house was selected. Therefore 74 households out of 370 households were the sample size of the study.

2.3. Method of data collection

Data were collected using an interview method and the tool was an interview schedule. The schedule was developed using information acquired from literature on millets. The schedule consists of open and close-ended questions to elicit the information and it is divided into four parts:

The first section consists of questions related to the demographic profile of the respondents: the name, gender, age, occupation, and number of members in the family.

The second section consists of questions related to the socioeconomic status of the respondents such as their economic status, source of annual income, and household financial management. The third section consists of respondents' knowledge of the health benefits of millets, the types of millets that they are familiar and the reasons for eating them. The fourth section consists of questions related to millet preparation methods, the types of millet dishes they prepared at home, and the frequency of consumption of millet by the family.

2.4. Validation of the interview scheduled

Face validation of the interview scheduled was done before the survey. The interview schedule was validated by two eminent experts in the field of Nutrition. Both eminent experts have a doctorate in nutrition with more than ten years of experience in research and academics.

2.5. Data entry and analysis

Data was entered in Microsoft Excel 2016 and IBM SPSS Statistics 23. Descriptive statistics was used to describe variables such as gender, age, occupation, sources of income, frequency of consumption of millets, varieties of millets that are known, and how well-informed people are about millets

Varieties of millets in Chingmei village

Figure 1. White sorghum locally known as *Thuibaibü* and scientific name is *Sorghum*

Figure 2. Red sorghum locally known as *Saklangbü aloto* and its scientific name is *Sorghum*

Figure 3. Non-sticky job’s tear is locally known as *Nyi akhanbü* and its scientific name is *Coix lacryma-jobi (Adlay)*

Figure 4. Sticky job’s tear is locally known as *Nyi khangbü* and its scientific name is *Coix lacryma-jobi (Adlay)*

Figure 5. Non-sticky foxtail millet is locally known as *Jei akhanbü* and its scientific name is *Setaria italica*

Figure 6. Sticky foxtail millet is locally known as *Jei khanbü* and its scientific name is *Setaria italic*

Figure 7. Little millet locally known as *Luo* and scientific name is *Panicum sumatrense*

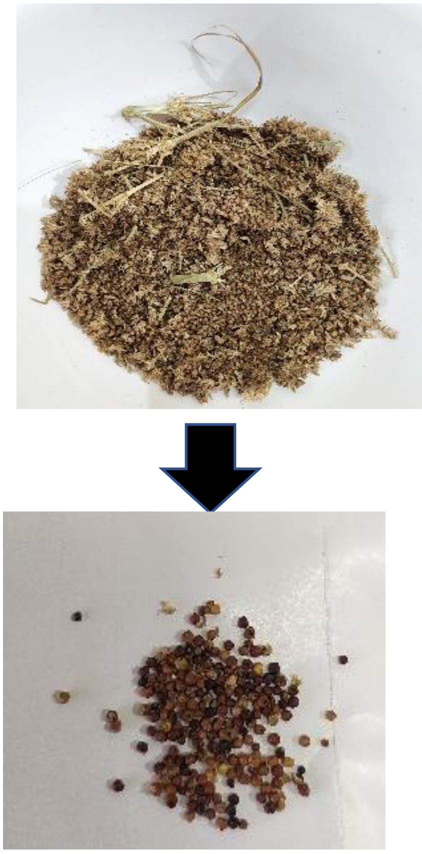

Figure 8. Finger millet locally known as *Launyak* and scientific name is *Eleusine coracana*

Figure 9. The traditional method of storing foxtail millet by the Chang Indigenous people in Chingmei village by tying and hanging the foxtail millet

3. RESULTS AND DISCUSSION

3.1 Demographic profile and socioeconomic status

Socioeconomic conditions are considered to have a significant impact on access to food and nutrition (Selvi, 2013). The demographic profile and socioeconomic information related to gender, age, occupation, source of income, and number of family members were clarified by the respondents using the interview schedule, and the results are provided in Table 1.

Table 1. Demographic profile and socioeconomic status

Demographic	Classification	Frequency (n)	Percentage (%)
Gender	Male	17	23
	Female	57	77
Age (WHO classification)	20-24	1	1
	25-29	3	4
	30-34	9	12
	35-39	7	9
	40-44	18	24
	45-49	10	14
	50 and above	26	35
Occupation	Farmer	55	74
	Wage laborer	15	20
	Skilled worker	3	4
	Govt. service	1	1
Source of income	Agriculture	49	66
	Dairy products	0	0
	Livestock	6	8
	Skill profession	19	26
Number of family members	<Two	2	3
	Three – five	30	41
	>Six	42	57

It could be seen that a large number of the respondents were female (77%) and slightly less than a quarter of the respondents were male (23%). Just over a third of the respondents were in the age group of 50 and above (35%), one-quarter of the respondents were between the age group 40-44 years (24%), less than a fifth were between 45-49 and 30-34 years (14% and 12%), a small number of the respondents were between 35-39 years (9%) and an insignificant number of the respondents were between the age groups 25-29 and 20-24 years (4% and 1%).

The occupations of family members have a significant impact on the family's food consumption habits and consequently, their health and nutritional status. A large number of the respondents were farmers (74%), a fifth were wage workers (20%), and an insignificant number of the respondents were skilled workers and government employees (4% and 1%).

The families' source of income and earnings are a significant indicator of their ability to manage their household resources. Access to income and stable employment are necessary for food access. Yearly income has a significant impact on the economic situation, which in turn affects household food insecurity. In this study, it was found that just over a fifth of the respondents' sources of income were labor jobs, carpentry, and government service (26%), a small number were from livestock sales (8%) and a large number of respondents' income were from agriculture (66%). According to the respondents, farmers get more profit by exporting their goods outside the other states and this made farmers more prosperous and well-off.

Regarding the household size, approximately half of the respondents consisted of six or more members (57%), more than two-fifths of the respondents consisted of three to five members (41%) and an insignificant number of the respondents had two or fewer members (3%).

3.2. Consumption of millet by Indigenous People

In Figure 10, all of the respondents were familiar with foxtail millet, and sorghum, and almost all were familiar with the job's tear. A large proportion of respondents were familiar with little millet (66%), and approximately half of respondents were familiar with the proso millet (54%). An insignificant percentage of the respondents were familiar with other varieties of millet such as barnyard (4%), finger millet (3%), and pearl millet (1%). Globally, the production of sorghum and pearl millets together is 92.6% (Prashanthi & Geetha Reddy, 2023). In India, sorghum and pearl millets are extensively cultivated (Bhat et al., 2023) but the country is the largest producer of other millet such as finger millets, kodo millet, and barnyard millet (Prashanthi & Geetha Reddy, 2023).

Just over a third of the respondents consumed millet every day (32%), just over a fifth of the respondents consumed weekly (28%), less than a fifth consumed monthly (15%), and one-quarter of the respondents rarely (24%) consumed millet. According to the study, there was not a single respondent in the village who did not consume millet (Figure 11).

In Figure 13, All respondents make traditional millet cake, and just under a third of the respondents make beverages, just under a fifth make *suji* (16%), *suji* is a famous Indian dish using semolina however, indigenous people in Chingmei substitutes semolina with millet. A small number of the respondents make porridge and roti (8% and 5%), and an insignificant number of the respondents make desserts (3%). Around the world, millets are prepared into porridge, baked products, and flatbread while in India, millets are made into traditional food (Tripathi & Vyas, 2023)

Even though there are a variety of millet products that the respondents made, a significant proportion of the respondents feel that they lack the expertise to prepare some other millet-based products and they express the need for a training program on the preparation methods of millet-based products. An interest in learning the development of new products from millets that are ready-to-eat and ready–to–serve was also reported by Priya et al., (2024) in their study in South India

More than two-fifths of the respondents eat millet because it is cultivated and is easily available at home (42%), just over a third of the respondents eat millet for health reasons (35%) and slightly less than a quarter eat it because it was healthy and easily available (23%). Similarly, this was seen in a study by Kane-Potaka et al., (2021) where people prefer eating millet for health reasons. Either because of disease conditions or because of losing weight.

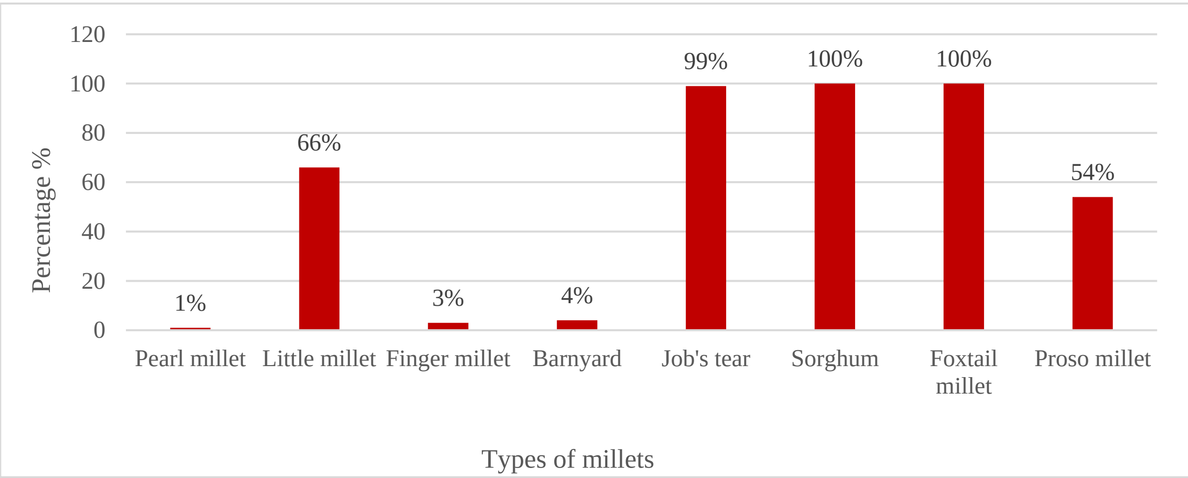

Figure 10. Types of millets known by the respondents

Four-fifths of the respondents were aware (80%) of the health benefits of millet while a fifth of the respondents were unaware (20%). From those who were aware, most of them knew that millets are good for one's health but only a few of them could describe the health benefits. These health benefits include the prevention of heart disease and controlling the blood sugar level of diabetes. All respondents had no idea about the nutritional benefits of millet.

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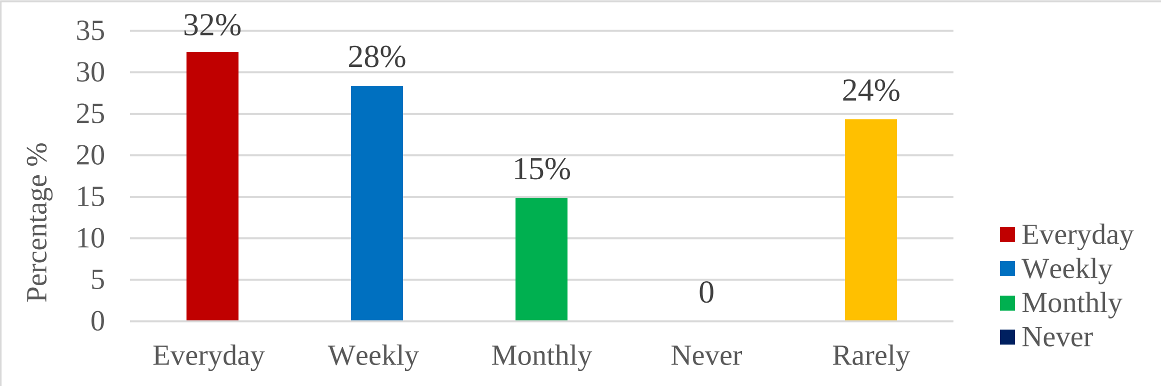

Figure 11. Frequency of consumption of millet

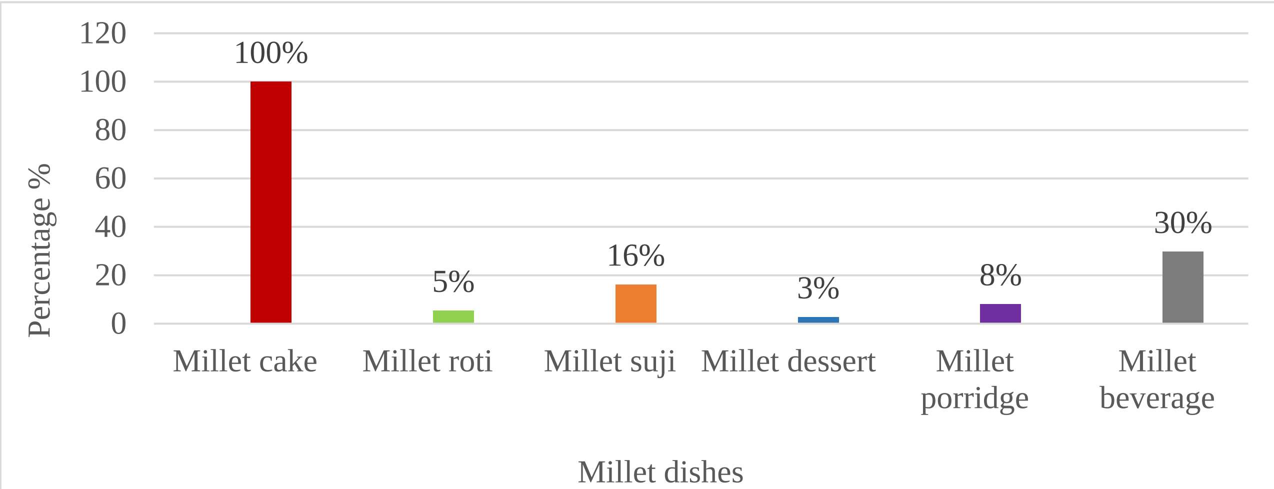

Figure 12. Millet-based dishes prepared by the Indigenous People

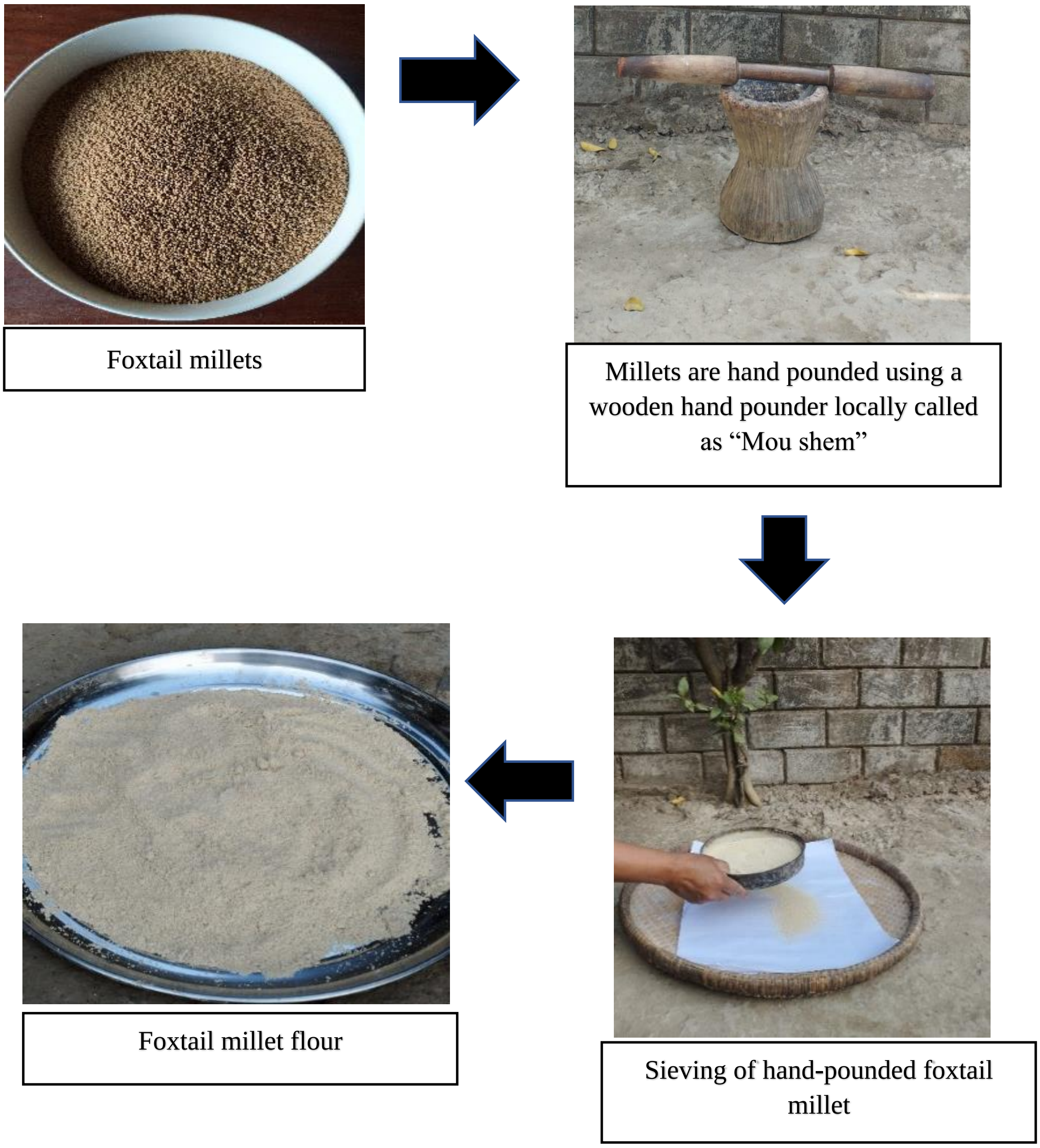

Figure 13. The traditional method of processing foxtail millet flour by the Chang Indigenous People.

4. CONCLUSION

The consumption of millet is common among the indigenous people in Chingmei village. Various types of millets are grown, including sorghum, job’s tears, foxtail millet, little millet, and finger millet. These millets are essential in the Indigenous diet and serve as a source of livelihood for the people. The Chang Indigenous people are most familiar with foxtail millet, sorghum, and job’s tears, locally known as *jei*, *aloto,* and *nyi*, which are widely used as staple foods in the area.

Millets are consumed because they are believed to have health benefits, particularly in controlling diabetes and preventing heart diseases. These grains are easily accessible, allowing Indigenous people to incorporate them into their diets.
They are eager to learn new methods for producing millet products, which could become an additional source of livelihood for

them if they can market such products. Moreover, there is a need for training programs on product development using millets and for raising awareness about the nutritional content of millets.

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FACTORS INFLUENCING THE ADOPTION OF CLIMATE SMART AGRICULTURAL PRACTICES IN SUNDARBAZAR, LAMJUNG

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ABSTRACT

This study focuses on discovering the factors that are responsible for the adoption of climate-smart agriculture practices in Sundarbazar Municipality of Lamjung district. A household survey was conducted to collect primary information through an interview schedule. A sample size of 100 farmers was taken using simple random sampling. An ordered logistic regression model was used to analyze the data. Sixty-eight percent of the farmers in the study area are using climate-smart agricultural practices for vegetable production. Major climate smart practices in the study area are vegetable cultivation inside the polyhouse, drip irrigation, mulching, IPM, improved varieties, and sprinkler irrigation system. The ordered logistic regression model shows factors influencing CSA adoption among various categories of adopters i.e., non-adopters, low, medium, and high adopters. Area of cultivation, subsidies obtained from organizations, age of household head, and number of active family members are determining factors for the level of adoption of CSA. Being a greater environmental advantage of adoption of climate smart agriculture, government should focus on increasing the climate smart agriculture practices throughout the agrarian communities of Nepal.

