Volume 9, Issue 1 June 2026 ISSN 2616-3926

Royal Government of Bhutan

BHUTANESE JOURNAL OF AGRICULTURE

Agriculture Research and Innovation Division
Department of Agriculture
Ministry of Agriculture and Livestock
Thimphu : Bhutan

Editorial

The Bhutanese Journal of Agriculture welcomes the submission of research articles and short communications. The article may be submitted to *bja@moal.gov.bt*. Please follow the journal format provided in the author guidelines, accessible from *www.bja.gov.bt* or *www.doa.gov.bt*. The online open access for the Bhutanese Journal of Agriculture is now available at *www.bja.gov.bt*.

Publisher
Agriculture Research and Innovation Division, Department of Agriculture
Ministry of Agriculture and Livestock, Thimphu, Bhutan
Post Box 113, Postal Code 11001
Contact no. +975-2-322228/331316

ISSN 2616-3926 (Print)
ISSN 2707-9821 (Online)

Part or whole of this publication may be reproduced or disseminated with proper acknowledgement to the above address. Readers are encouraged to send in comments or feedback on articles in this journal for future improvement.

Volume 9, Issue 1 June 2026 ISSN 2616-3926

BHUTANESE JOURNAL OF AGRICULTURE

Agriculture Research and Innovation Division
Department of Agriculture
Ministry of Agriculture and Livestock
Thimphu : Bhutan

*The Bhutanese Journal of Agriculture is registered with the ISSN International Centre,
Located in Paris 75003 (France), 45 rue de Turbigo.*

Editor-In-Chief

Mr. Tirtha Bdr. Katwal, Specialist I, National Centre for Organic Agriculture-Yusipang (NCOA), DoA

Editorial Board

i. Mr. Mahesh Ghimiray, Lecturer, College of Natural Resources (CNR)
ii. Dr. Ganja Singh Rai, Specialist, Agriculture Research and Development Centre (ARDC), Samtenling, DoA
iii. Dr. Norden Lepcha, Principal Agriculture Officer, NCOA-Yusipang, DoA
iv. Ngawang, Sr. Agriculture Officer, Agriculture Research and Innovation Division (ARID), DoA
v. Kuenzang Om, Principal Agriculture Officer, ARID, DoA

Member Secretary

Mr. SahaBir Rai (Member Secretary), ARID, DoA

Reviewers

1. Mr. Tirtha Bdr. Katwal, NCOA-Yusipang, DoA
2. Mr. Mr. Rinzin Wangchuk, Agriculture Production Division, DoA
3. Mr. Mahesh Ghimiray, College of Natural Resources
4. Mr. Chencho Dukpa, National Mushroom Centre
5. Mr. Passang Tshering, ARDC-Bajo
6. Dr. Ganja Singh Rai, ARDC-Samtenling, DoA
7. Dr. Norden Lepcha, NCOA-Yusipang, DoA
8. Mr. Suraj Chettri, National Soil Service Centre, DoA
9. Ms. Pema Wangmo, Agriculture Machinery and Technology Centre, DoA
10. Mr. Tshetrim, Agriculture Production Division, DoA
11. Ms. Kuenzang Om, ARID, DoA
12. Mrs. Sonam Lhamo, National Post Harvest Centre, DAMC
13. Ms. Passang Wangmo, NCOA-Yusipang
14. Mr. Ngawang, ARID, DoA

Editorial Assistant and Correspondence

Ms. Kuenzang Om, Principal Agriculture Officer, ARID, DoA

FOREWORD

The Department of Agriculture is delighted to publish the 9th Volume of the Bhutanese Journal of Agriculture (BJA). As the flagship scientific publication of the Department of Agriculture, the BJA continues to serve as an important platform for disseminating research findings, technological innovations, and practical experiences that contribute to the advancement of Bhutan's agriculture sector.

Agriculture remains central to the livelihoods, food security, and sustainable development of our nation. In an era marked by climate variability, evolving market demands, and emerging production challenges, scientific research and innovation are more important than ever. The continued publication of the BJA demonstrates our collective dedication to strengthening agricultural research, fostering knowledge exchange, and promoting a culture of scientific inquiry.

I would like to express my sincere appreciation to all authors for sharing their work, the reviewers for their constructive evaluations, and the editorial board for their professionalism and dedication in maintaining the quality and integrity of the journal. Their contributions are instrumental in ensuring that the BJA remains a credible and respected source of agricultural knowledge.

I hope that the knowledge shared in this issue will inspire further research, innovation, and collaboration for the benefit of Bhutanese agriculture.

Tashi Delek and Best Wishes!

YGyamtsho.

(Yonten Gyamtsho)
DIRECTOR

EDITORIAL

The Bhutanese Journal of Agriculture (BJA) published by the Department of Agriculture (DoA) encourages the publication of original research works undertaken by the Bhutanese scientists. The publication aims to generate scientific knowledge, information and technologies that contribute to the development of the Bhutanese agrifood sector. The publication of the research findings are ultimately expected to contribute to the transformation of the Bhutanese agrifood sector. The peer reviewed research papers published in the BJA provide the scientific basis for making informed decisions in agriculture. Although small in size, Bhutan has a diverse agro-ecology that is hugely influenced by the mountainous terrain. Adaptive research is thus very essential for the adaptation of specific technologies, skills, information, and knowledge to make agriculture sector more resilient and sustainable. The BJA serves a very good platform for the Bhutanese agriculture professionals to publish, present and share their outputs with the global agriculture professionals.

This edition Volume 9, Issue I of the BJA received more than 20 manuscripts which were reviewed by 14 experienced experts. The review reports were deliberated by a panel of reviewers in a three-day technical workshop. The review workshop is a special feature of the BJA as it ensures that the best articles are selected, and justice is done in accepting or rejecting the manuscript. Through a rigorous revision process including strict compliance with the journal guidelines, only 10 manuscripts were accepted for publication in this volume

The BJA Editorial team would like to thank all authors, reviewers, facilitators and the journal editorial board for their concerted effort and diligence in bringing out BJA Volume 9, Issue 1. On behalf of the editorial board, I would like to extend our sincere gratitude to all contributing institutions including the Agriculture Research and Development Centres at Wengkhar, Samtenling and Bajo, National Centre for Organic Agriculture, Yusipang. National Plant Protection Centre, Agriculture Machinery and Technology Centre (AMTC) and College of Natural Resources (CNR). I would like to put on record my appreciation to the Agriculture Research and Innovation Division (ARID) and the DoA for providing the resources required to conduct the research and to make this edition a success. We hope that all the agriculture professionals will appreciate the value of documenting evidence-based outputs, knowledge, information, and experiences for sharing with other peers through the BJA.

I wish you an intuitive reading.

Katwal

(Tirtha Bdr. Katwal)
Editor-In-Chief

Table of Contents

https://doi.org/10.55925/btagr.26.9101

Performance and Adaptability of Wine Grape Varieties in Bhutanese Agroecological Conditions

Pema Yangdon[1], Thinley Wangdi[1], Mandira Acharja[1], Kinzang Wangmo[1], Kinzang Thinley[1], Thinley Gyeltshen[1], Choki Wangchuk[1], Pema Dechen[2] and Kinley Gyeltshen[3]

Abstract

Grapes (Vitis vinifera L.) are among the most important temperate fruit crops worldwide, widely used for winemaking, fresh consumption, and processed products such as raisins. In Bhutan, however, grape cultivation remains limited due to heavy monsoonal rainfall and insufficient research. While a few table grape varieties have previously been tested, the cultivation of wine grapes is entirely new. To explore their potential for a niche wine industry, 18 wine grape varieties were introduced in 2018 through a Public–Private Partnership between the Department of Agriculture and Bhutan Wine Company. This study evaluated nine of these varieties across three agroecological zones, Lingmethang (600 masl), Bajo (1200 masl), and Paro (2400 masl) from 2019 to 2024, using a Completely Randomized Design with two replications. Data on fruit and yield traits, including cluster number, cluster weight, cluster dimensions, total soluble solids (TSS), berry size, and yield per vine, were analysed using ANOVA and Tukey's test in SPSS and R. The study found that all varieties contained TSS between 18% and 24%, the optimal range for winemaking, and yields between 2.9 and 4.9 kg per vine, highlighting their potential for commercial production. Significant genotype and location effects, along with strong genotype × environment interactions, were observed. Yield performance was site-specific, with Sauvignon Blanc and Pinot Noir performing best at Bajo, and Malbec and Cabernet cultivars excelling at Paro. Overall, all varieties produced superior yields and fruit quality at Paro, which is characterized by a warm climate with lower rainfall compared with the hot, wet conditions of Lingmethang. These findings confirm the feasibility of wine grape cultivation in Bhutan and highlight site-specific varietal suitability for commercial development. Consequently, all nine evaluated varieties were officially released for cultivation during the 27th Variety Release Committee meeting.

Keywords: *Wine grapes; TSS; Yield per vine; wine making*

Corresponding author: *pyangdon@moal.gov.bt*

[1] Agriculture Research and Development Centre, Wengkhar, Department of Agriculture, Ministry of Agriculture and Livestock

[2] National Seed Centre, Paro, Department of Agriculture, Ministry of Agriculture and Livestock

[3] Agriculture Research and Development Centre, Bajo, Department of Agriculture, Ministry of Agriculture and Livestock

1 Introduction

Grapes (*Vitis vinifera* L.), belonging to the family Vitaceae, are among the most important temperate fruit crops worldwide. In 2024, global grape production reached 28.87 million metric tons (MT), cultivated over approximately 7.1 million hectares (International Organisation of Vine and Wine, 2025; USDA, 2025). Grapes are classified as non-climacteric fruits and therefore must be harvested at full physiological maturity to attain the desired quality for fresh consumption or vinification (Venkitasamy et al., 2019). Of the total global production in 2022, approximately 50% was utilized for winemaking, 42% for fresh table grape consumption, and the remaining 8% processed into dried products such as raisins (Cosme et al., 2024). For wine production, grape berries with high acidity, moderate sugar content, and low pH are particularly valued, as these attributes play a critical role in determining wine quality and stability (Jones et al., 2014). Both yield and fruit quality are strongly influenced by climatic conditions and vineyard management practices (Almeida, 2017).