1. INTRODUCTION

Global temperature has been increasing. Annual temperature has increased at an average rate of 0.18°C per decade since 1981. The increasing rate was only 0.08°C per decade from 1880 to 1980. The year 2021 was the sixth warmest year on the globe. The nine years from 2013 through 2021 rank among the 10 warmest years on record. Parts of Northern Africa, Southern Asia, and Southern South America, as well as parts of the Atlantic and Pacific Oceans, felt record-high annual temperatures in 2021 (NOAA, 2022). IPCC (2022) reported that total net anthropogenic greenhouse gas emissions have continued to rise from 2010 -2019, as have cumulative net CO_2 emissions since 1850. Average annual greenhouse gas emissions during 2010-2019 were higher than in any previous decade. Nepal is vulnerable to the effects of climate change as majority of the people are dependent on agriculture. Nepal ranked fourth among the countries most affected by climate change in 2017 according to the Global Climate Risk Index (2019). A recent study by DHM Nepal (DHM, 2017) on climate trend analysis for 1975- 2014 showed a significant positive trend in annual maximum temperature data at the rate of 0.056 ℃/ year. This rate is remarkably high compared to the global scenario, where the rate is 0.18℃ per decade. This shows that Nepal is more vulnerable to the effects of climate change. Drought, floods, and landslides are increasing in Nepal. These climatic hazards account for approximately one hundred deaths annually and influence drinking water and agriculture patterns (Sudmeier-Rieux et al., 2012). Climatic hazards like floods, landslides, hailstorms, forest fires, heat waves, and cold waves are significantly increasing across the country (MoFE, 2021). The report also pointed out the increase in contextual droughts in specific regions and districts of the country. Changes in precipitation patterns increase the likelihood of short run crop failures and long run production declines (IFPRI, 2009). Bhusal (2019) discussed the loss of productive lands due to floods, landslides, and erosion. Climate smart Agriculture (CSA) is an approach for creating agricultural plans to ensure long-term food security in the face of climate change. Climate-smart agriculture must consider the local context, cultural norms, and social sensitivities and must listen to the local population about the strategy that best suits their situation (UNFCCC, 2021). Research conducted in Western Kenya (2021) with the use of multivariate probit analysis showed that the household head's gender, education, age, family size, contact with extension agents, access to weather information, ownership of arable land, and livestock, perception of climate change, infertile soil, and persistent soil erosion all had an impact on the adoption of CSA practices (Musafiri et al., 2021). According to the study performed in Ghana, access to agricultural extension services and membership in farmer-based organizations favorably impact farmers' willingness to participate in climate change capacity building training. Engagement in family labor, agricultural insurance, and capacity development training impacted farmers' CSA adoption intensity (Zakaria et al, 2020). Joshi and Joshi (2016) found that the adoption was influenced by the household head's education level, the size of the household, their ability to produce enough food for themselves, and the lack of adequate understanding of agricultural adaptation at the time. (Aryal et al., 2018) found that numerous elements, including demographics, farm plot characteristics, market accessibility, socioeconomics, climatic hazards, availability of extension services, and training, have an impact on the adoption of CSA. Farmers are more likely to embrace crop diversification and minimal tillage practices if they view elevated temperatures as a major climate risk factor. When faced with short winters, farmers are less likely to use site-specific nutrient management, yet they are more likely to use low tillage. The likelihood and intensity of CSA adoption were found to be positively impacted by training on agricultural issues. It is necessary to study the status of climate change in the country and the steps taken to overcome the effects of climate change in agriculture production system. Different climate smart agricultural practices that are suitable in Nepalese context and their adoption by farmers at grassroot level plays an important role in managing the programs related with the climate smart agriculture.The aim of the study was to identify different CSA practices adopted by vegetable farmers in Sundarbazar, Lamjung and to assess the factors influencing the adoption of CSA practices among vegetable farmers in Sundarbazar, Lamjung.

2. MATERIALS AND METHODS

The study was conducted in Sundarbazar Municipality of Lamjung district which lies in Gandaki Province, Nepal. Simple random sampling was used to select and collect the information of the household. A sample of 100 households was collected from different wards of the Sundarbazar Municipality. Data was collected by using both primary and secondary sources. Primary data were collected by directly visiting the respondent in each household in the study area. Descriptive statistics like mean, standard deviation, frequency, percentage, and range were used to analyze the data. The obtained result was expressed in a bar diagram and table. Age, gender, education, income, and holding variables were analyzed.

Ordered logistic regression model

We used the **ordered logit model** to study the effect of socio-demographic and economic factors of households on the adoption of climate-smart agriculture practices. In this study, we have ordered a categorical variable that categorizes households into non-adopters, low adopters, medium adopters, and high adopters based on the frequency of use of climate-smart agriculture practices. The coefficients obtained from the ordered logit model have no intuitive or practical explanation. We calculate marginal effects at the observed mean values following the concept given in Greene (2003) after ordered logit for a better explanation of results. The marginal effects measure the probability changes in adoption in response to the changes in explanatory variables.

In the framework of ordered logit, our estimation strategy is based on latent regression model given by following equations.

$$Y^* = X\text{'s}\ \beta + \varepsilon$$

Where Y* is a latent variable that depicts the climate-smart agriculture adoption categories

X's are explanatory variables selected in our model

B is the coefficient vector

Ɛ is the error term

We estimate probability as

$$Pr(Y_i < j) = Pr(B_1X_{1i} + B_2X_{2i} + \ldots + B_kX_{ki} + u_i \leq a_j)$$

$$Pr(Y_i < j) = Pr(u_i \leq a_j - B_1X_{1i} - \ldots - B_kX_{ki})$$

where there are j ordered alternatives, and $a_1 - a_{(j-1)}$ represent cutoffs or threshold parameters. It gives the (cumulative) probability that Yi falls in a category j and below. The slope coefficients of the X regressors are the same in each category; only their intercepts (cutoffs) differ. (Gujarati, 2011)

Where, $(Y_i < j)$ is a latent variable (0 if farmers use < 1 CSA practices, 1 if famers use ≤ 3 CSA practices, 2 if famers use ≤ 6 practices and 3 if farmers use ≥ 6 practices)

$Pr(Y_i < j)$ = Pr(Age, Gender, Education, Active family members, Subsidy, Total income, total cultivated area devoted to vegetables

Variables description

Table 1. Variables Description

Variable	Type	Description
Climate smart agriculture adoption	Binary	=1 if the farmer has adopted climate smart agriculture for vegetable production
Age	Continuous	Age of the household head
Gender	Binary	=1 if the gender of household head is male
Active family members	Continuous	Family members actively involved in the economic activity
Subsidy	Binary	=1 if the farmer has received subsidy on climate smart agriculture
Income	Continuous	Total annual income of the household
Area	Continuous	Total area of vegetable production of the household
		= 1 if the household head is literate only
Education	Ordinal	= 2 if the education level of household head falls between primary to SLC
		= 3 if the household head has gained education of intermediate level and above
		= Non-Adopter if farmer has adopted 0 CSA practices
		= Low Adopter if farmer has adopted 1 to 3 CSA practices
Level of adoption	Ordinal	= Medium Adopter if farmer has adopted 4 to 6 CSA practices
		= High Adopter if farmer has adopted more than 6 CSA practices

3. RESULTS AND DISCUSSION

Out of the one hundred respondents in the study area, 68% were adopting different climate-smart agriculture practices for vegetable production, but 32% were not adopting CSA practices**.** Most of the households (38%) are practicing commercial production systems followed by semi commercial (33%) and subsistence (29%). The majority (51.5%) of the households practicing commercial production system have adopted CSA practices. Among non-adopters, the majority (62.5%) are practicing subsistence production system. The major vegetables produced in the study area are tomato, cauliflower, onion, and potato.

3.1 Gender characteristics

The gender of the household head plays a key role in perceiving climate change and adopting **climate smart agricultural practices**. Most (64.7%) of households head were male among adopters and, 81.2% of households head were male among non-adopters.

Table 2. Gender characteristics of the household head in the study area

Gender of Household Head	Categories		Total
	Non-Adopter	Adopter	
Female	6(18.8%)	24(35.3%)	30(30%)
Male	26(81.2%)	44(64.7%)	70(70%)
Total	32(100%)	68(100%)	100(100%)

Source: Field survey, 2023

3.2 Education status

Education plays a key role in the adoption of the new practices. The education status of the household head is an important variable for adopting CSA practices. Illiterate, literate only, basic (primary + lower secondary), intermediate, and above are the four categories of the education level. Most (49%) of the household heads belong to the basic level category, followed by 26% literate only and 15% intermediate and above. Among CSA adopters household heads, 60.3% belong to basic level category, 19.1% belong to literate only, and intermediate and above.

Table 3. Education status of household head

Education level	Non-Adopter	Adopter	Total
Illiterate	9(28.1%)	1(1.5%)	10(10%)
Literate only	13(40.6%)	13(19.1%)	26(26%)
Basic (Primary + Lower secondary)	8(25%)	41(60.3%)	49(49%)
Intermediate and above	2(6.3%)	13(19.1%)	15(15%)
Total	32(100%)	68(100%)	100(100%)

Source: Field survey, 2023

3.3 Age of household head

The average age of the household head was 46.04 years. Among CSA adopters, the average age of the household head was 42.15. The average age of CSA non-adopters was 54.31 years. From the table below, it is visible that CSA adopters were younger than non-adopters. This may be due to risk-bearing capacity and affection towards the new practices.

Table 4. Age composition of the household head

Adoption category	Age of Household Head	Minimum	Maximum
Non-Adopter	54.31	35	72
Adopter	42.15	24	65
Overall	46.04	24	72

Source: Field survey, 2023

3.4 Active members

The sampled population was divided into economically active and dependent populations. Economically active represents the working population and dependent represents the population that is dependent on other family members or the government. A majority (65.86%) of the household members were economically active, and 34.14% of family members were dependent on other family members. Among CSA adopters, 68.22% of family members were economically active, and 31.78% were dependents. Similarly, among non-adopters, 60.29% were economically active, and 39.71% were dependents, as shown in table 5.

Table 5. Dependents and economically active population of the sample household

Population type	Non-Adopter	Adopter	Total
Economically active	82(60.29%)	219(68.22%)	301(65.86%)
Dependent	54(39.71%)	102(31.78%)	156(34.14%)
Total	136(100%)	321(100%)	457(100%)

Source: Field survey, 2023

3.5 Income status

The annual income of sampled households was categorized into farm, off-farm, and remittance income. CSA adopters were the highest earners annually and major contribution was from farm income, including crops, livestock, and bee farming. Similarly, 0ff-farm income includes pensions, government services, other services, labor, and business Details of income source and its relationship with adoption of CSA practices as shown in Table 6.

Table 6. Income status of sampled household

Income source	Non-Adopter	Adopter	Overall
Farm income (NPR)	122812.50 (72043.194)	308970.59 (171474.202)	249400.00 (170708.979)
Off-farm income (NPR)	159125.00 (174741.053)	243382.35 (275066.398)	216420.00 (249653.707)
Remittance (NPR)	145937.50 (304714.138)	138529.41 (339412.419)	140900.00 (327186.028)
Total income (NPR)	427875.00 (275934.629)	690882.35 (472205.797)	606720.00 (435832.956)

**Parenthesis represents standard deviation*

3.6 Area under vegetables

The average area under vegetables was higher (2.18 ropani) in CSA adopters as compared to non-adopters (0.78ropani). The area ranged from 0.5-8 ropani among adopters, whereas it ranged from 0.3-2 ropani among non-adopters as shown in below table 7.

Table 7. The area under vegetables of the sampled household.

Vegetable cultivation	Non-Adopter	Adopter	Overall
Area(ropani)	0.78	2.18	1.73
Standard deviation	0.49	1.46	1.39
Range	0.3-2	0.5-8	0.3-8

Source: Field survey, 2023

3.7 Climate smart agricultural practices adopted by the farmers for vegetable production

Out of 68 farmers adopting CSA practices for vegetable production, 55 farmers are growing vegetables inside polyhouse during the winter and rainy seasons. Tomato and cauliflower are two major vegetables inside the polyhouse. Forty-two farmers are using improved varieties of tomato, cauliflower, onion, potato, and other vegetables. Likewise, fourty farmers use a drip irrigation system to irrigate tomatoes, cauliflower, and cabbage. Similarly, 38 farmers use mulching for vegetables like tomatoes, potatoes, and chilly. Thirty-six farmers are using Integrated Pest Management strategies to overcome the pests of potatoes, tomatoes, crucifers, and cucurbits.

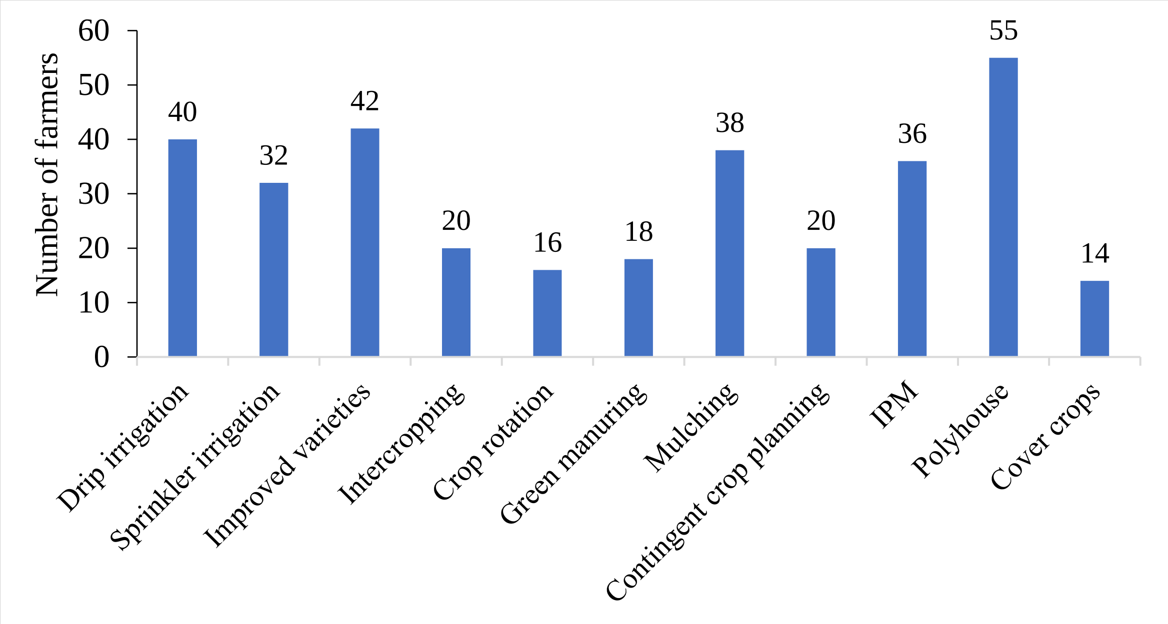

Figure 1. Different CSA practices adopted by the farmers

Likewise, 32 farmers use a sprinkler irrigation system to irrigate crucifers, potatoes, onion, and garlic. Twenty farmers adopt each intercropping and Contingent crop planning mostly in onion, radish, peas, and crucifers. Among the CSA adopters, 18 farmers are using green manure, 16 are using crop rotation and 14 are using cover crops in the vegetable cultivated land.

3.8 Government subsidy on CSA practices

Local government as well as the province government are providing subsidy programs on different practices to the farmers of study area. Fourt-eight farmers have received subsidies for the construction of polyhouse. Similarly, 38 farmers have received subsidies on drip irrigation, 35 on improved varieties, 34 on mulching materials and 18 on sprinkler irrigation system.

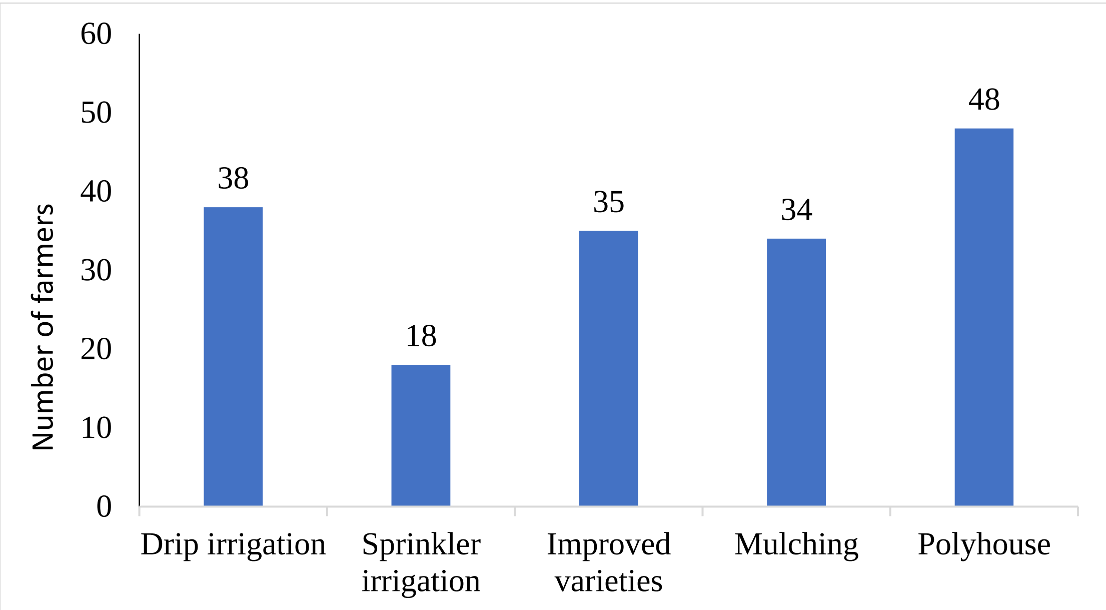

Figure 2. Subsidy on different practices received by the farmers

3.9 Factors influencing the level of climate-smart agriculture adoption

We use the ordered logit regression model to analyze the factors affecting the adoption of CSA practices in different categories. The Pseudo R squared value was 0.382, meaning 38.2% of the variation in the dependent variable is explained by the independent variables of the regression equation. Among the tested variables, the age of the household head, subsidy, number of active family members, and total cultivated area devoted to vegetable production were found to be significant in different categories.