Although grapes are predominantly cultivated in temperate regions, they are also well adapted to subtropical and tropical agro-climatic zones extending into the warm temperate belt (Ghule et al., 2021). Grapevines perform best under a Mediterranean-type climate characterized by long, warm, dry summers and cool, moist winters. Successful grape production requires two distinct seasonal phases: a growing season with adequate warmth and sunlight to support photosynthesis and sugar accumulation in berries, and a winter dormancy period induced by cooler a temperature that allows vines to rest and prepare for the subsequent growing cycle. Optimal growth generally occurs within a temperature range of 15ºC–40 °C and under annual rainfall of around 900 mm.

In Bhutan, however, grape cultivation remains limited compared to other fruit crops, largely due to agro-ecological constraints. The predominance of heavy monsoonal rainfall across most regions coinciding with the harvest, poses significant challenges to grape production, including increased disease pressure and difficulties in vineyard management. Consequently, national grape production in 2024 was only 4.25 MT, accounting for merely 0.0096% of the country’s total fruit production and ranking the lowest among 31 fruit commodities (National Statistics Bureau, 2025). While a few table grape varieties have been evaluated in the past, the cultivation of wine grapes is relatively new in Bhutan.

Recognizing the potential for developing a niche wine industry, the Bhutan Wine Company, in collaboration with the Department of Agriculture (DoA), introduced 18 wine grape varieties from California in 2018. This initiative marked the beginning of a systematic evaluation of wine grape adaptability under Bhutanese conditions. Since 2019, varietal trials have been established under a Public-Private Partnership (PPP) framework at multiple locations, including the Agriculture Research and Development Sub Center (ARDSC) Lingmethang, Agriculture Research and Development Center (ARDC) Bajo, National Seed Center (NSC) Paro, and the National Center for Organic Agriculture (NCOA) Yusipang, with further expansion to ARDC Samtenling in 2022. In parallel, the Bhutan Wine Company has developed commercial vineyards at Pinsa, Gortshalu, and Norjinthang since 2021, covering a total area of approximately 35 acres.

Until 2025, wine production in Bhutan relied entirely on imported grapes. However, with the launch of the country's first wine produced from locally cultivated grapes, *SER KEM Wine*, by the Bhutan Wine Company in 2024, Bhutan entered a new phase in its viticulture and winemaking history. These ongoing varietal trials therefore represent pioneering efforts to assess the feasibility of grape cultivation and wine production under Bhutan's unique agro-climatic conditions. Systematic evaluation of the adaptability, productivity, and fruit quality of introduced wine grape varieties is essential for identifying cultivars suitable for commercial cultivation and for supporting the sustainable development of a domestic wine industry.

The main objectives of this study were to:

a. Assess the adaptability and performance of wine grape varieties under varying agro-climatic conditions; and
b. Identify the most promising wine grape varieties for commercial production in Bhutan.

2 Materials and methods

2.1 Study area

The evaluation trial was initiated in 2019 at four locations representing distinct agroecological zones of Bhutan: ARDSC Lingmethang (600 m above sea level, masl), NCOA Yusipang (2600 masl), NSC Paro (2400 masl), and ARDC Bajo (1200 masl). These sites were selected to assess the performance and adaptability of wine grape varieties across different agroecological zones. However, from 2023 onwards, the trial at Yusipang was discontinued because the center's mandate as the national organic coordinating center does not permit the spray schedules required for grape production. Consequently, data from Yusipang were excluded from the present study.

Climatic conditions varied markedly among the three locations over the past two decades (National Center for Hydrology and meteorology, 2024). Lingmethang received an annual rainfall ranging from 673 mm to 1,185 mm, with mean maximum temperatures of 20ºC–25 °C and minimum temperatures of 11ºC–15°C (Figure 1). Similarly, Bajo experienced annual rainfall between 400 mm and 900 mm, with maximum temperatures ranging from 24 ºC to 27 °C and minimum temperatures from 13°C to 16 °C (Figure 3). In contrast, Paro, located at a higher altitude, received comparatively lower annual rainfall (300 mm–700 mm) and was characterized by cooler temperatures, with maximum temperature of 19ºC–21 °C and minimum temperatures ranging from 5ºC to 11°C (Figure 2).