Table 8. Marginal effects of factors influencing the level of CSA adoption

Variable	Marginal effects			
	Non-Adopter	**Low Adopter**	**Medium Adopter**	**High Adopter**
Gender of HH	0.083	0.020	-0.097	-0.006
Literate only	-0.129	0.052	0.074	0.004
Basic level	-0.185	0.045	0.132	0.007
Intermediate and above	-0.121	0.051	0.067	0.003
Age of HH	0.008*	0.001	-0.008*	-0.000
Active members	-0.068**	-0.005	0.069**	0.004
Subsidy	-0.628***	0.187*	0.411***	0.030*
Income	0.017	0.001	-0.017	-0.001
Area under vegetables	-0.076**	-0.006	0.077**	0.005*

*** p<0.01, ** p<0.05, *p<0.1

Number of observations	100
Pseudo R squared	0.382
Chi-square	95.986
Prob > Chi-square	0.000

The age of household head was negative and statistically significant (p<0.1) for medium adopters. With the increase of the household head age by one year, the probability of CSA adoption decreases by 0.8%. This may be due to the attraction of youths and younger people to the new practices. This aligns with the findings of (Justin et.al 2017). Surprisingly, among non-adopters, the age of the household head was positive and statistically significant (p<0.1). An increase in the age of the household head by a year increases the probability of CSA adoption by 0.8%. This may be due to their experience in farming, and they want to try new practices to get more than traditional ones. This result for non-adopters of climate-smart agriculture is also like (Justin et al. 2017), who found that the probability of adoption increases with the increase in age among farmers adopting zero practices.

Number of active family members was statistically significant (p<0.05) for both medium adopters and non-adopters. An increase in active family members by a number increases the probability of CSA adoption by 6.9% for medium adopters but decreases the probability of adoption by 6.8% for non-adopters. The addition of active members allows the family to try different new practices. But it also makes room for income opportunities from sources other than agriculture, reducing adoption and farming. Asfaw et al. (2017) found that an active family labor force had a significant and positive effect on the level of adoption. The subsidy was positive and statistically significant at a 10% level of significance for low and high adopters. The subsidy was negative for non-adopters and positive for medium adopters, both significant at a 1% significance level. Receiving a subsidy increases the probability of CSA adoption by 3%, 41%, and 18.7% for high, medium, and low adopters, respectively. Ouédraogo et.al (2019) found that access to subsidies had a positive and significant impact on adopting climate smart agriculture. Among non-adopters, receiving a subsidy decreases the probability of adoption by 62.8%. Partial subsidy programs were run in the study area, so a certain percentage of the money should come from the farmers as well. Non-adopters might be hesitant to invest some amount into the new practices by themselves instead they continue with their own practices. The vegetable cultivation area was positive and statistically significant at 10% and 5% significance for high and medium adopters, respectively. An increase in the area by one unit increases the probability of CSA adoption by 0.5% and 7.7% for high and medium adopters, respectively. Vegetable cultivation area was negative and significant (p<0.05) for non-adopters. An increase in area by one unit decreases the probability of adoption by 7.6% for non-adopters. An increase in area helps in adopting new practices, but in the case of non-adopters, the probability of adoption decreases. Farmers not adopting CSA practices may not be sure about the cost of cultivation and the yield, so they might not want to risk their farms and adopt the new practices. Justin et al. (2017) showed that an increase in the area of land decreased the probability of adopting climate-smart agriculture as small land-sized farmers may not be able to recover the additional costs that they bear with an increase in land size.

4. CONCLUSION

The study focused on the factors influencing adopting CSA practices on vegetable production. CSA adopters were comparatively younger than non-adopters. There was no association between gender and the adoption of CSA practices. CSA adopters were also higher earners than non-adopters. The average cultivated land and area of vegetables were also higher for adopters. The major CSA practices the farmers adopt are polyhouse farming, improved varieties, drip irrigation and mulching. Receiving a subsidy increases the probability of CSA adoption by 3%, 41%, and 18.7% for high, medium, and low adopters, respectively. Among non-adopters, receiving a subsidy decreases the probability of adoption by 62.8%. Other variables influencing CSA adoption among four categories are the household head's age, active family members and area under vegetables.

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EVALUATING THE EFFICACY OF POTENTIAL FUNGICIDES FOR THE MANAGEMENT OF SOUTHERN LEAF BLIGHT (*Bipolaris maydis*) OF MAIZE

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ABSTRACT

Southern Leaf Blight (SLB) is one of the most damaging diseases, reducing maize productivity by up to 50%. One of the primary causes of Nepal's low maize yield is a lack of understanding, which leads to incorrect fungicide selection at the inappropriate doses. The in vitro study evaluated seven fungicides at four distinct doses: 50 ppm, 100 ppm, 150 ppm, and 200 ppm. The fungicides examined were hexaconazole, tricyclazole, azoxystrobin + tebuconazole, azoxystrobin + difenoconazole, azoxystrobin + propiconazole, copper oxychloride, and mancozeb, all of which are routinely used in Nepal to control fungal infections of crops. All tested fungicides considerably reduced ($P < 0.001$) the pathogen's mycelial growth in the poisoned culture. hexaconazole, azoxystrobin + propiconazole, and azoxystrobin + tebuconazole all reduced Bipolaris maydis mycelium development to the greatest extent possible. mancozeb showed reduction in inhibition percentage with lowering in concentration similar in the case for azoxystrobin + difenoconazole and copper oxychloride.

1. INTRODUCTION

Although agriculture plays a crucial part in Nepal's economy, its current state is not conducive to poverty reduction. Nepal's agriculture industry, although lacking modernization and mechanization, is the country economy's driving force. It contributes about 23.95% of GDP and about 65% of the economically active population involved in this sector. Among the total land, 30,91,000 ha of land is cultivated, and 10,30,000 ha of agricultural land is uncultivated (MoALD, 2022). It is grown as second most important cereals crop after rice in Nepal and is considered one of the major staple foods for Nepalese. Maize is a versatile crop that can be used for human consumption, animal feed, and industrial purposes. It is a good source of dextrose, starch, syrups, oils, and linoleic acid (Saritha et al., 2020). Maize covered a total area of 9,85,565 ha with a production of 31,06,397 mt with a productivity of 3.15 mt per ha (MoALD, 2022).

Southern leaf blight (SLB) (*Bipolaris maydis*) of maize is also known as maydis blight. Earlier, the disease was considered minor for maize, but now it has assumed the status of a major disease in the world. It is a very devastating fungal disease that attacks the maize crop at all stages (Atif et al., 2019; Rehman et al., 2021). SLB is considered to be one of the most devastating diseases as it reduced grain yield by maize by 28-91% (Payak & Sharma, 1980). The yield loss in certain states in the United States and Ontario, Canada, ranged from 7.5 to 13.5% of grain production between 2012 and 2015 (Mueller et al., 2016). In the United States, the annual yield loss has been reported from 2 to 15% (Munkvold & White, 2016). It can cause significant damage to maize plants, particularly during periods of high humidity and warm temperatures.it manifests symptoms initially took the form of spindle-shaped lesions, usually measuring 2 to 6 mm in length. In later instances, brown, chocolate-colored lesions that ranged from 2.5 to 20 cm.

The burning regions on the older leaves are oval-shaped and extra-long (Akonda et al., 2015). The losses due to southern leaf blight can be managed through the foliar application of chemical fungicides. Utilization of plant extracts, bio agents and essential oils in disease management is considered as eco-friendly, without any environmental pollution. Maximum yield of maize was recorded in *Trichoderma viride* (39.16 q/ha), Lemon grass oil @0.2% (37.98 q/ha), Neem oil @ 0.1% (34.33 q/ha), Lantana camara @15% (32.42 q/ha), Neem leaf extract @10% (30.83 q/ha), and Onion bulb @ 10 % (30.50 q/ha) as compared to the untreated control (25.00 q/ha) by botanical management. (Vardhan et al., 2020). Chemical fungicides have been widely used to control the disease, but there is a lack of information on the efficacy of different chemical fungicides in Nepal. This made farmers use different types of chemicals haphazardly leading to high cost of cultivation, and environmental hazards while controlling this disease.

Effective disease management strategies are becoming more and more necessary since foliar diseases of corn have become a bigger issue in recent years (Wise & Mueller, 2011). Cultural practices often do not provide adequate management and also resistant cultivars are not available. Available cultivars have low tolerant capacity for the disease and introducing varieties may not be long lasting. Hence, there is a need to find an appropriate, efficient and economically chemical control method. Chemical fungicides are commonly used to control SLB. Continuous use of a single fungicide can lead to the development of resistance. Therefore, several options of chemical fungicides with different modes of action, help to prevent the development of resistance with a single fungicide.

Targeting certain cell organelles and interfering with their cellular processes, each chemical fungicide or active ingredient has a unique mechanism of action and target location that prevents the growth of fungal diseases (Hermann & Stenzel, 2019; Hu & Chen, 2021), including those selected for this study (Table 1). To disrupt fungal cell membranes, for instance, Triazoles (propiconazole, hexaconazole, tebuconazole, difenoconazole, and tricyclazole), are systemic fungicides and demethylation inhibitors (DMI) of sterol biosynthesis, oxidizes the side chain attached to the dioxolane ring enzymatically and deketalisation the fungal cell by losing the dioxolane moiety, thereby blocking demethylation. azoxystrobin belongs to the class of quinone outside inhibitor (QoI) fungicides, which also have systemic properties. These fungicides inhibit mitochondrial respiration at the QoI-site of cytochrome b, which is a component of the cytochrome bc1 complex (complex III), which prevents the production of adenosine triphosphate (ATP), and ultimately impede the growth and germination of spores in fungal pathogens (Rodriguez-Morelos et al., 2021; Andrade et al., 2022). The effectiveness of triazoles and strobilurins against key types of plant pathogenic fungi is well established; however, little is known about how they affect bacteria (Yen et al., 2009). Copperoxychloride mode of action is based on the presence of Cu^{2+} ions in aqueous solution. Cu^{2+} ions are absorbed by the pathogen until the accumulation collapses its cells by blocking the respiratory process, inhibiting protein synthesis and reducing membrane activity. The mancozeb interferes with enzymes containing sulfhydryl groups, disrupting several biochemical processes within the fungal cell cytoplasm and mitochondria. (FRAC Code List, 2023).

The disease's severity has been increasing, leading to substantial yield losses in various regions. While SLB management in Nepal involves the use of traditional fungicides, our study focuses on evaluating the efficacy of newly registered fungicides. This research addresses a crucial gap in understanding SLB management, providing valuable insights for sustainable maize cultivation practices.

2. MATERIALS AND METHOD

2.1 Experimental site and design

The *in-vitro* experiment was conducted in the laboratory of plant protection, Jhumka, Sunsari in a Completely Randomized Design (CRD) with 7 chemical fungicides and 4 doses (50 ppm, 100 ppm, 150 ppm and 200 ppm) repeated for 5 times. The experiment was done during March to May, 2023. The details of treatments and fungicides are presented in Table 1.

2.2 Sample collection, isolation, purification, and maintenance of the pathogen cultures

Maize leaves with the typical lesions of SLB were obtained from the farmer's field. The diseased leaves were air-dried, kept in a paper bag, and refrigerated at 4^0 C upon arrival in the laboratory for subsequent isolation of the pathogen. The isolation of fungus, *B. maydis* was made from the diseased leaf samples using the isolation technique. (Shekhar & Kumar 2012). A small portion of infected leaf tissues with some adjacent healthy tissue of approximately 5 mm × 5 mm were cut using the sterilized knife. Surface sterilization was done to kill all the undesirable surface microbes by immersing the leaf portions in 75% ethanol for 30 seconds and 1 % sodium hypochlorite solution for 1 minute followed by 3 successive rinsing with distilled water. The leaf portions were blotted in sterile filter paper to absorb moisture. The leaf portions were placed in the sterile petri plate with three layers of moistened blotting paper to prepare a moist chamber intended for fungal sporulation at 25±2°C in the bio-oxygen demand (BOD) incubator for 24 h.

Single spore isolation technique was followed to pick up the spore with the help of a fine flattened needle under the stereomicroscope (Olympus/ SZX16) and placed it on the water agar (20 g agar per litre of distilled water) aseptically. After the germination of the spore in 24 h, a single spore was again transferred from water agar to separate culture tubes of potato dextrose agar (PDA) (200 g potato infusion, 20 g dextrose, 20 g agar for 1 l) slants with the help of stereomicroscope and inoculating needle under laminar flow chamber aseptically. To prevent the unwanted growth of bacteria, streptomycin sulfate of 50 ppm was added to the PDA. The tubes were incubated in an incubator at 25± 2°C for 12 days to get pure monoconidial isolate. For short-term preservation, PDA slants were maintained at 4°C in the refrigerator as the pure stock culture for further investigation.

2.3 In-vitro assay of different chemical fungicides against Bipolaris maydis

In-vitro assay of seven different fungicides was done against *B. maydis* by employing "poisoned food techniques" (Schmitz, 1930) at four different concentrations (50 ppm, 100 ppm, 150 ppm, and 200 ppm). To make respective concentrations of each fungicide, a 10,000-ppm stock solution on the base active ingredient of each fungicide was formulated, and the requisite quantity of each chemical was aseptically inserted in 100 ml of sterilized PDA medium. To prevent bacterial contamination, 50 ppm of streptomycin sulfate was applied when the PDA medium was around 45-50°C using a micropipette. After solidification, a 5 mm diameter mycelial disc was cut from the periphery of a 7-day-old culture with the help of a sterile cork borer and inoculated onto the plates. Simultaneously, the fungus was grown on a chemical-free PDA medium to serve as a check and incubated at 25±2°C in the BOD incubator. The colony diameter of each fungus was measured until the Petri plates (90 mm diameter) under control were filled with the fungus mycelial growth. The mean values of the fungal colonies' longitudinal growth were determined using the measuring scale from two different angles

2.4 Assessment of percent inhibition (PI)

The inhibition percent for mycelial growth was calculated by applying the formula given by Vincent, 1945.

$$PI = \frac{(C - T)}{C} X\ 100$$

where, PI = Percent Inhibition (%), C = colony growth in the control plate, and T = colony growth in the treated plate.

Table 1. Details of treatments (fungicides) used in the experiment.

S.N.	Trade name	Common name	Pesticide type	Chemical group*	FRAC-MoA Group Names*
1	Titan	hexaconazole 5% EC	Systemic	Triazole	DMI
2	Trip	tricyclazole 75 % WP	Systemic	Triazole	DMI
3	Apollo	azoxystrobin 7.1% + Propiconazole 11.9 % SE	Systemic	Methoxyacrylates + Triazole	QoI and DMI
4	Godiwa super	azoxystrobin 18.2%+ difenoconazole 11.4% SC	Systemic	Methoxyacrylates + Triazole	QoI and DMI
5	Raptor	azoxystrobin 11% + tebuconazole 18.3% SC	Systemic	Methoxyacrylates + Triazole	QoI and DMI
6	Nagcoper	copper oxychloride 50% WP	Non-Systemic	Inorganic	Inorganic
7	Indofil M-45	mancozeb 75% WP	Non-Systemic	Dithiocarbamates and relatives	Dithiocarbamates and relatives
8	Control (Without any pesticide)				

* Source: FRAC Code List, 2023

2.5 Statistical Analysis

Microsoft Excel was used to enter and save the experiment's data. R and its packages, agricolae and ggplot2, were used to construct graphs, analysis of variance (ANOVA), a post hoc test using DMRT, and a 5% level of significance for the data.

3. RESULTS AND DISCUSSION

3.1 Inhibition percentage of mycelial growth

The efficacy of seven fungicides were tested at different concentrations against the growth of pathogens by the poisoned food technique. The percent inhibition of growth of the test fungus at different concentrations over control was calculated. At 50 ppm concentration, hexaconazole, azoxystrobin + propiconazole and azoxystrobin + tebuconazole showed 94.44 percent inhibition of mycelial growth followed by azoxystrobin + difenoconazole and mancozeb with 81.77 percent and 73.88 percent growth inhibition respectively, and least inhibition of mycelial growth was recorded in Tricyclazole with 19.53 percent. At 100 ppm concentration, hexaconazole, azoxystrobin + propiconazole and azoxystrobin + tebuconazole showed 94.44 percent inhibition of mycelial growth followed by azoxystrobin + difenoconazole and mancozeb with 84.11 percent and 78.88 percent growth inhibition respectively, and minimum inhibition of mycelial growth was recorded in tricyclazole with 32.86 percent. At 150 ppm concentration, hexaconazole, azoxystrobin + propiconazole, and azoxystrobin + tebuconazole showed 94.44 percent inhibition of mycelial growth followed by mancozeb and azoxystrobin + difenoconazole with 86.88 percent and 85.22 percent growth inhibition respectively, and minimum inhibition of mycelial growth was recorded in tricyclazole with 47.77 percent. At 200 ppm concentration, hexaconazole, azoxystrobin + propiconazole, azoxystrobin + tebuconazole, and mancozeb showed 94.44 percent inhibition of mycelial growth followed by azoxystrobin + difenoconazole and copper oxychloride with 87.55 percent and 70.33 percent growth inhibition respectively, and minimum inhibition of mycelial growth was recorded in tricyclazole with 60.55 percent.

Among all the tested fungicides, hexaconazole, azoxystrobin + propiconazole, and azoxystrobin + tebuconazole were found highly effective with cent percent inhibition of mycelial growth of *B. maydis* at the concentrations (50, 100, 150, and 200 ppm). The mancozeb showed 94.88 percent inhibition at the concentration of 200 ppm at par with hexaconazole, azoxystrobin + propiconazole, azoxystrobin + tebuconazole at 50 ppm concentration (i.e., 94.88) followed by azoxystrobin + difenoconazole (87.55 percent at 200 ppm and 85.22 percent at 150 ppm). Minimum inhibition of mycelial growth was recorded in tricyclazole (19.53 percent at 50 ppm).

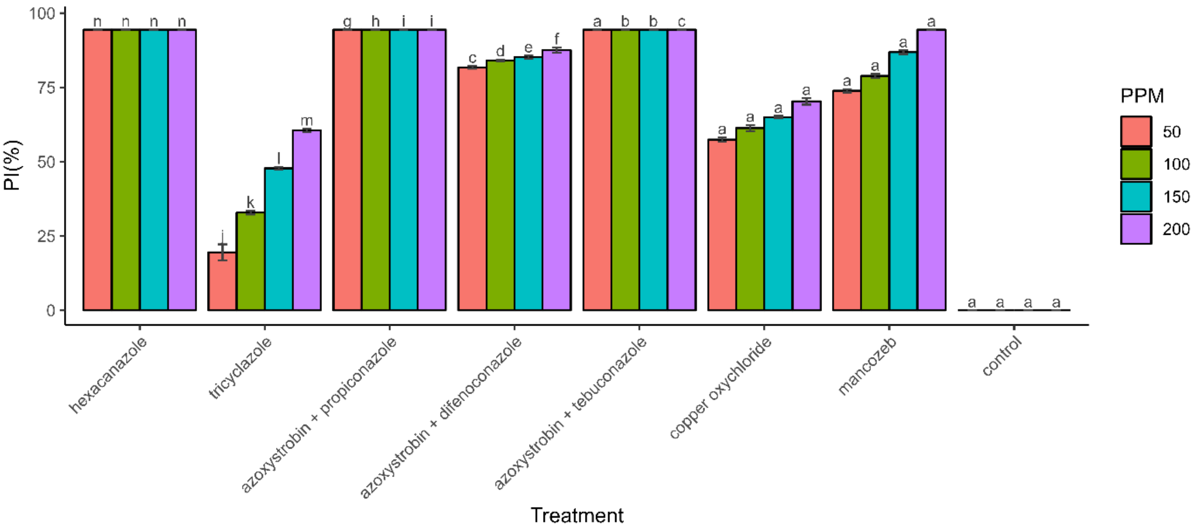

Fig 1. Effect of different fungicides on percentage inhibition of mycelial growth of *B. maydis* at 15 days after inoculation

3.2 Diameter growth of mycelium

Diameter of mycelium ranged from 5 to 71.5 mm in which control plates had the highest value (90 mm). The lowest mycelium diameter was found in hexaconazole, azoxystrobin + propiconazole and azoxystrobin + tebuconazole. Lowest diameter was recorded in 200 ppm only of mancozeb. The hexaconazole, azoxystrobin + tebuconazole, and azoxystrobin + propiconazole at all tested concentrations and mancozeb at 200 ppm were found effective inhibiting mycelial growth of *B. maydis*.
Kumar et al, 2019 & Kumar et al, 2021 also noticed the mycelial growth of *B. maydis* completely inhibited by propiconazole at 50, 150, 200, and 250 ppm concentrations and mancozeb demonstrated 93.88 percent inhibition at 250 ppm and found effective and considerably superior. Numerous other researchers have also realized that chemicals, like carbendazim, chlorothalonil, mancozeb, and propiconazole, are effective against H. maydis (Kumar et al., 2009). The application of propiconazole as a foliar spray and seed treatment has boosted maize yield and reduced southern corn leaf blight (Kumar, 2010). The most successful fungicide for southern leaf blight on maize was propiconazole (Tilt 25% WP). The least successful treatment for the condition was copper oxychloride 50%WP (Shah et al., 2023). The propiconazole was found to be more effective against the pathogen than mancozeb, carbendazim, chlorothalonil, and copper oxychloride in vitro at 50, 100, 150, 200, and 250 ppm concentrations (Bharti et al., 2020).