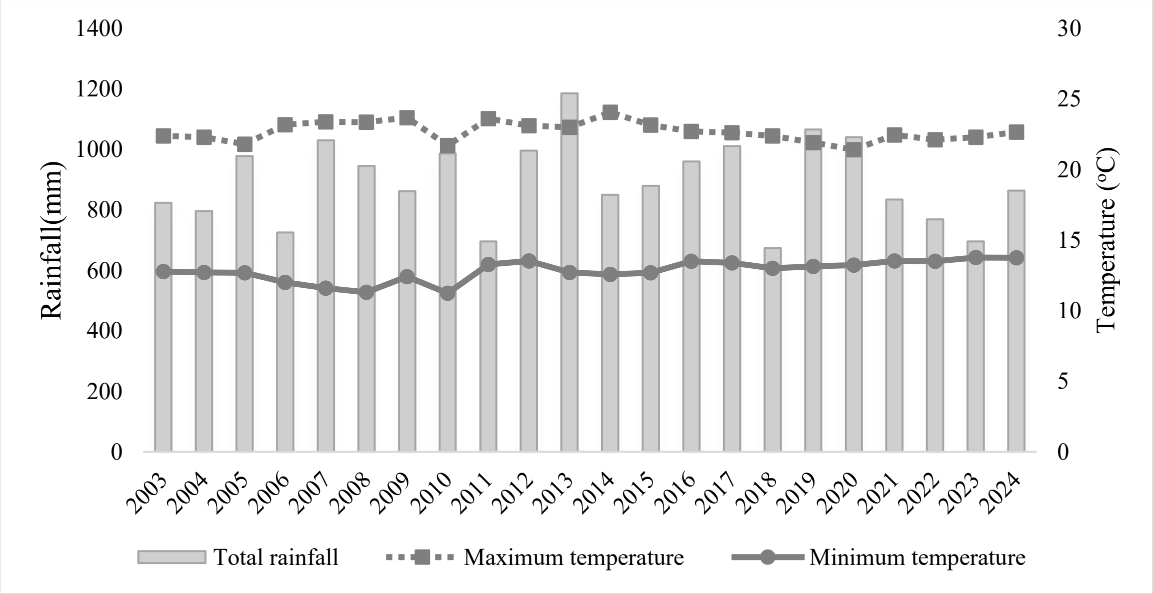


Figure 1. Climate data of Lingmethang

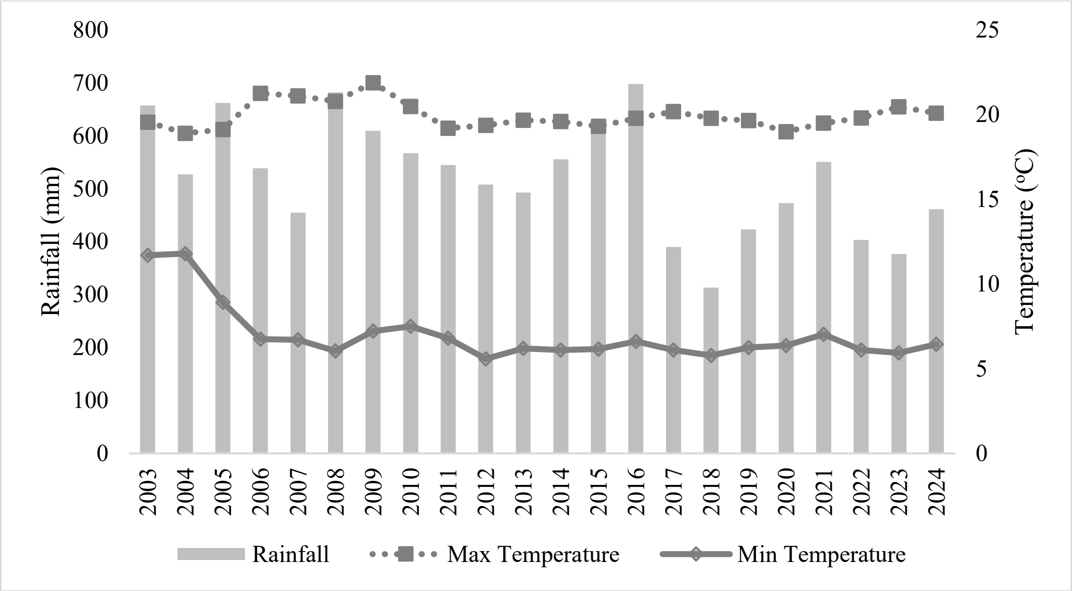


Figure 2. Climate data of Paro

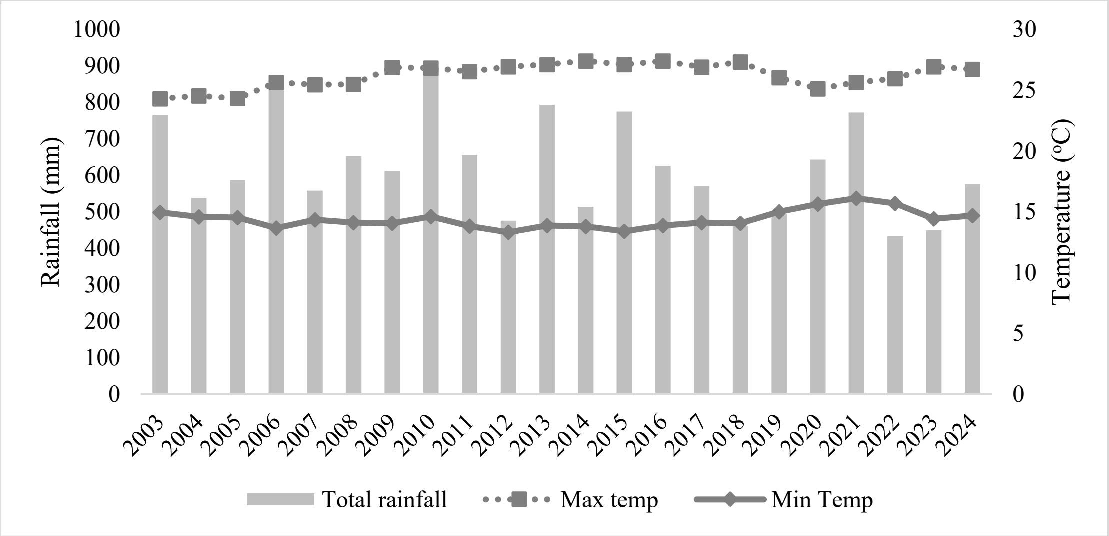


Figure 3. Climate data of Bajo

2.2 Study Design

The study was conducted using nine treatments with three replications in a Completely Randomized Design (CRD), comprising a total of 27 vines at each location. The treatments consisted of nine wine grape varieties which were introduced to Bhutan from California by the Bhutan Wine Company and subsequently shared with the ARDCs and the NSC for evaluation. Among these, three were white grape varieties, while the remaining six were red varieties that develop black coloration upon ripening (Table 1).

Table 1. List of nine varieties and their colour

Treatment	Variety	Berry colour
T1	Cabernet Franc	Red
T2	Cabernet Sauvignon	Red
T3	Chardonnay	White
T4	Malbec	Red
T5	Merlot	Red
T6	Petit Manseng	White
T7	Pinot Noir	Red
T8	Sauvignon Blanc	White
T9	Syrah	Red

Planting pits measuring 40–50 cm in depth were prepared and filled with a mixture of well-decomposed farmyard manure (FYM) and topsoil. A planting mound of 15–20 cm height above ground level was formed, and the seedlings were planted at the center of the mound. To facilitate proper vine growth and canopy development, a Kniffin trellis system was established. Under this system, two primary leaders were trained on either side of the main stem, and the lateral shoots arising from these leaders were tied vertically to the upper wire.

Standard vineyard management practices were followed throughout the study period. Annual pruning was carried out during the dormant season (December–January). Irrigation was supplied through a drip irrigation system to ensure timely and uniform water application, and each vine received 10–15 kg of FYM annually. From fruit set until harvest, a weekly spray schedule was followed using Mancozeb and sulphur @ 2g/lt of water applied alternately to manage downy mildew and powdery mildew. Additionally, insect-proof netting was installed to protect grape clusters from damage by birds and wasps.