A disease is an outcome of the interaction of the host, pathogen, and environment. The result is based on aseptic conditions provided by the laboratory only for the pathogens whereas the complete interaction is missing and can be comprehended on field experiments. Therefore, fungicides that were successful in this study in inhibiting the growth of *B. maydis* should undergo additional testing in the field to validate their efficacy and to establish the best dosages for use. Further study with other lower doses of i.e., hexaconazole, azoxystrobin + propiconazole, and azoxystrobin + tebuconazole less than 50ppm needs to be carried out so that we can use the minimum concentration of hexaconazole, azoxystrobin + propiconazole, and azoxystrobin + tebuconazole for the management of this pathogen which is also cost-effective for the farmers.

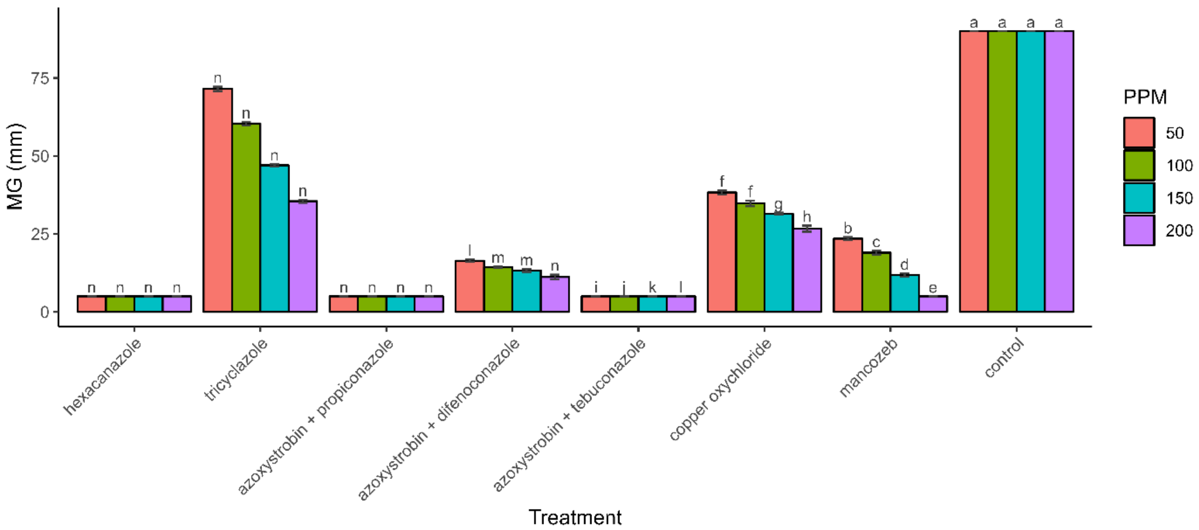

Fig 2. Effect of fungicide (different concentration) in the diameter growth of mycelium of *B. maydis* at 15 days after inoculation

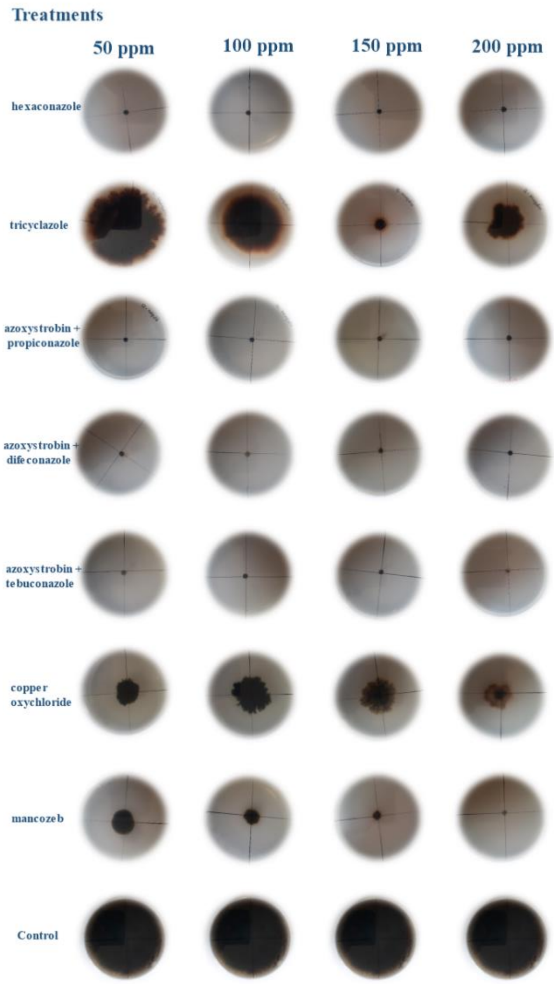

Fig 3. *In-vitro* performance of chemical fungicides on the mycelial growth of *B. maydis* in different concentrations

4. CONCLUSION

Based on the current experimental research, chemical fungicides such as hexaconazole, azoxystrobin + tebuconazole, and azoxystrobin + propiconazole have the ability to manage SCLB. These fungicides, after proper in-vivo testing, can be recommended for usage in farmer's fields.

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EVALUATION OF DIFFERENT WEED MANAGEMENT PRACTICES ON THE PHONOLOGY OF SPRING MAIZE IN GAURADAHA, JHAPA

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ABSTRACT

Maize is highly sensitive to weed competition, especially during early growth stages. A field experiment on "Evaluation of different weed management practices on the phonology of spring maize in Gauradaha, Jhapa" was conducted in Randomized Complete Block Design with three replications, comprising 8 treatments, control, cover crops, hand weeding (30,45 and 60 DAS), botanical weedicides, inorganic weedicides, black plastic mulch, straw mulch, narrow inter-row spacing at the arm of the Gauradaha Agriculture Campus, Jhapa during the spring season of 2022. Arun-2 maize variety was used. The findings indicated that, across treatments, the number of days before emergence was not significant. Among the different treatments, black plastic mulching recorded higher leaf area index i.e., 1.58, 2.72, 2.34 at 45,60 and 75 DAS respectively. Plant height was recorded higher in black plastic mulch (136.75 cm, 234.43 cm and 229.10 cm) at 45 ,60 and 75 DAS. Similarly, leaf number was also significantly affected and found to be highest in black plastic mulch i.e., 11, 12, 11 at 45 ,60 and 75 DAS respectively. According to the study, phonological performance was better in black plastic mulching. But since it produced results on par, straw mulching can also be employed as an alternative.

1. INTRODUCTION

Maize (*Zea mays*) is the second-most significant cereal crop after rice in Nepal (Kandel, 2021). An area of 979,776 ha of land covered by maize farming gives total production of 2,997,733 MT having average productivity of 3.05 mt/ha. Maize contributes 6.83% in agricultural GDP(MoAD,2022). It is the most imperative and primary cereal crop in the hills of Nepal, where the grain is used for human consumption and the stover for animal fodder (Gaihre and Adhikari, 2018). Maize requires a balance dose of organic and inorganic fertilizer for increased productivity (Adhikari *et al.*, 2016). Maize, around the world, cultivated on nearly 201.98 million ha yielding 1162.35 million mt with mean of 5.75 t/ha (FAOSTAT, 2020). 32% of global maize production and 34.1 % of total maize producing area is covered by Asia. Even so, demand surpasses local production of maize due to low rate of growing poultry and dairy farming (Osti, 2019). About 17-18 % area coverage is in terai and inner terai which accounts for 18-19 % of production (MoAD,2020). The province with the largest area and production of maize, province 1, has 289714 ha under cultivation and 884659 MT of production, whereas province 2 has the highest productivity i.e., 3.48 mt/ha. Jhapa district ranks highest among the 14 districts in province 1, grows 159105 MT of maize on 39385 ha of land, with productivity of 4.04 mt/ha (MoAD,2022). There are many production constraints behind the lower productivity of maize in the country. Among them, weed infestation has been a serious problem limiting the maize production globally. Corn has a steady initial growth rate and also extensive row spacing due to which weed intrusion is becoming a severe problem (Sharma and Rayamajhi, 2022). Maize plants are more sensitive to the competition during critical period of weed control i.e., between 4-7 weeks after sowing (Shrestha *et al.,* 2019).

Nascent stages of maize development would increase plant-to-plant variability in plant dry-matter accumulation due to weed interference, result in a reduction of grain yield at maturity (Cerrudo *et al.,* 2012). Total maize yield reduction of 58–62% in winter and 67–79% in summer was documented from unrestricted weed growth, including an average of 65% plant height reduction under the same weedy conditions (Mukhtar *et al.,* 2007). Weeds compete with main crop, suppress the crop plant and ultimately influence their yield and quality (Webster, 2003). Weeds compete with crops for nutrients, moisture, light and space and also possess allelopathic effects on crops (Walia and Walia, 2015). Primarily, Farmers are practicing maize cultivation along with applying high inputs for higher production that has enhanced the weed infestation. The extensive use of chemical fertilizers, reiterated irrigation and wide spacing between maize rows provide feasible environment for weed establishment, growth and enhancing the yield loss (Fanadzo *et al*., 2007) and (Bajwa *et al.,* 2014). As Kakade *et al.* (2020) reported that different species of maize associated weeds were *Cyperus rotundus L.* under sedges. Broad-leaved weeds include *Euphorbia spp., Corchorus fascicularis L., Parthenium hysterophorun, Amaranthus viridis, Commelina benghalensis L., Acalypha indica L., Abutilon hirtum, Phyllanthus niruri, Boerhavia coccinea, Argemone Mexicana L., Achyranthus aspera L., Celosia argentea L., Cardiospermum helicacabum L., Euphorbia geniculata Orteg., Xanthium strumarium L.* supervened by grassy weeds like *Cynodon dactylon L., Eriochloa spp.* and *Sorghum halepense L.* According to Naik et al., (2018) *Cyperus rotundus* dominated other weeds such as grasses like *Cynodon dactylon* and among broad leaf weeds *Phyllanthus niruri, Digera arvensis, Trianthema portulacastrum* and *Cleome viscosa. Cyperus rotundus* being most observed in field creates competition as they have rapid emergence and higher growth rate. Hand weeding is the most efficient and widely adopted practice of weed control but due to back breaking, labor intensive, time consuming and costly, it narrowed down the profits of the cultivation. Manual labor is becoming scarce with the gradual increase in indication of our country, coupled with the raising standard of living and literacy.

Chemical method of weed control has become ineluctable in the context of high cost of labor during peak season and labor shortage for crop properly to reduce the weed competition at critical period of weed interference without much disturbance to crop growth and soil health (Muoni *et al.,* 2013). Bu *et al*. (2013) recounted the highest growth rates, greater leaf area index in maize field mulched with film plastic compared to the non-mulched crop field. There is a big gap in the performance of maize in Nepal, as affected by various technological and socio-economic factors (Kc *et al.,* 2015). For this reason, an effort was made to know the effect of weed management technique on the phenology of spring maize while keeping the aforesaid points in mind. So, there is a necessity to make a comparative study of different weed management techniques to develop an innovative weed management approach for better performance in maize. Consequently, the present exhaustive research was undertaken to study the effect of weed management practices on phonology of spring maize. Thus, the research was conducted by using different weed management techniques with the objectives to determine the best method of controlling weed in context of spring maize in Jhapa.

2. MATERIALS AND METHODS

i. Experimental site

The experiment was conducted at Gauradaha, Jhapa, Eastern Nepal during spring season of 2022. The area is situated in eastern terai, located at 26.56° N latitude, 87.72° E longitude and at altitude of 79 m above sea level.

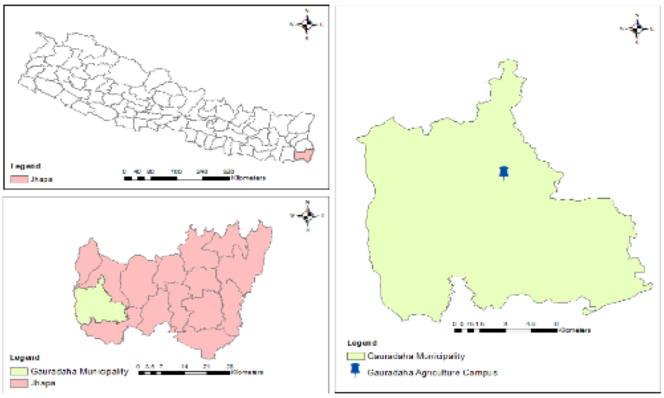

Figure 1. Map of experiment site

The graph shows maximum temperature (˚C), minimum temperature (˚C), relative humidity (%) and total precipitation (mm/day) of Gauradaha, Jhapa during spring season of 2022.

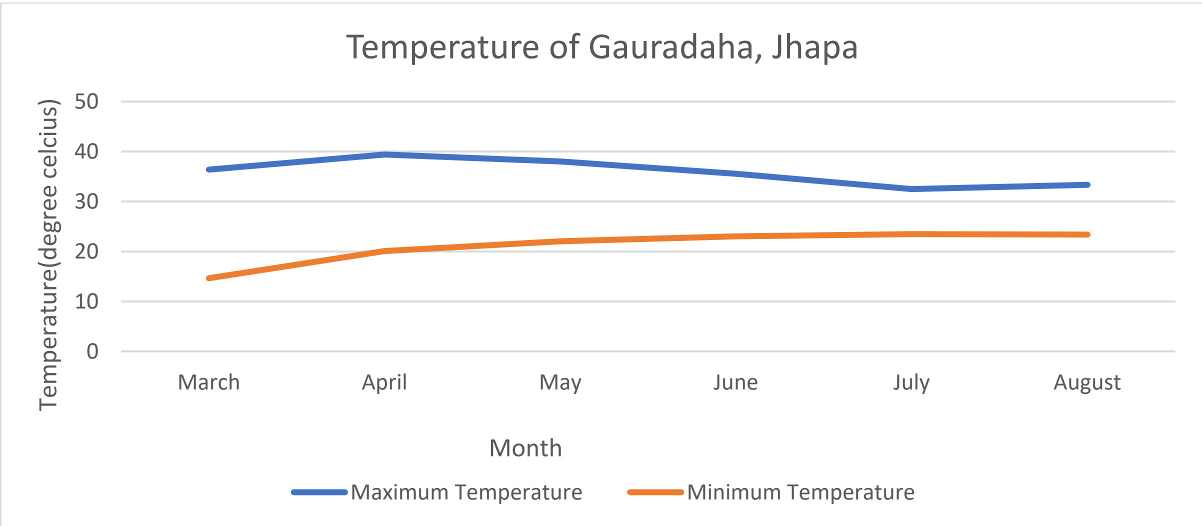

Figure 2. Maximum and minimum temperature of Gauradaha, Jhapa, 2022.

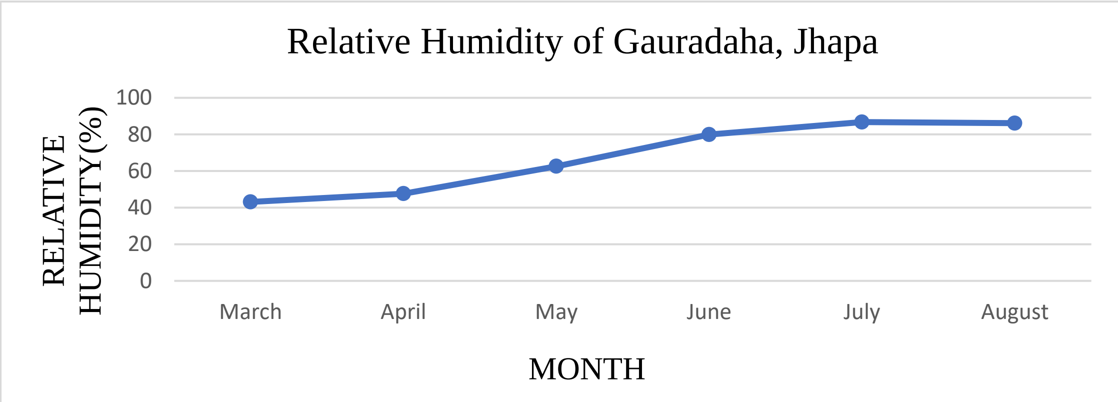

Figure 3. Relative Humidity (%) of Gauradaha, Jhapa, 2022

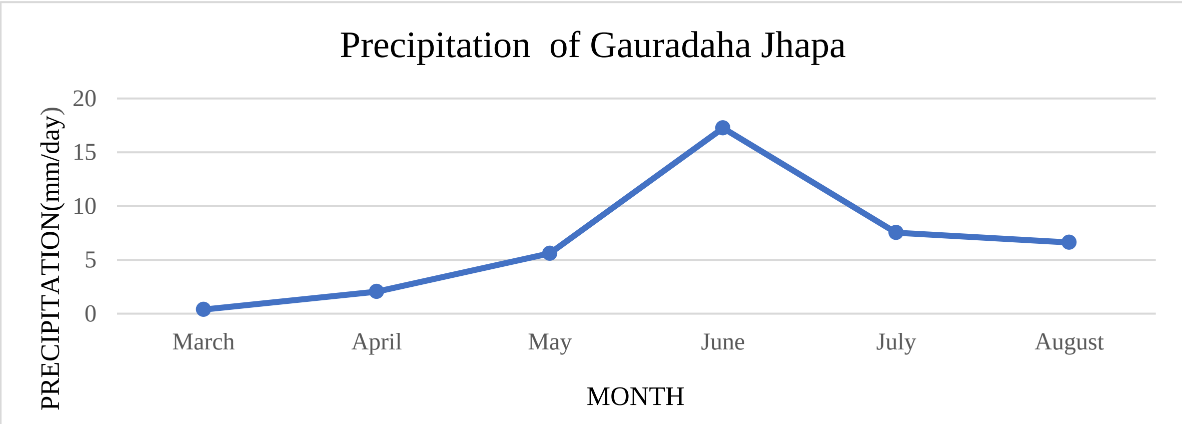

Figure 4. Precipitation(mm/day) of Gauradaha, Jhapa, 2022.

Source: Department of Hydrology and Meteorology

2.2 Treatment details

Treatment	Treatment details
T1	Control
T2	Cover crop (cowpea 312 variety)
T3	Hand weeding (30DAS,45DAS,60DAS,75DAS)
T4	Botanical weedicides (5ml/15lit,30DAS, 45DAS,60DAS, 75DAS)
T5	Inorganic weedicides Atrazine: 2.1gm/42m2 (3DAS) 2-4D 58%Sl:7.26ml/42m2 (20DAS)
T6	Plastic mulch
T7	Straw mulch
T8	Narrow inter-row spacing Plant-plant: 20cm Row-row:60cm

2.3 Experimental design and crop management

The experiment was laid out in Randomized Complete Block Design (RCBD) comprising of 8 treatments and 3 replications. The size of individual plot was 4m×3.5m. Distance between plot was 0.5m and between replication was 1m. The entire field was divided into flat beds of workable length and width, and shallow furrows were opened. On the 11th of Falgun (23 feb ,2022), the Arun-2 variety was sown. Before seed sowing, the land was ploughed. 600kg of well-decomposed FYM @ 6 t ha^{-1} was applied as organic fertilizer on the research field. The field was fertilized with common inorganic fertilizer, namely nitrogen, phosphorus, and potash, at a rate of 120:60:40 kg ha^{-1}.

During final land preparation, urea((4.38kg), phosphorous(12.6kg), and potassium(2.24kg) were applied, followed by ploughing just before sowing. The remaining half dose of N was side dressed at 45 days after sowing. 182.5gm of Urea, 525gm of SSP and 93gm of MOP was applied per plot. A single maize seed was sown 3-4 cm deep and gently covered with soil at a distance of 25cm between plants and 70cm between rows in all plots giving 96 plants but in T8, at a distance of 20 cm between plants and 60 cm between rows giving 120 plants. Seed rate of 25-30 kg ha^{-1} was used. Irrigation was done at 2-3 days interval and completely stopped after the silking stage. Furrow irrigation was done in the field based on moisture conditions to support crop growth. Earthing up was done in all plots except black plastic mulch at 45 DAS after the side dressing was completed. To control the infestation of FAW (*Spodoptera frugiperda*), application of Emamectin benzoate @10gm/16lit was done at 45DAS.

2.4 Observations and measurements

Phonological stages were measured in term of days to emergence, plant height (cm), Leaf number, Leaf Area Index at different days after sowing. Days of emergence was considered when 80% plants were emerged. Phonological data from 10 plants were collected at 45, 60 and 75 DAS using measuring tape and scale from 2^{nd} and 5^{th} rows of each plot.

Leaf Area Index: Leaf area index is defined as one sided green leaf area per unit ground surface area. It was calculated by dividing the leaf area per plant by the land area occupied by single plant (Sestak *et al.*, 1971).

LAI=A/P

Where, LAI = Leaf area index 21 A = Leaf area (cm2) P = Unit land area (cm2)

2.5 Statistical analysis

The data collected from the experiment at germination, plant height, leaf number, Leaf Area Index were first tabulated in Microsoft excel (2016). Analysis of Variance (ANOVA) for all parameters was analyzed by using R Stat version 4.2.1 at 5% level of significance. Duncan Multiple Range Test (DMRT) was used for mean separation as prescribed by (Gomez, & Gomez, 1984).

3. RESULTS AND DISCUSSION

3.1 Days to Emergence

As shown in Table no 1, Effect of different types of weed management practices on days to emergence was nonsignificant but least days to emergence was observed in black plastic mulch (30.66) as compared to control plot (42.66). Successful emergence is a combination of three key factors i.e., environment, genetics, and seed quality. As single variety was used and experiment was done in same land, this might be reason for getting non-significant results in days of emergence.

3.2 Leaf Area Index

The LAI in the experiment was significantly influenced by weed management practices at all dates of observation (Table no.1). Statistically highest leaf area index was observed in black plastic mulch 1.58, 2.72, 2.34 at 45,60 and 75 DAS respectively. At 45 and 75 DAS, black plastic mulch was at par with narrow inter-row spacing (1.29, 2.01, 1.72). These results are in agreements with Bu *et al.* (2013) and Kole, (2010). The higher leaf area index in the polythene mulch might be attributed to their weed control, thus providing favorable conditions also accelerated vegetative growth in polythene mulches while higher LAI in high density planting might be due to a greater number of plants per unit area attributing more leaf area per unit land area with slight reduction in leaf area per plant (Gul *et al.,* 2009).

Table no.1. Effect of different weed management practices on days to emergence and Leaf Area index of Maize in gauradaha, Jhapa 2022.

Treatment	Days to emergence	Leaf Area index 45 DAS	Leaf Area index 60 DAS	Leaf Area index 75 DAS
Control	42.66 ± 5.36	0.747^{cd}±0.026	1.46^{cde}±0.211	1.33^{b}±0.302
Cover crop	48.00 ± 8.51	0.678^{d}±0.095	1.15^{de}±0.298	1.04^{b} ±0.297
Hand weeding	34.66 ± 0.33	0.907^{bcd}±0.0987	1.72^{bc}±0.151	1.54^{b} ±0.157
Botanical weedicides	39.00 ± 4.93	0.734^{cd}±0.116	1.09^{e}±0.164	1.05^{b} ±0.128
Inorganic weedicides	41.66 ± 8.21	0.842^{cd}±0.15	1.53^{cd}±0.064	1.64^{ab}±0.16
Plastic mulch	30.66 ± 3.48	**1.58^{a}±0.207**	**2.72^{a}±0.202**	**2.34^{a}±0.356**
Straw mulch	33.32 ± 2.40	1.17^{abc}±0.03	1.84^{bc}±0.143	1.65^{ab}±0.082
Narrow inter-row spacing	40.00 ± 8.89	1.29^{ab}±0.215	2.01^{b}±0.196	1.72^{ab} ± 0.327
Grand mean	38.75	0.99333	1.690833	1.539167
C.V	25.54	23.26135	18.89	25.5788
M. Error	98.02	0.05339	0.1021	0.1550
LSD	17.33	0.4046396	0.559	0.6894524
F Value	0.972ns	**5.795^{**}**	**8.047^{***}**	**3.368^{*}**

Note: C.V.: Coefficient of Variation, ns: not significant, LSD: Least Significant Difference, (***): Significant at 0.1% level of significance, (**): Significant at 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 Plant height(cm)

Effect of different types of weed management practices on plant height and leaf number was shown in Table no.2. Plant height was recorded significant in 45, 60 and 75 DAS. At 45 DAS, Highest plant height was recorded in black plastic mulch (136.75 cm) which was at par with straw mulch (120.26 cm). Plant height was observed highest in black plastic mulch (234.43 cm, 229.10 cm), followed by Hand weeding (183.46 cm, 184.43 cm) at 60 DAS and 75 DAS respectively.

These results are in agreement with findings of (Mahajan *et al*., 2007) and (Hussain *et al.*, (2022). The increased plant height in polythene mulch was attributed to excellent weed control, high water use efficiency along with early and vigorous growth of plants (Mahajan *et al.,* 2007). Plastic mulch raised soil temperature, which might have accelerated maturity of maize plants. These results for mulches effect are in line with the work of Yonge, (1994) that plastic mulch significantly raised soil temperature, kept soil water content stable and enhanced microbial activity as a result crops grew faster. Plastic mulching helps to adjust soil environment by subsequent increase in soil temperature, reduction in evaporation, weed competition, soil compaction and soil erosion which promote water and nutrient absorption ability of root. This might be the reason for increased plant height in plastic mulch treated plot (Clark *et al.,* 2003). Similarly, plastic mulching results a change in microclimate due to reduction in nitrate leaching which indirectly increases nutrient availability in plastic mulch treated plot (Bandyopadhyay *et al.,* 2018). The reduction of nitrate leaching results in optimum utilization of applied Nitrogen which might be the reason for highest plant height in plastic mulch treated plot (Qin *et al.,* 2015).

3.4 Leaf Number

There was a significant effect of different weed management practices on Leaf number at 45,60 and 75 DAS. At 45 DAS, maximum number of leaves was observed in black plastic mulch (10.83). Similarly, Leaf number of 11.91 i.e., in black plastic mulch was recorded which was at par with hand weeding (10.43), straw mulch (10.33), Inorganic weedicides (10.33), narrow inter-row spacing (10.12) at 60 DAS. Likewise, Black plastic mulch showed highest leaf number of 11.26 at 75 DAS. Prevented contact between the soil and dry air which reduced water loss into the atmosphere through evaporation, reduced surface runoff, and reduced moisture depletion all together increases soil moisture content and showed better results in terms of the number of leaves (Sharma *et al.*,2022).

Table no. 2. Effect of different weed management practices on Plant height(cm) and Leaf number of Maize in gauradaha, Jhapa 2022

Treatments		**Plant height (cm)**			**Leaf number**	
	45 DAS	**60 DAS**	**75 DAS**	**45 DAS**	**60 DAS**	**75 DAS**
Control	9.01^{c}±0.8	135.06^{b}±35.2	171.06^{bc}±2.19	7.47^{bc}±0.38	10.06^{ab}±0.37	9.23^{b}±0.43
Cover crop	94.33^{c}±5.84	145.30^{b}±18.4	182.70^{b}±11.7	7.33^{bc}±0.55	8.90^{b}±1.05	9.63^{b}±0.29
Hand weeding	12.68^{bc}±4.80	183.46^{b}±9.22	184.43^{b}±9.49	8.80^{b}±0.35	10.43^{ab}±0.27	9.80 ^{b}±0.4
Botanical weedicides	9.39^{c}±0.55	149.76^{b}±9.99	153.02^{c}±8.07	7.23^{c}±0.32	8.80^{b}±1.16	8.53 ^{b}±0.91
Inorganic weedicides	06.41^{bc}±6.12	177.13^{b}±0.41	179.33^{bc}±0.83	8.13^{bc}±0.65	10.33^{ab}±0.57	9.86 ^{b}±0.47
Plastic mulch	**36.75^{a} ±7.01**	**234.43^{a}±4.59**	**229.10^{a}±5.84**	**10.83^{a}±0.06**	**11.91^{a}±0.31**	**11.26^{a}±0.3**
Straw mulch	20.26^{ab}±3.97	178.21^{b}±3.86	182.70^{b}±1.35	8.60^{bc}±0.17	10.33^{ab}±0.52	9.70 ^{b}±0.26
Narrow inter-row spacing	06.35^{bc}±8.69	171.60^{b}±11.1	176.76^{bc}±9.51	7.63^{bc}±0.52	10.12^{ab}±0.37	9.56 ^{b}±0.27
Grand Mean	109.40	171.90	182.37	8.25	10.08	9.7
C.V	8.95	16.54	8.03	9.27	9.80	7.99
MS Error	95.9	809.3	214.6	0.58	0.97	0.60
LSD	17.14	49.81	25.65	1.34	1.73	1.35
F Value	**00234****	**3.520***	**3.00***	**7.28****	**2.88***	**2.92***

Note: C.V.: Coefficient of Variation, LSD: Least Significant Difference, (***): Significant at 0.1% level of significance, (**): Significant at 1% level of significance, (*): Significant at 5% level of significance, Figure after ± indicate standard error, Same letter signify no significant difference between treatments.

4. CONCLUSION

This study showed that plastic mulching had a considerable impact on plant height, leaf number and Leaf Area Index. The various weed management practices did not significantly affect Days of emergence. Overall, the use of plastic mulching can be an effective strategy for enhancing the phonological performance of spring maize in Jhapa. For small scale farming, straw mulching can be the best alternative.

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Authors' contribution

K. Khanal: Designed & conducted experiment, data collection & analysis, and manuscript writing

R. Limbu Sanwa: Designed experiment, and data analysis

S. Baral: Data analysis, and manuscript writing

G. Dhital: Designed experiment, and data analysis

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FARMERS' KNOWLEDGE AND MANAGEMENT PRACTICES OF FALL ARMYWORM IN MAJOR MAIZE-PRODUCING DISTRICTS OF NEPAL

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ABSTRACT

Spodoptera frugiperda (J. E. Smith), commonly known as Fall Armyworm (FAW), poses a significant threat to maize crops globally as well as in Nepal. A household survey was conducted in July 2023 at Lamahi, Dang and Raptisonari, Banke using a purposive sampling of 120 maize farmers to investigate the farmers' knowledge on FAW and its management. The survey revealed that 65% of respondents in Dang and 76.67% in Banke perceived FAW as the most problematic insect pest. Chemical pesticides were predominantly used for FAW management by 90% and 75% of respondents in Dang and Banke, respectively. The order of management strategy preference was chemical>mechanical>cultural methods. The farmers in Banke exhibited a higher pesticide usage (10%) compared to Dang (3.33%). Furthermore, the frequency of pesticide application and the types of management practices varied between the two districts, reflecting regional differences in agricultural practices. In both regions, farmers perceived chemical pesticides as the most effective method, whereas botanical and cultural practices were considered less effective. However, the study revealed some concerning practices among farmers, such as the misuse of chemical pesticides without reading labels, inadequate protective measures during pesticide application, and not following the waiting period instructions. A significant number of farmers (43.33%) relied on progressive farmers for technical assistance, highlighting the importance of improving extension services and promoting integrated pest management (IPM) knowledge. The findings emphasize the need for enhancing farmers' realization of the harmful effects of chemical pesticides, promoting the use of protective gear, and the judicious use of chemicals as a last resort. In conclusion, improving extension services and disseminating IPM knowledge are crucial for sustainable pest management in maize.

1. INTRODUCTION

Agriculture plays a pivotal role in sustaining economic prosperity and supporting the livelihoods of the majority, contributing to inclusive economic growth through scientific advancements (NPC, 2019). In Nepal, about 62% of the population relies on agriculture (National Statistics Office, 2023). Half of the total agriculture-dependent population are small-scale farmers, who face unique challenges due to their limited land holdings (Kafle, 2018). Maize production contributes to 25.02% of total cereal production (Pandey & Basnet, 2018) and 7.6% of the Agricultural

Gross Domestic Product (AGDP) (MoALD, 2078/79). Hence, maize production has emerged as a crucial agriculture sub-sector with a significant contribution to the nation's GDP.

Maize (*Zea Mays* L.) holds a central position in agriculture as the most extensively grown highland cereal and a staple food crop in many developing nations (Dawadi & Sah, 2012). In Nepal, maize ranks second in both production (3106397 MT) and acreage (985565 ha) among staple foods, with rice holding the top position (AITC, 2023). Maize is cultivated across diverse ecological zones from the Terai to the high hills for food, feed, and fodder production (Poudel & Paudyal, 2001). Maize is produced in hilly regions predominantly as a staple food crop, whereas in the Terai, the poultry and livestock feed industry is the major sink (Ransom *et al.,* 2003). The districts of focus in this survey are also decent producers of maize. Dang and Banke produced 77,999 and 55, 747 Mt maize respectively in the fiscal year 2020/21 (MoALD, 2022). PMAMP's Project Implementation Unit, Dang established the maize superzone in 2016/17 covering 3100 ha of hand and it has served about 121 groups/cooperatives/farms until now (PMAMP, 2024). In a report by the Prime Minister Agriculture Modernization Project's (PMAMP) maize super zone, it has been stated that they had established 19 custom hiring centres, 3 silage industries, 1 pallet industry, 1 maize flour and grits mill and 1 seed processing unit by 2020/21 (Regmi, 2021). In the same report, it has been stated that the combined efforts from PMAMP and CSISA (Cereal Systems Initiative for South Asia) had been able to introduce Nepali maize hybrid RH10 in Dang (175 ha) within the same time point. The report adds that the coverage of spring maize in Dang had been increased from 300 ha to 2000 ha. The activities of PMAMP, Banke have also been effective in the sense that the maize cultivated area has doubled in the two years of implementation with a total of 40 producer-farmer groups (Setopati, 2018). These reports suggest that Dang and Banke are doing better in maize production with the help of various government and non-government organizations.

There is a huge global demand for maize because it has diverse applications in industrial processes (Andorf *et al.*, 2019). Beyond its role as food and feed, maize is a crucial raw material for diverse products such as corn oil, margarine, corn syrup, and sweeteners, and it has other applications in ethanol production, plastics, and high-quality paper industries (Gamage *et al.*, 2022; Kaul *et al.*, 2019; Naz *et al*., 2019). Biotechnological advancements in maize have facilitated the development of elite cultivars with traits like drought tolerance, herbicide resistance, and high protein content to meet the escalating demand (Malenica *et al.*, 2021). However, the introduction of invasive alien species (IAS) poses a substantial threat to agricultural productivity, biodiversity, and food security globally (Meyerson & Reaser, 2003). In developing countries like Nepal, the invasion of IAS has intensified due to increased global trade, changing land-use patterns, and climate change (Thapa *et al.*, 2018). Among these invasive species, the Fall Armyworm (FAW) *Spodoptera frugiperda* (J. E. Smith) is a lepidopteran insect indigenous to the Americas, has emerged as a significant economic pest capable of causing widespread economic damage to crops (Bateman *et al.*, 2018). This pest poses a serious threat because of its voracious feeding on cereals which may potentially lead to complete crop loss if not managed effectively (Beshir *et al.*, 2019; Frerot *et al.*, 2017; Pogue, 2002).

The sudden appearance of FAW in Africa (2016) and its subsequent spread to sub-Saharan Africa within a year underscored its potential to threaten global food security (Day *et al.*, 2017; Goergen *et al.*, 2016). In Nepal, the first detection of the FAW occurred in the Nawalparasi district in 2019, and it was locally named "*American Phaujikira*" (Pokharel, 2019). Given the potential consequences of the FAW invasion, effective management strategies are essential. Integrated Pest Management (IPM) emerges as a comprehensive approach, incorporating cultural control, biological control, and judicious pesticide application (Khanal *et al.*, 2021) to mitigate the impact of this invasive pest (Day *et al.*, 2017). As Nepal grapples with the challenges posed by FAW, understanding farmers' perceptions and practices is crucial for devising sustainable and context-specific management strategies. This research aims to investigate the current status of FAW in maize farming and to assess the management practices adopted by farmers in the Dang and Banke districts in the Lumbini Province.

2. MATERIALS AND METHODS

2.1. Site

The survey covered Dang and Banke districts in Nepal, specifically Lamahi municipality, Dang (Lamahi-7: 27°52'15.1" N 82°27'46.9" E) and Raptisonari rural municipality, Banke (Raptisonari-1: 28°00'32.5" N 82°05'34.5" E and Raptisonari-3: 28°01'47.2" N 81°55'52.4" E). These municipalities were selected due to their heavy reliance on agriculture, particularly maize cultivation. Lamahi and Raptisonari were purposively chosen based on the significant impact of FAW on the livelihoods of the communities residing there.