2.3 Data collection

For this study, yield and fruit quality data collected over three consecutive years (2022–2024) were used for analysis. Harvesting time varied by location, with wine grapes harvested in July at Lingmethang and Bajo, whereas harvesting at Paro, took place in September. Data on both fruit quality and yield parameters were recorded at all experimental sites.

Fruit quality parameters included berry weight, berry diameter, berry length, total soluble solids (TSS), and number of seeds per berry. Yield parameters comprised number of clusters per vine, cluster weight, cluster length, cluster diameter, number of berries per cluster, and yield per vine. For the assessment of fruit quality, ten berries were randomly selected from harvested clusters of each variety, and their weight, diameter, length, TSS, and seed number were recorded. For yield-related measurements, all harvested clusters per vine were counted, and five clusters were randomly selected from each variety to determine cluster length, cluster diameter, and number of berries per cluster. Total yield per vine was calculated by weighing all clusters harvested from each variety and replication using a digital weighing balance. TSS was measured using a digital refractometer. Berry length and diameter were measured using a vernier caliper, while cluster length and diameter were measured using a 30 cm measuring scale.

2.4 Data Analysis

The average fruit and yield parameters of nine varieties across three locations were analysed using one-way ANOVA, while the interaction effects between location and variety were evaluated through two-way ANOVA. All statistical analyses were carried out in SPSS and R software at a 95% confidence level, and Tukey's HSD test was employed for post hoc comparisons.