2.2. Survey design

A structured non-disguised questionnaire with closed-ended questions was used in the survey. The questionnaire was pretested in 10% of the total sample frame to optimize the questionnaire. Respondents were selected by purposive sampling to make the survey representative and inclusive as much as possible. The study targeted individuals above 25 years of age with at least 10 years of residency in these localities, ensuring a sample capable of providing valuable insights into past climatic hazards. A total of 120 households, 60 from each municipality, were randomly selected to make an inclusive sample frame. Respondents were surveyed using a one-to-one interview method. The information from respondents was validated simultaneously.

2.3. Data entry and analysis

The data collected from both primary field surveys and secondary sources underwent coding, tabulation, and analysis using the Statistical Package for the Social Sciences (SPSS Version 25) and Microsoft Excel. Various socio-demographic factors, pest management practices in maize, and economic variables, including the age of respondents, education level, land holdings, ethnicity, and main occupation, were characterized using basic descriptive statistics such as frequency count, percentage, mean, and chi-square analysis. The findings were visually represented through pie charts, bar diagrams, and tables for effective presentation and interpretation.

3. RESULTS AND DISCUSSION

3.1. Demographic and socioeconomic characteristics of respondents

The demographic and socioeconomic status of the respondents is mentioned in Table 1. The majority (55.83%) of respondents were male. However, there was no distinct dominance of one gender in any survey district. 46.67% of the respondents were between the age of 30-45. The literacy rate of the respondents was on the higher side of the spectrum with 72.5% representation. The study was inclusive with 85% of the respondents being *Janajatis* (Indigenous groups) or *Dalits*. These ethnic groups belong to the marginalized and the underprivileged in Nepal (Gurung, 2009). About 84.17% of the respondents possessed more than 5 kattha land implying that most of the farmers had a decent land size. 71% of these farmers had been continuing agriculture for a decade; 67.5% of all respondents accepted that agriculture was their main occupation but only half of the total farmers were able to produce crops commercially.

Table 1. Demographic and socioeconomic characteristics of the respondents

Attributes	Frequency (%)			χ^2	P-Value
	Dang	Banke	Aggregate %		
Gender				0.85	0.36
Male	36(60)	31(52)	55.83		
Female	24(40)	29(48)	44.17		
Age group				4.86	0.18
Below 15	0(0)	1(1)	0.83		
15-30	22(36)	15(25)	30.83		
30-45	29(49)	27(46)	46.67		
Above 45	9(15)	17(28)	21.67		

Level of education				13.29	<0.01
Illiterate	17(28)	16(26)	27.50		
Literate	5(8)	9(15)	11.67		
Primary Level	25(42)	16(26)	34.17		
Secondary level	5(8)	17(29)	18.33		
Higher Secondary	8(13)	2(3)	8.33		
Ethnicity				58.68	0.00
Brahmin	0(0)	2(3)	1.67		
Kshetri	0(0)	16(26)	13.33		
Janajati	59(99)	19(31)	65.00		
Dalit	1(1)	23(39)	20.00		
Land holdings (Kattha)				17.51	0.00
Below 5	2(3)	17(28)	15.83		
6 to 10	13(21)	17(28)	25.00		
11 to 15	11(18)	7(11)	15.00		
16 to 20	11(18)	6(10)	14.17		
Above 20	23(40)	13(23)	30.00		
Purpose of agriculture				14.70	0.00
Subsistence	19(31)	40(67)	49.17		
Commercial	41(69)	20(33)	50.83		
Experience of farmers				7.21	0.07
At least 1 year	1(1)	0(0)	0.50		
2-5 years	10(16)	3(5)	10.50		
6-10 years	13(21)	9(15)	18.00		
Above 10 years	36(62)	48(80)	71.00		
Occupation				3.67	0.30
Agriculture (Primary)	41(69)	40(68)	67.50		
Agriculture (Secondary)	10(16)	16(26)	21.67		
Returnee migrants	1(1)	1(1)	1.67		
Jobs/services	8(13)	3(5)	9.17		

3.2. The current status of maize production

The most popular maize variety in the survey area was Rampur composite followed by a local variety called Baijapure. Half of the farmers accepted that insect was the major problem in their farm. Only 10.83% of the farmers reported that unavailability of inputs was the major problem, whereas, 20.83% of farmers reported that unpredictable weather was a bigger problem than the unavailability of inputs. Before the invasion of FAW in Nepal (Bajracharya et al., 2019), 46.67% of respondents reported no pest infestation in their maize fields, whereas, the rest 53.33% reported maize stem borer was the most notorious pest. However, 70.83% of respondents think that FAW is the most harmful maize pest after its invasion. 27.5% of the farmers report more than half of their crop was lost due to FAW infestation and 44.17% of the farmers report damage between 30-50%. Most of the farmers gained their knowledge on FAW from progressive farmers and agro vets. A similar dependency of farmers on agro-vets was found in Chitwan, (Rijal *et al.*, 2018) and Surkhet (Lamsal *et al.*,2022)too.

Table 2. The current status of maize production

Attributes	Frequency (%)			$\chi 2$	P-Value
	Dang	Banke	Aggregate %		
Varieties of maize				45.95	0.00
Arun 2	3(5)	11(18)	11.67		
Rampur Composite	32(55)	17(28)	40.83		
Rajkumar	4(6)	0(0)	3.33		
Local (Baijapure)	6(10)	32(54)	31.67		
Tenveten	6(10)	0(0)	5.00		

Subarna	4(6)	0(0)	3.33		
Shankar	5(8)	0(0)	4.17		
Major problems of maize farming				29.41	0.00
Insect	25(40)	35(61)	50.00		
Disease	9(17)	1(1)	8.33		
Unpredictable weather	12(20)	13(21)	20.83		
Unavailability of inputs	12(20)	1(1)	10.83		
Wild animals	0(0)	10(16)	8.33		
Rodents	2(3)	0(0)	1.67		
Pest problems before 2019				1.21	0.27
Maize stem borer	35(60)	29(480	53.33		
No pest before	25(40)	31(52)	46.67		
Ranking of FAW				7.06	0.29
Most problematic of all	39(65)	46(77)	70.83		
Least problematic	2(3)	6(10)	6.67		
As problematic as other insect pest	19(32)	8(130	22.50		
% Yield loss due to FAW				0.60	0.90
Relatively fewer quantity (10-15%)	4(6)	6(10)	9.80		
Average (15-30%)	12(20)	12(20)	20.00		
Relatively larger quantity (30-50%)	28(48)	25(42)	44.17		
Very large quantity (>50%)	16(26)	17(28)	27.50		
Most problematic season				53.11	0.00
Rainy	5(8)	43(72)	40.00		
Winter	12(20)	8(13)	16.67		
Spring	43(72)	9(15)	43.33		
Source of information about FAW				13.61	0.09
Training	3(5)	3(5)	5.00		
Agrovet	26(43)	14(23)	33.33		
I/NGO	3(5)	7(11)	8.33		
Progressive Farmers	27(46)	25(43)	43.33		
Villagers	1(1)	11(18)	10.00		

3.3. *Knowledge of the stage of infestation and symptoms of FAW*

The majority of respondents identified the vegetative stage as the most critical for FAW damage (94.17%), followed by the reproductive stage (3.33%). A study reports how FAW larvae attack the leaves resulting in sequential loss of photosynthetic area on leaves and a significant reduction in growth and development of the crop (Chimweta *et al.*, 2020). Equal percentages of respondents considered damage near the seedling, pre-tassel, and post-tassel stages (0.83% each). The majority of respondents noted leaf damage as the predominant symptom of FAW (54.17%), followed by damage to the infested whorl (35.83%). High temperature and low rainfall were considered the primary abiotic factors influencing FAW infestation by the majority of respondents (99.17%).

Table 3. Knowledge of the stage of infestation and symptoms of FAW

Attributes	Frequency (%)			χ^2	P-value
	Dang	Banke	Aggregate %		
Stage of damage				4.08	0.40
Seedling	0(0)	1(1)	0.83		
Vegetative	58(98)	55(96)	94.17		
Pre-tassel/tassel	0(0)	1(1)	0.83		
Reproductive	1(1)	3(3)	3.33		
Post-tassel	1(1)	0(0)	0.83		
Damage symptoms				6.13	6.13
Leaf damage	37(64)	28(47)	54.17		
Windowpane effect	3(5)	3(5)	5.00		
Defoliation	1(1)	0(0)	0.83		
Infested whorl	17(28)	26(43)	35.83		
Ear/ cobs damage	1(1)	3(5)	3.33		
Feed on tassels	1(1)	0(0)	0.83		
Abiotic factors influencing infestation				1.01	0.32
Low relative humidity	0(0)	1(1)	0.83		
High temperature and low rainfall	60(100)	59(99)	99.17		

3.4. Practices of FAW management

The majority of respondents employed plant diversity as a preventive measure against FAW (33.33%), followed by the use of quality seeds (30.83%) and 27.5% of the respondents were unable to employ any preventive measures. Farmers reported that the change in shifting of maize planting dates did not affect the FAW infestation. However other studies suggest that early planted maize, particularly in the early rainy days of the season, observes a higher damage from FAW since maize is the sole food available to the first batch of hatching larvae (Mbaidiro *et al.*, 2023). The larvae could still get washed off the crop during heavy rain and consequently become more easily available to their natural enemies (Zalucki *et al.*, 2002). Mbaidiro *et al.* (2023) explain that rainfall-induced mortality of FAW larvae is lower in the early days of the rainy season because of lower precipitation. In contrast, Sowmiya *et al.* (2022) observed higher yields in early-planted maize under similar conditions. 82.5% of farmers used chemical pesticides against FAW and this rate was up to 90% in Dang alone. It could also be because there are intensive input subsidy-based programs concentrated in Dang for it has been categorized as the maize super-zone by the PMAMP. Only 5% of farmers were found to have applied pesticides pre-pest-emergence, whereas, 80.83% of farmers applied pesticides after the pest-emergence. About 84% of the respondents accepted that they had used pesticides, at least once, during the crop cycle against FAW.

Table 4. The current FAW management practices used at the study areas

Attributes	Frequency (%)			χ^2	P-value
	Dang	Banke	Aggregate %		
Preventive measures				31.56	0.00
Quality seed	9(10)	31(52)	30.83		
Plant diversity	23(38)	17(28)	33.33		
Planting time	4(7)	6(10)	8.33		

none	27(45)	6(10)	27.50		
Management practices				9.07	<0.03
Cultural	3(5)	1(2)	3.33		
Botanicals	0(0)	1(2)	0.83		
Mechanical	3(5)	13(22)	13.33		
Chemical pesticides	54(90)	45(75)	82.50		
Timing of pesticide applications				4.04	0.13
No application	6(10)	14(23)	16.67		
After pest emergence	52(87)	45(75)	80.83		
Before pest emergence	2(3)	1(2)	5.00		
Frequency of pesticide applications				4.21	0.24
No use of pesticide	6(10)	14(23)	16.67		
One time use	28(47)	23(38)	42.50		
Two times use	20(33)	16(27)	30.00		
Three times use	6(10)	7(12)	10.83		

3.5. Safety measures to minimize hazard

The majority (86.67%) of farmers were found unaware of pesticide hazards. Though 81.67% of farmers reported that they measured pesticides during spray solution preparation, it is difficult to trust the responses since we could not find supportive documentation for such high pesticide measurement compliance in Nepal. Only 34.17% of respondents said that they were aware of the waiting period for pesticides. The same number of farmers reported that they were used to reading labels on pesticides. The farmers who follow the waiting period are bound to be lower than those who are aware of it. 72.5% of farmers reported that they wear masks while applying pesticides. However, an inadequate safety measure was highlighted when only 20.83% of farmers reported that they used gloves. Only 10.83% of farmers reported that they used personal protective equipment (PPE) during pesticide spraying. A similar result was reported by Thapa *et al.* (2021) who conducted a similar study in Bhaktapur, Nepal. Compliance with safety precautions is low also because farmers unaware of pesticide hazards tend to neglect the usage of proper PPEs in addition to the discomfort they feel while wearing them (Singh & Gupta, 2009).

Table 5. Pesticide use and application of safety measures

Attributes	Frequency			χ^2	P-value
	Dang	Banke	Aggregate%		
Knowledge of hazard level of pesticides				0.29	0.59
Known	9(15)	7(12)	13.33		
Unknown	51(85)	53(88)	86.67		
Pesticides measurement practices				3.56	<0.06
Yes	53(88)	45(75)	81.67		
No	7(12)	15(25)	18.33		
Knowledge of the waiting period				1.82	0.18
Known	17(28)	24(40)	34.17		
Unknown	43(72)	36(60)	65.83		
Use of PPE				0.78	0.78
Yes	8(13)	5(8)	10.83		
No	52(87)	55(92)	89.17		

Use of mask				0.38	0.54
Yes	45(75)	42(70)	72.50		
No	15(25)	18(30)	27.50		
Use of gloves				0.46	0.50
Yes	11(18)	14(23)	20.83		
No	49(82)	46(77)	79.17		
Awareness of labels and instructions				0.93	0.34
Yes	23(38)	18(30)	34.17		
No	37(62)	42(70)	65.83		

3.6. Changes in FAW management approaches

About 64% of respondents reported that they had been using chemicals rationally. Only 35% of farmers agreed that they had been employing IPM strategies in their farms. Less than 1% of farmers turned towards lures and traps for pest surveillance. 74.17% of farmers reported that they started using a higher quantity of pesticides to counter FAW. Agriculture professionals advocated that technical training and subsidized pesticides were the most effective ways to fight against FAW infestation. Atreya (2007) advocates the positive impacts of pesticide training on the usage of PPEs by farmers. Many studies suggest that several positive impacts are observed in farming activities when farmers receive training regularly (Gautam *et al.*, 2017; Huang *et al.*, 2012; Noor & Dola, 2011).

Table 6. FAW management practices and expectations from agriculture professionals

Attributes category	Frequency (%)			χ^2	P-value
	Dang	Banke	Aggregate %		
New practices of management				6.00	<0.05
Rational use of pesticides	44(74)	33(55)	64.17		
Use of lures and traps	1(1)	0(0)	0.83		
IPM strategies	15(25)	27(45)	35.00		
Differences between conventional and new practices				9.59	<0.02
Increased use of pesticides	39(66)	50(84)	74.17		
Decreased use of organic fertilizer	2(3)	0(0)	1.67		
Increased use of chemical fertilizers	8(13)	8(13)	13.33		
Increased use of hybrid seed	11(18)	2(3)	10.83		
Expectations from agriculture-related personnel				17.41	0.00
Training	31(52)	49(82)	66.67		
Quality seed	7(11)	1(1)	6.67		
Irrigation	4(6)	0(0)	3.33		
Free pesticides	15(25)	6(10)	17.50		
Organic pesticides	0(0)	1(1)	0.83		
Regular field visit	3(5)	3(5)	5.00		

4. CONCLUSION

Fall Armyworm has been a devastating pest in maize fields since it invaded Nepal, and farmers have very limited knowledge of FAW management. They mainly depend on agro-vets and agriculture professionals for technical support. During the initial years after the invasion, even the service providers lacked proper information to manage FAW. Hence, a tendency to recommend strong chemical pesticides arose with increasing panic among farmers concerning the damage in their maize fields. This survey collected information from 120 respondents to find out the knowledge and practices among maize farmers about FAW management. Results show that FAW is the major problem in maize after 2019. About 72% of the respondents reported that FAW can cause at least 30% damage in maize cropping. The majority of respondents believed that a combination of high temperature and rainfall enhances FAW infestation and resulting damage. More than 80% of farmers applied synthetic pesticides against FAW infestation. There is a lack of awareness of pesticide hazards among farmers. Even among those who are aware, the use of good pesticide application practices is rare. There are various underlying causes of the FAW outbreak and the consequent crop damage. Hence, it needs further research and extension on FAW management technologies.

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IMPLEMENTATION STATUS OF FOOD SAFETY MANAGEMENT SYSTEMS IN NEPALESE FOOD INDUSTRIES

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ABSTRACT

Food industries in Nepal are now becoming a crucial part of the food delivery system where the final quality of the food products is largely influenced by the operations carried out in the food industry. In order to protect public health from food related hazards, there is need for the food industries to have food safety management systems that assure the safety of the processed/final food products. The current study was conducted to explore and evaluate the status of the food safety and quality management systems of the food manufacturing industries across Nepal along with their challenges in its implementation. The method of data collection was primary in nature. Questionnaires were self- administered to the owners, managers/ supervisors of the food manufacturing industries and to the officials working in the field of food safety from the Government of Nepal. Food Safety management systems were found to be gradually improving but not at a satisfactory level as there are no mandatory legal provisions for the system certification of food industries till now. Therefore, food manufacturing industries in Nepal needs to be strengthened by implementing a broader concept of HACCP and other food safety management systems along with pre requisite programs to assure safe food delivery.

1. INTRODUCTION

In recent times, there has been a surge in global consumer concerns about the safety of food products. This is mainly due to a series of food scandals that have taken place in the last decade, and the perceived lack of progress in preventing their frequency of occurrence. As a result, the food industry is now expected not only to produce safe food, a role that has always been recognized, but also to be transparent in demonstrating how food safety is planned and ensured. (Fotopoulos et al., 2009)

Consumers are susceptible to food-borne hazards that may occur during manufacturing processes, and therefore a basic requirement for any food processing operation is a Food Safety and/or Quality Management System. Examples of such Systems are HACCP and IS0 22000. These are certification systems which are implemented to prevent the risk caused by physical, chemical, and microbiological hazards during food processing and distribution (Arvanitoyannis & Traikou, 2005). Food safety and quality has received attention in the agri-food sector and is basis of all initiatives taken on different activity levels starting from farm to enterprises as a whole on regional, national, and international levels. A hybrid of the ISO 9001, Quality Management System and Hazard Analysis and Critical Control Point (HACCP), ISO 22000 has been developed as an international solution for assuring the food safety (Panghal et al., 2018). Food safety management systems such as HACCP, GHP and GMP have been developed over recent decades to provide the industry with excellent tools for the control of food safety. The framework of risk analysis has been proposed as the common way to evaluate risks and determine appropriate risk management interventions relating to societal public health goals (Gorris et al., 2006).

The potential for food and beverages cross-contamination may occur at any and all food processing steps including transportation from the farm fields to processing facilities, processing steps in the food manufacturing facilities (due to deficient SSOPs, lack of personnel training, deficient GHPs, deficient good process practices (GPP), food packaging and storage. Foodborne illness outbreaks are the result of the likely risk of contamination of food products during processing and packaging and storage activities. Therefore, the identification of the root causes of contamination is crucial to understand the likely sources and paths of contamination of foodborne outbreaks and product recalls (Lee et al., 2021).

Food industries in Nepal are scattered in the various parts of the country. The small scale industries with less capital and simpler technology generating employment at local level are based on processing of fruits and vegetables, milk and milk products, meat and meat products, grain mill products, bakery products, oil and fats, beverages, spices, noodles, tea and confectionaries, etc. The medium and large scale industries are based on mineral and processed drinking water, instant noodles, alcoholic and non-alcoholic beverages, biscuit, fruits and vegetables, ghee and oil etc. The global food trade has entered into a new era after establishment of World Trade Organization. The introduction of SPS and TBT Agreement has made imperative for the need of quality assurance system to be replaced by preventive and proactive approaches such as GMP and HACCP. (Shrestha & Joshi, 2007).

In context of Nepal, still a large number of food business operators haven't successfully implemented food safety and quality management systems within their organizations, as a result, food industries are underperforming due to their inability to assure quality food product and are unable to achieve the desired market share, competitive advantage, growth and profitability. Thus, the Food safety management system employed by the food industries in Nepal needs to be strengthened through capacity and infrastructural investment to assure safe production and delivery of the food. Furthermore, government needs to promote the certification systems in food businesses to reduce the issues imposing risk on food safety.

2. MATERIALS AND METHODS

This study was conducted from April to May 2022 to investigate the food safety status and implementation of Food Safety Management Systems in various food production companies across seven provinces of Nepal. The study employed a cross-sectional survey design.

Data collection was primarily conducted through self-administered questionnaires that consisted of both open and closed-ended questions. The questionnaires were distributed to the owners, managers/supervisors of the food manufacturing industries, and face-to-face interviews were conducted with the officials working in the field of food safety from the Government of Nepal. The questionnaire was designed based on basic current food safety status and implementation of Food safety management systems. The questions focused on various aspects of food safety, including food handling practices, quality control procedures, and compliance with food safety regulations.

3. RESULTS AND DISCUSSION

3.1 Global context of food safety certification and Nepal

Global consumers are worried about the safety of their food which is mostly caused by a continuing sequence of food scandals and incidents in the food business. In response, consumers are calling for high quality food, food integrity, safety guarantees and transparency. Similarly, governments are imposing new laws and legislations and retailers are imposing new demands on their supply chains. Food supply chains react by implementing systems to improve the product's quality and guarantee its safety, at the same time making it more transparent and reliable (Beulens et al., 2005).

In this era of globalization, food supply chains extend beyond national borders, evolution partly facilitated by new processed food and an environmental policy that is friendlier towards international food trade. While public standards, considered to be minimum standards of quality, protect the consumer, they often do not allow food industry companies to differentiate their products on the basis of quality characteristics to protect and gain market share when competing in national and regional markets (Smith, 2009). As a result, private standards and certification systems have emerged to bridge this gap and respond to regulatory developments. Generally, they are based on coordinated systems to meet consumer demand for quality characteristics based on product differentiation and market segmentation (Fulponi, 2006).

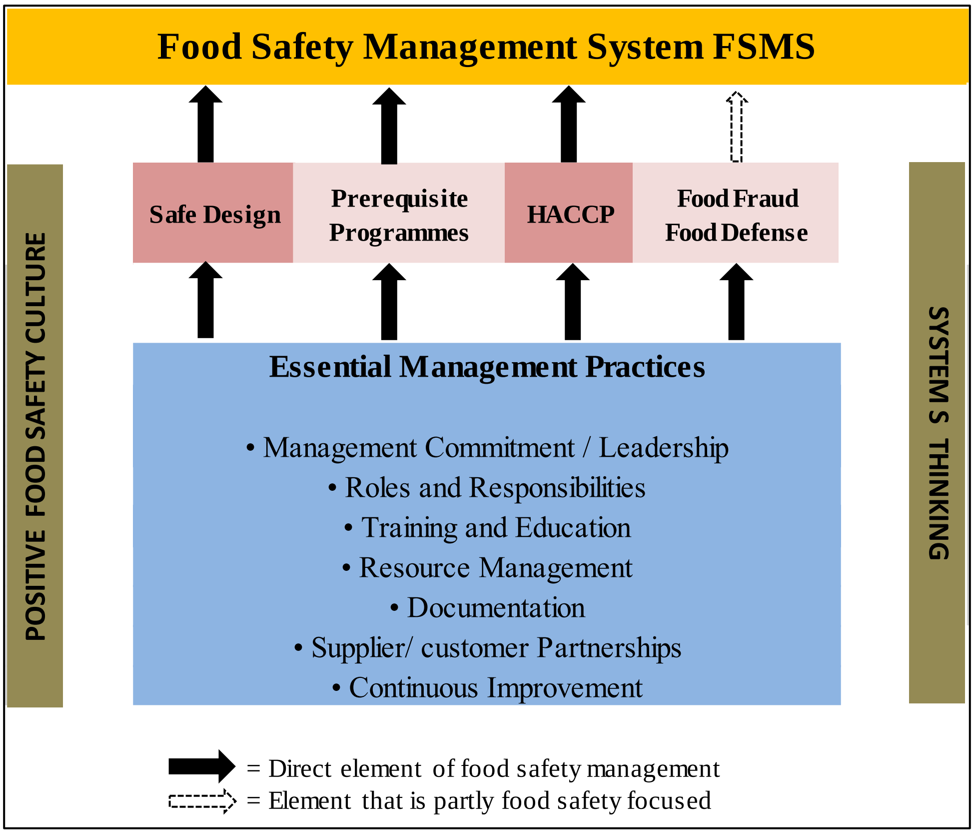

Figure 1. The interaction between FSMS, necessary good management practices, positive food safety culture and systems thinking (Wallace et al., 2018)

In order that a food company is able to conform to food safety specifications and requirements, food safety systems (FSS) have been created and launched worldwide. More specifically, in the food industry, FSS that are based on the HACCP principles (Hazard Analysis and Critical Control Points) or on the more recently launched ISO 22000 standard. These are applied to ensure food safety, to prevent liability claims and to build and maintain the trust of consumers (Kafetzopoulos et al., 2013; Motarjemi & Mortimore, 2005).

Ensuring food safety in today's complex food chain is a daunting task, and is possible only with the concerted effort of all sectors including government, consumer organizations and industry. Some quality assurance systems such as GMP, HACCP, ISO, BRC and GFSI, frequently found in the food industry, are obligatory by law, and others are implemented voluntarily by the food chain members (Sikora & Strada, 2005).

The concept of processed food industry is evolving in Nepal but is still at developing stage adapting indigenous technology in most of the business units. In Nepal, most of the available processed foods consists of cereal foods (processed rice, milled rice, flour, beans, pulses), dry foods (nuts, spices, tea, pickles, biscuits, snack foods, sugar, confectionery) and wet acidic foods (canned fruits, fruit juices, beer, wine, soft drinks, yoghurt, jam, jelly, squash and sauces). For quality assurance of their products many food industries elsewhere have started to adopt different food safety management methods. Good manufacturing practices (GMP) serves as the backbone in food safety and is gaining popularity among Nepalese food manufacturing sectors. To provide greater assurance of food safety, hazard analysis and critical control points (HACCP) program is also introduced in some industries and few are assigned with quality standard mark as NS. Apart from that, new concept of quality management like ISO 22000 is also adapted in some food industries.

3.2 Overall status of food safety management system in Nepal

Implementation of food safety programmes has been difficult for small and medium sized food companies (SMEs) in Nepal, taking into consideration specific practices witnessed amongst Nepali food producers. Small and medium sized food business operators tend to have a poor understanding of food management systems and limited adoption and implementation. Most of the food processing industries are scattered in various parts of the country with less capital, simpler technology and without any food safety management systems. Most of them are based on processing of fruits and vegetables, milk and milk products, meat and meat products, grain mill products, bakery products, oil and fats, beverages, spices, noodles, tea and confectionaries, etc. Lack of knowledge, lack of product specific guidelines, limited investment potential and lack of motivation for upgrading the existing quality status are some of the main challenges for the food industries in Nepal to lead them for a certification system. Likewise, most of the industries in Nepal run with limited capital which hinders them from investing both time and cost for installing food safety management systems. Considering the fact that many food processing industries in Nepal even do not meet the criteria to get a food license from DFTQC, implementing a food safety management system would require a huge effort in the present scenario. However, there are few large scale food based industries as well which are based on mineral and processed drinking water, instant noodles, beer, biscuit, ghee and oil etc where food safety management systems (HACCP/ISO 2200/ GMP) or Nepal Standard (NS) are implemented to assure the quality of finished products which can be listed as below in Table 1.

Table 1. List of major food industries implementing food safety management systems

S.N.	Name/Address of Food Industry	Food product
1.	Super Quality Meat Processing Industries P. Ltd., Butwal, Rupandehi	Meat and meat products
2.	Barun Beverage (Nepal) P. Ltd., Ramgram, Nawalparasi	Carbonated beverage, Processed drinking water
3.	Ten star Food Products, Devadaha, Rupandehi	Chowmein, Sauce
4.	Mega Food and Beverage P. Ltd., Siddarthanagar, Rupandehi	Processed drinking water
5.	Yasodha Foods P. Ltd., Tilottama, Rupandehi	Noodles, Cheese balls, Potato cracker
6.	S.R. Food P. Ltd, Omsatiya, Rupandehi	Wheat flour, Maida
7.	Kwality Food and Snacks Industries P. Ltd., Duhabi, Sunsari	Biscuit, Confectionery, Wafers etc.

8.	Kwality Diet and Food Products P. Ltd., Duhabi, Sunsari	Biscuit, Confectionery, Wafers etc.
9.	Mist Valley Tea Industries P. Ltd., Deumai, Ilam	Tea
10.	Nepal small Tea Producers Ltd., Fikkal, Ilam	Tea
11.	Gorkha Tea Estate P. Ltd., Fikkal, Ilam	Tea
12.	Nepal Green and Speciality Tea P. Ltd., Pashupatinagar, Ilam	Tea
13.	Himal Chiya Udhyog P. Ltd., Biratnagar, Morang	Tea
14.	Siddhibinayak Tea Industries P. Ltd., Fikkal, Ilam	Tea
15.	Swastik Oil Industries P. Ltd., Tankisinwari	Oil
16.	Vikas Food Products P. Ltd., Kohalpur, Banke	Wheat flour
17.	C.G Food Enterprises, Basgadhi, Bardiya	Noodles
18.	Sitaram Gokul Milks P. Ltd., Kathmandu	Milk and milk products
19.	Sundarijal Mineral Water Plant, Gokarneshwor, Kathmandu	Mineral water
20.	Himalayan Spring Water Inc., Mulpani, Kathmandu	Mineral water
21.	Swastik Oil Industries, Hattimuda, Morang	Oil

3.3 Legal provisions and past efforts to promote food certification in Nepal

In Nepal, there are no mandatory legal provisions for the system certification of food industries till now, however, Government of Nepal has enforced various acts, regulations, directives, guidelines which are still functional for assuring safety of the food products. For instance, Food Act- 2023, Food Regulations- 2027, Directives on Export Import Inspection and Quality Certification System in Nepal- 2007, Dietary Supplement Regulation Guidelines- 2016, Directives on Meat Products Processing Industry- 2017, Directives on Processed Drinking Water Industries- 2017, Directives on Categorization of hotels, restaurants, canteens based on food safety- 2017, Directives on Safety of Milk and Milk Products- 2018, NepalGAP Implementation Directives- 2019 etc. are some legislations formulated by Government to assure food safety and quality. Department of Food Technology and Quality Control (DFTQC) is the major governmental institution responsible for the food safety and quality management in Nepal. Recently, the Government of Nepal enacted the new Food Safety and Standards Act, 2081, set to replace the outdated Food Act, 2023. This new legislation aims to modernize and strengthen the regulatory framework for food safety, ensuring higher standards and better compliance across the food industry.

Apart from this, GMP and GHP’s are mandatory in certain commodities like processed drinking water, dairy industries, meat processing industries and in hotel and restaurants. Furthermore, Food Safety Policy-2019 has been brought into action by the Government of Nepal since 2019 which has envisioned to promote food safety in Nepal by upgrading all concerned organizations including the Government Authority, Organizations in the food chain and the consumers. The main purpose of the Policy is to develop a regulatory system to monitor and control food safety at all levels and protect the right of the people to get safe and quality food. Similarly, it will help to develop the organizations in the food chain to ensure food safety and quality of their products which will ultimately help to decrease the import of food products and support the export.

3.4 Factors influencing food quality management system implementation

Many researchers have revealed the factors that influenced positively or negatively the food quality management system implementation in food production industries (Vladimirov, 2011). These factors were typically two groups – internal and external to firms (FAO/WHO, 2006), and could be regarded simultaneously as incentives and barriers. To the internal factors belong the good knowledge and information about requirements, appropriate personnel training and motivation, availability of qualified specialists to deal with a great amount of documentation, or to overcome difficulties with validation and verification procedures after the system implementation (Fotopoulos et al., 2009). According to Wallace and Powell (2005), the HACCP system can prevent the food-borne cases only if it is implemented appropriately, e.g. if it works efficiently. At the same time the introduction of a quality management system requires a considerable extra work by all staff, which determines very often its unwillingness to accept standards. (Fotopoulos et al., 2009) summarized some common internal barriers the food companies faced as a lack of financial resources, motivated and trained personnel, skills, time restrictions, resistance on behalf of both top management and employees to change their way of working, etc.

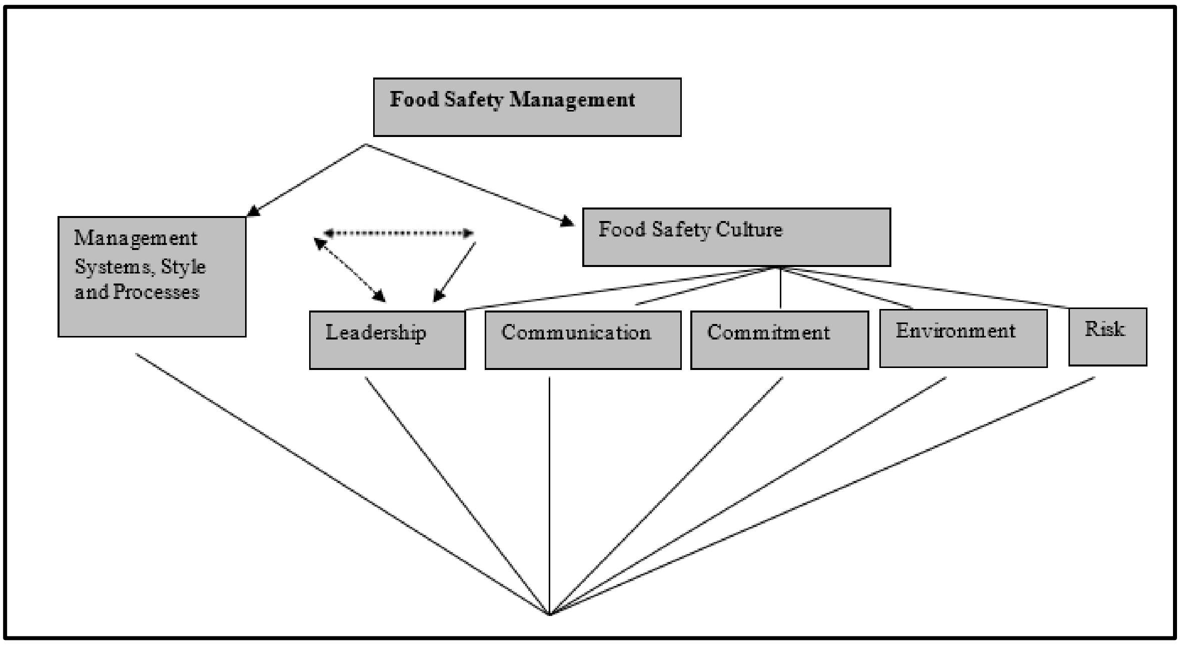

Figure2. Factors influencing Food Safety Management System (Pandi & Watson, 2018)

External to companies factors usually refer to the capacity of consultancy agencies to guide the implementation of food quality systems trust to and effects of the official control, information, environment, local infrastructure, etc. Researchers have agreed that in every country, region or food chain, the implementation of the food quality and safety system is determined by a complex set of factors, and each study could analyze only some of these factors (Vladimirov, 2011). The studies demonstrated also that firms respond differently to the obligation of the food quality system adoption. These differences persist among food sectors and subsectors, food retailers and catering, as well as among SMEs and large enterprises. That is why the significance of factors for the food safety system adoption depends on the stage of the development of firms, sectors, and even countries.

3.5 Benefits and challenges in implementing FSMS

Implementation of appropriate food safety management system is decided by the food manufacturers depending upon the specific situations which when implemented effectively not only assures the safety of the food but also show compliance with regulatory and customer requirements at each step in the food production chain (Trienekens & Zuurbier, 2008). As any other systems, there are benefits and challenges in installing Food safety management systems which can be described in Figure 3.

Benefits

- Increases food safety and quality
- Increases customer satisfaction
- Increases product shelf life
- Production cost reduction
- Improvement of employee motivation
- Access to international trade
- Sales volume improvement
- Management image improvement
- Employee skill improvement
- Reduces food waste
- Offer a defense against a lawsuit
- Business results improvement

Challenges

- Implementation costs
- Legal requirements
- Increased employee number
- Volume of paperwork
- Employee qualification
- Time limitation
- Internal resistance to change
- Difficulties in applying FSMS tools and methodologies
- Interpretation requirements
- Training requirements
- Top level management commitment

Figure 3. Benefits and Challenges of implementing FSMS.

3.6 Way Forward

In case of Nepal, there are still many areas to improve like infrastructure development, upgrading of laws/regulations, awareness of people to ensure food safety. Considering the current situation of Nepal, resources such as technical, financial and infrastructure are the major factors serving as barriers in the implementation of Food safety management systems in food industries. From government's perspective, it seems very important to make legal compulsions of ISO 22000, GMP and HACCP systems implementation for food industries considering product safety and their export potential. Likewise, general as well as product specific guidelines can be prepared for adoption in food industries which could guide for the implementation of GMP. After certification of GMP from DFTQC or sub-ordinate offices, the provision of renewal of food license would ultimately bring the uniformity as well as convenience in food control system. Similarly, guidelines adhering to HACCP or ISO principles can be adopted and customized for Nepalese food industries, serving as a foundation for the implementation of Food Safety Management Systems.

Apart from this, government can provide special subsidies and specific FSMS training programs to the industries interested in implementing FSMS. Fundamental to food safety management is the training and education of the staff. Training and education are the stepping stones in food safety management: they link theory to practice. From employees to managers, perception of risk, attitude, and skills are key determining factors in their practice. The same is true in relation to consumers, regulatory authorities, and other stakeholders in society who have a role in food safety. None of the scientific expertise, management systems, regulatory requirements, and experiences will be useful in ensuring safety, if the people who have to implement them are not knowledgeable, trained, motivated, or empowered to do so (Lelieveld et al., 2016).

Furthermore, government can make policies to encourage ISO/GMP/HACCP consultation and certification service providers to extend their service in cost effective manner to support small and medium scale food industries.