3 Results and Discussion

3.1 Overall fruit and yield parameters

3.1.1 Fruit parameters

To assess the overall fruit parameters of nine wine grape varieties, one way ANOVA was conducted. Significant variation was observed among the wine grape varieties for berry length, TSS, and seed content, while differences in berry weight and berry diameter were not statistically significant (Table 2). Berry weight ranged from 1.62 g in Petit Manseng to 2.64 g in Malbec, with a mean value of 2.10 g. Similarly, berry diameter varied from 1.34 cm in Cabernet Sauvignon to 2.16 cm in Petit Manseng with no significant differences among the varieties.

In contrast, berry length differed significantly among varieties ($p < 0.001$). Sauvignon Blanc recorded the longest berries (1.51 cm), followed by Malbec, Chardonnay, and Petit Manseng, while Petit Manseng exhibited the shortest berry length (1.23 cm). These differences in berry length may influence berry morphology and skin-to-pulp ratio, which are important determinants of wine quality attributes such as phenolic extraction and flavor intensity.

TSS content showed highly significant variation among varieties ($p < 0.001$), ranging from 18.85% in Pinot Noir to 23.30% in Petit Manseng, with an overall mean of 20.18%. The significantly higher TSS observed in Petit Manseng indicates superior sugar accumulation potential, which is desirable for producing wines with higher alcohol content or balanced sweetness. Varieties such as Chardonnay and Sauvignon Blanc also exhibited relatively high TSS values with fewer seed content, suggesting good adaptability and favourable ripening behaviour under the experimental conditions. Importantly, all evaluated varieties fall within the widely accepted optimal range for winemaking (18% to 24%), suggesting their suitability for wine making under Bhutanese growing conditions (Yuyuen, Boonkerd and Wanapu, 2015).

Seed content per berry differed significantly among varieties ($p < 0.01$), varying from 1.87 in Chardonnay to 2.70 in Cabernet Franc. Higher seed numbers, as observed in Cabernet Franc and Malbec, may contribute to increased tannin extraction during vinification, potentially influencing wine astringency and structure (Rousserie et al., 2020). Although Malbec produced berries with relatively higher berry weight, it was characterized by lower TSS and higher seed content. These attributes may increase production costs due to the potential need for sugar supplementation during fermentation and additional processing to manage excessive seed-derived tannins. Conversely, lower seed content in Chardonnay may be advantageous for producing wines with softer mouthfeel.

Overall, the results indicate that while berry size traits such as weight and diameter were relatively stable across varieties, berry length, TSS, and seed content exhibited strong varietal influence. The superior TSS in Petit Manseng and favorable berry dimensions in Sauvignon

Blanc and Malbec highlight their potential suitability for quality wine production under the studied agro-climatic conditions.

Table 2. Overall fruit parameters of nine winegrape varieties

Varieties	**Berry weight (g)**	**Berry diameter (cm)**	**Berry length (cm)**	**TSS (%)**	**Seed content (no)**
Cabernet Franc	2.02	1.36	1.32 ^{abc}	19.41	2.70 ^{a}
Cabernet Sauvignon	1.85	1.34	1.28 ^{bc}	19.09	2.15 ^{ab}
Chardonnay	2.01	1.43	1.45 ^{ab}	21.09	1.87 ^{b}
Malbec	2.64	1.70	1.46 ^{ab}	19.58	2.62 ^{a}
Merlot	2.09	1.44	1.34 ^{abc}	19.78	2.29 ^{ab}
Petit Manseng	1.62	2.16	1.23 ^{c}	23.3	2.26 ^{ab}
Pinot Noir	2.33	1.47	1.30 ^{abc}	18.85	2.31 ^{ab}
Sauvignon Blanc	2.35	1.41	1.51 ^{a}	20.55	2.24 ^{ab}
Syrah	1.97	1.53	1.32 ^{abc}	19.81	2.48 ^{ab}
Mean	2.10	1.56	1.37	20.18	2.32
StdErr	0.31	0.44	0.06	0.86	0.21
p-Value	ns	ns	***	***	**

**P<5%, ** P<1%, and ***P<0.1%, ns- not significant, StdErr- Standard Error*

3.1.2 Yield parameters

Significant varietal differences were observed for the number of clusters per vine, while cluster morphology traits and yield per vine did not differ significantly among varieties (Table 3). The number of clusters per vine varied significantly among varieties ($p < 0.001$), ranging from 12.08 in Chardonnay to 32.50 in Sauvignon Blanc, with a mean of 23.59 clusters per vine. Sauvignon Blanc recorded the highest cluster number, followed by Cabernet Sauvignon (30.58), but these varieties have lower TSS which need the addition of sugar while making wine. On the other hand, Petit Manseng and Chardonnay have higher TSS but the yield and cluster numbers are comparatively lower.