4. CONCLUSION

In Nepal, still a large number of food industries haven't implemented food safety management systems which is necessary for ensuring food safety and facilitating the international trade due to various reasons such as: cost of implementation, lack of knowledge of benefits of FSMS, lack of training and qualification, volume of paper work, lack of PRPs (pre-requisite programs), not enough support from competent authorities etc. Therefore, in order to promote the adoption and implementation of food safety management systems in food industries (especially small and medium) Government should formulate food safety policies in favor of educating senior managers the potential benefits of food safety management systems for their firms. Additionally, Government should disseminate information on the successful adoption of such systems and the need to focus Government resources on assisting smaller firms for the adoption and implementation of food safety management systems. Gradually, food industries should be enforced to improve the necessary infrastructure to maintain Good Hygienic Practices during pre-inspection for license for new industries and license renewal for running industries. Likewise, food industries should be enforced to recruit competent manpower to implement such systems. Additionally, private labs should be encouraged to be established and accredited which should be able to analyze critical food safety hazards like pesticides, veterinary drugs, mycotoxins and heavy metals. Additionally, various government institutions for monitoring and controlling food safety at different levels should coordinate and interact with institutions that are working in the area of food production, quality certification and food safety.

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INTEGRATED APPROACHES FOR THE MANAGEMENT OF ROOT KNOT DISEASE OF TOMATO IN KASKI, NEPAL

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ABSTRACT

Root-knot nematode (RKN) is increasingly becoming a major disease found in tomatoes grown in protected environments in Nepal. A study conducted at Horticulture Development Resource Center, Kaski district from March to July 2023 aimed to assess efficacy of Mustard cake, Neem cake, Fosthiazate, Paecilomyces lilacinus, mustard cake and P.lilacinus, grafted Srijana (F1) on Solanum sisymbriifolium including control in mitigating root knot nematode (Meloidogyne spp.) infestation under field conditions. In the field experiment, seven treatments were replicated three times in a randomly complete block design, while the pot culture involvedfive treatments replicated four times with varying initial nematode egg densities (20, 40, 60, 80, 100) arranged in completely randomized design. Key parameters, including root length, weight, volume, number of galls, nematode eggs, adult nematodes and yield, were assessed and statistically analyzed. A galling index ranging from 0 to 10 was used to categorize the severity of gall formation. The results revealed significant differences among treatments for key parameters studied. Grafted Srijana tomato on S. sisymbriifolium demonstrated superior performance, exhibiting the lowest galling index (0) and favorable root length. P. lilacinus emerged as the most effective treatment, showing significant differences in multiple parameters compared to others, though comparable to Fosthiazate. In the pot experiment, increasing nematode egg densities correlated directly with gall formation, root weight, root volume, nematode eggs and adults, and inversely with plant height and root length. The study suggests P. lilacinus as the most suitable method for managing root knot nematode infestations in tomatoes, while grafted S. sisymbriifolium shows promise, particularly in reducing gall formation. Further research is recommended to comprehensively assess the yield potential of grafted tomato seedlings on S. sisymbriifolium in nematode-infested environment

1. INTRODUCTION

Tomato (*Lycopersicon esculentum M.*) occupies fourth and fifth position among vegetables in terms of production and area respectively in Nepal with a total production of 432,616 Mt and productivity of 19.14 mt/ha. (Statistical information in Nepalese Agriculture,2078/79). Recently, tomato cultivation under plastic tunnel is gaining popularity in Nepal, especially in hilly areas due to higher productivity and income as compared to an open field production. Due to monoculture practice in tomato, problem of various disease and insect infestation is becoming severe, among which, the problem of root knot nematode (RKN) is the major one identified through farmers field survey.

Mainly, four species of *Meloidogyne* i.e., *Meloidogyne incognita*, *M. javanica*, *M. arenaria,* and *M. hapla* are primarily responsible for yield reduction in tomato (Bhardwaj & Hogger, 1984). The incidence of RKN was first recorded in Nepal on tomato, eggplant, okra, and chilli (Amatya & Shrestha 1969). *M. incognita* is reported to be the dominant species responsible for excessive loss in tomato (Bhardwaj & Hogger, 1984). A total of 1100 plant diseases have so far been reported from Nepal; about 91 % are caused by fungi and the remaining 9 % by nematodes, bacteria, and viruses (Mathur et al., 1992). Root knot nematode is also responsible for increasing the severity of other diseases and pests. Incidence and severity of Fusarium wilt in several plant species (tomato) is found to increase when the plants are affected by RKN. Similarly, Verticillium wilt, Pythium damping off, Rhizoctonia and Phytophthora root rot are increased due to root knot nematode infection (Agrios, 2005). The problem of RKN in tomato is reported early from the seedling stage and intensifies later as the plant continues to grow causing severe damage at flowering and fruiting stage (Schomaker,C. H. and Been,T. H.,2013). To control RKN problem in tomato, various attempts have been made. It is suggested to change the place of cultivation if nematode problem is seen. Similarly, various chemicals/nematicides such as methyl bromide, chloropicrin, fenamiphos, fosthiazate, aldicarb etc. have also been used with limited efficacy (Chen, J., Li, Q. X., & Song, B. 2020).

This study aims to identify a combination of easily available materials and other options for which locally available materials such as mustard cake and neem cake along with the use of bio nematicide, *P. lilacinus*, grafted *S. sisymbriifolium* and fosthiazate as a chemical nematicide. Having data on nematode population, host plant interaction and economic losses caused by nematode infestation in tomato, this study intends to develop detailed root knot nematodes management strategies that are both cost effective and sustainable, contributing to improved crop yields and livelihood in Nepal.

2. RESEARCH METHODOLOGY

The whole experiment was carried out in two sections. Firstly, pot experiment was carried out to assess the effect of inoculum (RKN eggs) density on gall formation in tomato plants. Secondly, a field experiment was conducted to assess the effect of mustard cake, neem cake, *P. lilacinus,* grafted tomato seedlings, and Fosthiazate in managing RKN populations in field conditions.

2.1. Pot Experiment

2.1.1. Site selection

This study was conducted in a polyhouse tunnel (size: 18m* 6-m) at Horticulture Development Resource Center (HDRC), Malepatan, Kaski district from Jan-July,2023. The site is situated at Latitude: 28.2639° N, Longitude: 83.9721° E, with an altitude of approximately 862 masl. Selection of this site was prompted by previous reports of nematode infestation, which had led to significant production losses. The study aimed to verify the presence of nematode, evaluate their impact on crops in this controlled environment, and investigate potential management practices. . The average temperature in the summer reaches 27 degrees Celsius, while it drops to 15 degrees Celsius in the winter. Monthly average rainfall was around 500 mm.2.1.2. Soil collection and sterilization Approximately 60 kilograms of soil from a uniform experimental site within a polyhouse tunnel was systematically collected. The soil sampling process involved thorough removal of extraneous materials such as debris, pebbles, and plant roots. Following the collection process, the soil samples were subjected to sterilization in an autoclave under controlled conditions. Sterilization was achieved by subjecting the soil to a temperature of 212°F (100°C) and a pressure of 22 pounds per square inch (PSI) for a duration of 2 hours (Razavi Darbar, S.,2007). This rigorous sterilization procedure was employed to ensure the elimination of potential contaminants and maintain the soil's purity for subsequent experimentation.

2.1.2. Inoculum preparation

For collection of Meloidogyne eggs, root samples from tomato plant were collected from premises of HDRC. The samples were thoroughly washed and subjected to light maceration of 10-second beats repeated three times using a regular kitchen mixture. The mixture was then passed through sieves of varying mesh sizes (250, 100, 50). The final solution was decanted to obtain a concentrated solution. The number of eggs was counted by taking 1 ml sample on glass slide under a binocular microscope. These prepared Meloidogyne eggs were used accordingly in experiments.

2.1.3. Seedling preparation and transplantation

One hundred tomato seeds were sown in a germinating tray (50 cells), using a mixture of sterilized soil and cocopeat in the ratio of 1:2. Regular and timely watering was administered to prevent wilting, ensuring successful germination and early growth of tomato seedlings. Following a 21-days period of nurturing tomato seedlings in a controlled environment, robust and well-developed seedlings were transplanted into individual poly bags. Each poly bag contained 1 kilogram of sterilized soil, ensuring a favorable growing medium for the continued growth and development of the tomato plants.

2.1.4. Experimental Design

Five different RKN egg densities were used for the experiment. The whole experiment was set up in a Completely Randomized Design (CRD) with 4 replications, each containing 3 polybags.

Table 1. Different treatments used in pot experiment.

Treatments	Egg densities
T_1	20 Eggs
T_2	40 Eggs
T_3	60 Eggs
T_4	80 Eggs
T_5	100 Eggs

2.1.5. Infecting Seedlings with Root knot nematode eggs

Following the transplantation of healthy tomato seedlings into an individual poly bag of size 25 cm*20 cm, a seven-day period was allowed for seedling acclimatization in polytunnel condition. Subsequently, varying densities of RKN eggs were inoculated into the soil. The poly bags were grouped into five treatment levels, with egg densities of 20, 40, 60, 80, and 100 eggs per poly bag. Each treatment level consisted of four replications, facilitating a comprehensive investigation of the impact of RKN density on tomato plant development.

2.1.6. Observations of Biometrical parameters

To assess the effect of RKN on tomato plant growth and development and the presence of nematode in soil, different parameters such as root length, root weight, root volume, number of galls on root, number of eggs and adults in soil were measured 30 and 60 Days after Transplantation (DAT) using destructive methods. Root length, root weight, and root volume were measured using a ruler, weighing balance and water displacement method respectively. The total number of galls on plant roots was counted and Galling Index was calculated. The Galling Index was assigned 0-10 which represented 0%, 20%. 40%, 60%, 80%, 100% of infestation. To assess the presence of RKN eggs and adults in soil, 4 grams of roots from each treatment were weighed and subsequently ground to separate nematode eggs and adults. The resulting suspension underwent filtration through micro test sieves of varying sizes (250, 100, and 50 micrometers) to concentrate the sample. Finally, a 1-milliliter concentrated sample was subjected to stereoscopic microscopy for the observation and enumeration of different life stages of root-knot nematodes.

2.2. Field Experiment

2.2.1. Field layout

This experiment was conducted in a polyhouse tunnel (size: 18m* 6-m) at Horticulture Development Resource Center (HDRC), Malepatan, Kaski district from Jan-July,2023. The total number of treatments were 7 and the treatments were replicated 3 times. The treatments used are as follows:

Table 2. Different treatments used in the field experiment.

Indication	Treatments
T1	Mustard Cake
T2	Neem Cake
T3	Fosthiazate
T4	*P. lilacinus*
T5	Mustard Cake+ *P. lilacinus*
T6	Grafted Srijana Tomato with *S. sisymbriifolium*
T7	Control

2.2.2. Cultural practices

2.2.2.1. Growing Seedling

Healthy seeds of Srijana variety of tomato were used for growing seedling. The germination process involved the careful preparation of a growth medium consisting of soil, well-rotted farmyard manure (FYM), and cocopeat in a ratio of 2:1:1. Three tomato seeds were sown in each hole within a germinating tray, followed by diligent watering and attentive care to ensure the successful germination and health of the seedlings.

2.2.2.2. Grafting of Srijana tomato on Solanum sisymbriifolium

The process of grafting includes *S. sisymbriifolium* as the rootstock with Srijana variety (F1) as the scion. The grafting was done at 3 weeks age of the tomato seedlings using cleft grafting technique. Grafting was achieved with a good setup of the grafting chamber. The grafted plants were placed inside a transparent bucket with a lid and moisture was maintained by applying water continuously. After a week of grafting, the bucket was introduced to sunlight for one hour daily and slowly increased to full sunlight. After successful grafting, the grafted plants were transplanted into the main field.

2.2.2.3. Field preparation and fertilizer application

The field preparation process began with primary tillage followed by two rounds of secondary tillage to ensure optimal soil texture. The soil was subsequently finely pulverized. To enhance the soil's fertility and structure, well-rotten FYM was incorporated at a rate of 15 tons per hectare. Thorough mixing was carried out to ensure even distribution of FYM throughout the field. The prepared land was then methodically organized into three identical strips, each representing a replication. Within each replication, a further division took place, resulting in seven distinct plots.

Following this soil preparation, the recommended dose of fertilizers, including Urea (46%N), DAP (18%N, 46% P_2O_5) and MOP (60%K_2O) was applied in the field at the rate of 200:150:100 kg N, P, K per hectare. This application was conducted promptly after the field preparation to ensure that the soil was well-nourished and ready for the upcoming cultivation activities.

2.2.2.4. Application of substances according to treatments

Treatments, including Mustard Cake, Neem Cake, Fosthiazate, and *P. lilacinus*, were applied at recommended dosages, with precise rates for each. Mustard Cake and Neem Cake were spread across the plots at 80gm/m2 and 50gm/m2 respectively while Fosthiazate was applied at the rate of 3 kilograms per hectare. *P. lilacinus* was used in root dipping at a concentration of 5 milliliters per liter of water. Use of mustard Cake in combination with *P. lilacinus,* each at half the recommended amount, is used to investigate the potential effects.

2.2.2.5. Transplantation of the seedling.

After three weeks of careful nurturing in germinating trays, the tomato seedlings had grown into robust and healthy plants, ready for transplantation into the main treatment plots. In addition to the seedlings, the grafted *S. sisymbriifolium* were also transplanted into their designated treatment plots.

2.2.2.6. Weeding, Training and Pruning

Weeding activities were carried out at 25 DAT and again at 60 DAT to eliminate weed species that could hinder tomato growth. The training of plants involved the use of UV stabilized polyropes and clips, allowing for single-stem growth. It was carried out when plants were grown up to knee high stage. Additionally, pruning was done to remove excess tomato shoots and leaves, ensuring that nutrients were channeled to fruit development, optimizing crop yield. Pruning was carried out throughout the growing season.

2.2.2.7. Harvesting

The culmination of the tomato crop's growth cycle arrived with the long-awaited harvesting stage. Harvesting took place at 120 DAT, comprising three distinct harvest sessions. . Ripe, red-colored tomatoes were selectively hand-picked, and comprehensive data records were maintained to capture the outcome of each harvest.

2.3. Statistical analysis

All the recorded data was arranged systematically treatment-wise under three replications based on various observed parameters in MS-Excel 2016 data work sheet. Different statistical tools such as Excel and R studio were used for the analysis of variance and other related data parameters. 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 2016 and SPSS were used for tabular and graphical representation.

3. RESULTS AND DISCUSSION

3.1. Pot Experiment

The study found that different densities of nematode eggs had a noticeable effect on plant height. Plants grew tallest at the lowest egg density of 20 eggs per 400 g of soil. In contrast, the shortest plants were seen at the highest density of 100 eggs. On average, plant height across all treatments was 97.25 cm. As nematode egg density increased, root length decreased, with an overall average of 17.65 cm. Root weight initially dropped with higher nematode densities up to 40 eggs, but then it increased, likely due to the development of enlarged cells. Similarly, root volume decreased with rising nematode density in the first two treatments, but increased at higher densities. There was also a clear difference in gall formation depending on egg density. The highest number of galls, 176.75, occurred at the 100-egg density, while the lowest, 48, was observed at the 20-egg density.

Table 3: Effects of different egg densities on plants' biometrical parameters of tomato and different stages of nematode numbers in a pot in Kaski,2023.

Egg density	Plant height (cm)	Root length(cm)	Root weight(g)	Root volume(cm^3)	No. of galls	No. of Eggs	No. of Adults
20 Eggs	123.25[a]	25.25[a]	22.25[a]	21.75[a]	13.50[e]	48.00[e]	4.25[e]
40 Eggs	111.75[b]	20.00[b]	21.75[a]	19.50[b]	23.75[d]	72.50[d]	9.00[d]
60 Eggs	92.75[c]	16.75[c]	19.2[b]	17.25[c]	44.50[c]	97.50[c]	14.00[c]
80 Eggs	83.50[d]	14.75[c]	18.50[b]	17.00[cd]	61.75[b]	133.25[b]	18.50[b]
100 Eggs	75[e]	11.50[d]	17.25[b]	15.75[d]	80.75[a]	176.75[a]	21.75[a]

3.2. Field Experiment

The data shows differences in gall formation among various treatments for root-knot nematodes. The control group had the most galls at 244.33, whereas grafted tomatoes showed complete resistance with no galls observed. Egg counts further illustrate these differences, with the control group recording the highest egg count at 352, indicating greater susceptibility to nematode infection. In contrast, grafted *S. sisymbriifolium* had the lowest egg count of only 5, demonstrating strong resistance to RKN. Treatments with *P. lilacinus* and fosthiazate also showed reduced egg counts, with 146 eggs recorded.

For adult nematodes, the control group had the highest count of 42.33, while adult nematodes were not extracted from grafted tomatoes further confirming their full resistance. Root length measurements showed that grafted *S. sisymbriifolium* had the longest roots at 36 cm, indicating effective resistance to nematode-induced damage, while the control group had shorter roots of 22.67 cm, reflecting the impact of nematode infestation on root growth. Root weight varied across treatments, with grafted plants having the highest at 59.67 gm. However, the control group had even higher root weight of 68.67 gm, likely due to hypertrophied root galls. The highest root volume was recorded in grafted tomatoes at 69.67 cm^3, suggesting that the robust root system of the rootstock and resistance to root-knot nematodes contributed to this outcome. Inversely, the control group had a lower root volume of 54.33 cm^3, as nematode infestation limited the root growth. In terms of yield, *P. lilacinus* demonstrated the highest yield of 316gm per plant. Despite having the largest root volume, *S. sisymbriifolium* produced the lowest yield of 75 gm per plant. This could be due to the factors such as graft incompatibility, improper grafting techniques, or ongoing issues related to nematode infestation.

Table 4: Effects of different treatments on biometrical parameters of tomato and different stages of nematodes in field conditions in kaski,2024.

Treatments	Root length(cm)	Root weight(g)	Root volume(cm^3)	No.of galls	No.of Eggs	No.of Adults	Yield (g)
Mustard Cake	26.333[c]	49.67[c]	54.33[b]	184.00[b]	287.67[b]	32.67[ab]	324.33[a]
Neem Cake	24.33[cd]	42.00[d]	46.00[c]	153.67[c]	202.33[d]	24.33[bc]	236.50[ab]
Fosthiazate	27.00[c]	29.67[e]	32.67[d]	48.00[d]	146.00[e]	14.33[cd]	268.67[ab]
Paecilomyces lilacinus	31.67[b]	32.00[e]	32.33[d]	32.67[e]	119.67[f]	9.33[de]	316.00[a]

Mustard cake+ *Paecilomyces*	23.33^{d}	43.00^{d}	46.67^{c}	181.00^{b}	228.67^{c}	31.00^{ab}	180.67^{bc}
Solanum sisymbriifolium	36.00^{a}	59.67^{b}	66.00^{a}	0.00^{f}	5.00^{g}	0.00^{e}	75.00^{c}
Control	22.67^{d}	68.67^{a}	69.67^{a}	244.33^{a}	352.00^{a}	42.33^{a}	261.67^{ab}

4. CONCLUSION

The results clearly indicate that higher nematode egg densities have a detrimental effect on plant growth, as evidenced by the reduction in plant height, root length, root weight, and root volume. These findings suggest that nematode infestation severely limits the plant's ability to uptake nutrients and water, leading to stunted growth. It also showed that the tomato grafted in S. sisymbriifolium displayed outstanding root development, in contrast to the controlled group with the most galls. The biological control agent P. lilacinus emerged as the most effective control agent in terms of overall yield. With further multilocation confirmation trials the grafted tomato with S. sisymbriifolium and biological control agent (P. lilacinus) should be promoted for the control of root knot nematodes in field level.

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