Cluster weight varied among varieties, ranging from 86.93 g in Petit Manseng to 156.67 g in Malbec, with an overall mean of 109.31 g. Although Malbec exhibited the highest cluster weight, its overall yield remained comparatively lower due to a reduced number of clusters per vine. In contrast, cluster diameter and cluster length did not differ significantly among varieties, with mean values of 6.37 cm and 11.25 cm, respectively. These findings suggest that, under the prevailing experimental conditions, cluster size and shape were relatively stable traits and less responsive to genetic variation among the evaluated wine grape varieties.

The number of berries per cluster ranged from 67.57 in Pinot Noir to 92.42 in Petit Manseng, with a mean of 77.86 berries per cluster. Despite numerical differences, the variation among varieties was non-significant, implying that berry set per cluster was relatively uniform across varieties and possibly more influenced by environmental conditions during flowering and fruit set than by varietal effects.

Yield per vine varied from 1.75 kg in Syrah to 3.69 kg in Sauvignon Blanc, with a mean yield of 2.49 kg per vine. These results differ from previous findings in other regions, where Sauvignon Blanc produced lower yields in Telangana and Syrah exhibited moderate yields in Australia (Dry et al., 2010; Joshi, 2022). The higher yield observed in Sauvignon Blanc can be largely attributed to its significantly higher number of clusters per vine rather than differences in cluster size or berry number. Given that cluster number is a key determinant of yield, higher cluster counts are generally associated with increased productivity (Fataliyev et al., 2025).

Overall, the results suggest that cluster number per vine is the primary yield-determining trait among the evaluated wine grape varieties, while cluster morphology traits showed limited varietal differentiation. The superior cluster production and higher yield potential of Sauvignon Blanc highlight its adaptability and productivity under the studied conditions, whereas varieties such as Chardonnay and Syrah may require improved management practices to enhance reproductive performance.

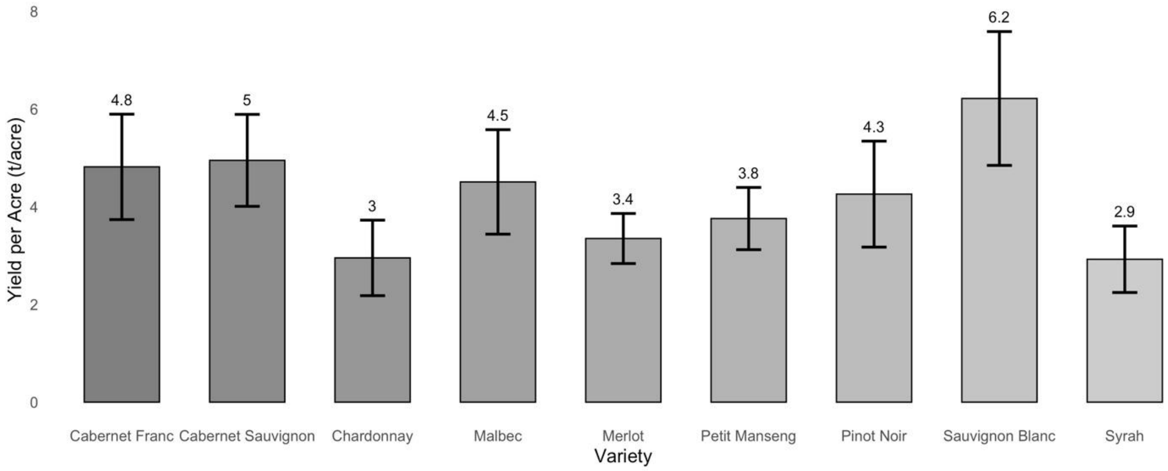


Figure 4. Overall yield/acre of nine winegrape varieties

Table 3. Overall yield parameters of nine winegrape varieties

Varieties	Cluster (no)	Cluster weight (g)	Cluster diameter(cm)	Cluster length (cm)	Berries/cluster (no)	Yield/vine (kg)
Cabernet Franc	23.75 ^{abc}	99.92	6.11	11.67	72.53	2.85
Cabernet Sauvignon	30.58 ^{ab}	98.13	6.36	12.03	80.65	2.94
Chardonnay	12.08 ^{c}	127.39	6.57	12.15	76.08	1.76
Malbec	22.33 ^{abc}	156.67	7.17	12.41	86.83	2.67
Merlot	25.33 ^{abc}	91.82	6.06	11.83	76.57	1.99
Petit Manseng	27.42 ^{abc}	86.93	6.48	10.11	92.42	2.24
Pinot Noir	23.42 ^{abc}	95.49	5.95	9.29	67.57	2.53
Sauvignon Blanc	32.50 ^{a}	112.52	6.34	10.23	69.45	3.69
Syrah	14.92 ^{bc}	114.94	6.33	11.5	78.62	1.75
Mean	23.59	109.31	6.37	11.25	77.86	2.49
StdErr	5.49	24.02	0.8	1.08	14.45	0.79
p-Value	***	ns	ns	ns	ns	ns

**P<5%, ** P<1%, and ***P<0.1%, ns- not significant, StdErr-Standard Error*

3.2 Interactions between locations and varieties

3.2.1 Fruit parameters

The two-way ANOVA showed significant effects of both location and variety on berry weight, diameter, TSS, and seed content ($p < 0.05$), while berry length remained unaffected (Table 4). The significant Location × Treatment interaction highlights that a strong varietal performance varied across agroecological zones.

At Bajo, berries were generally larger, with Pinot Noir recording the highest weight (3.58 g), and Petit Manseng showing the highest TSS (25.45%). TSS was higher across all varieties in Bajo, likely due to warmer temperatures promoting sugar accumulation (Shikhamay & Srinivasulu, 2014). In Lingmethang, berry weights were lower, ranging from 1.17 g to 2.04 g, but Petit Manseng yielded higher TSS of 20.32% followed by 20% in Chardonnay, reflecting adaptability to warm and humid climate (Ting et al., 2025). Similarly, at Paro, berries were small in size, but Petit Manseng once again excelled in sugar content, registering a TSS of 24.12%. This performance is likely linked to its distinct morphological traits, such as small, thick-skinned berries and loosely packed clusters, which allow for extended hang-time on the vine, leading to greater sugar accumulation (Moccio et al., 2023). Seed content was relatively stable across sites, though slightly higher in Paro and Lingmethang. The overall trends confirm that varieties such as Petit Manseng and Chardonnay consistently showed superior

TSS across sites, while Syrah and Malbec performed well in warmer environments, suggesting their potential for site-specific varietal recommendations.

Table 4. Fruit parameters of nine winegrape varieties in three multilocation

Location	**Varieties (Treatment)**	**Berry weight (g)**	**Berry diameter (cm)**	**Berry length (cm)**	**TSS (%)**	**Seed content (no)**
Bajo	Cabernet Franc	3.15 ^{abc}	1.20	1.30	20.80 ^{d}	2.50
Bajo	Cabernet Sauvignon	2.55 ^{cd}	1.12	1.17	21.43 ^{cd}	2.00
Bajo	Chardonnay	3.15 ^{abc}	1.35	1.32	22.15 ^{c}	2.5
Bajo	Malbec	3.38 ^{ab}	1.40	1.42	21.18 ^{cd}	2.25
Bajo	Merlot	3.23 ^{abc}	1.32	1.4	20.88 ^{d}	2.75
Bajo	Petit Manseng	2.65 ^{bcd}	4.05	1.32	25.45 ^{a}	2.25
Bajo	Pinot Noir	3.58 ^{a}	1.05	1.32	20.40 ^{d}	2.00
Bajo	Sauvignon Blanc	3.40 ^{a}	1.33	1.42	23.27 ^{b}	2.50
Bajo	Syrah	2.30 ^{d}	1.23	1.32	21.30 ^{cd}	2.50
Lingmethang	Cabernet Franc	1.47 ^{de}	1.47 ^{bc}	1.25 ^{bc}	17.63 ^{ab}	2.80 ^{a}
Lingmethang	Cabernet Sauvignon	1.53 ^{cde}	1.53 ^{abc}	1.24 ^{bc}	16.82 ^{b}	1.85 ^{ab}
Lingmethang	Chardonnay	1.38 ^{de}	1.52 ^{abc}	1.50 ^{a}	20.00 ^{ab}	1.25 ^{b}
Lingmethang	Malbec	1.97 ^{ab}	1.98 ^{ab}	1.27 ^{abc}	19.74 ^{ab}	2.80 ^{a}
Lingmethang	Merlot	1.60 ^{bcd}	1.60 ^{abc}	1.19 ^{bc}	16.78 ^{b}	2.20 ^{ab}
Lingmethang	Petit Manseng	1.17 ^{e}	1.17 ^{c}	1.09 ^{c}	20.32 ^{a}	1.92 ^{ab}
Lingmethang	Pinot Noir	1.67 ^{abcd}	1.67 ^{abc}	1.18 ^{bc}	16.70 ^{b}	2.80 ^{a}
Lingmethang	Sauvignon Blanc	1.90 ^{abc}	1.40 ^{c}	1.40 ^{ab}	19.73 ^{ab}	2.02 ^{ab}
Lingmethang	Syrah	2.04 ^{a}	2.04 ^{a}	1.35 ^{ab}	17.50 ^{ab}	2.12 ^{ab}
Paro	Cabernet Franc	1.42 ^{bc}	1.40 ^{bc}	1.40 ^{bcd}	19.80 ^{bc}	2.80 ^{a}
Paro	Cabernet Sauvignon	1.48 ^{bc}	1.38 ^{bc}	1.42 ^{bcd}	19.05 ^{bc}	2.60 ^{a}
Paro	Chardonnay	1.50 ^{bc}	1.42 ^{bc}	1.52 ^{abc}	21.68 ^{ab}	1.85 ^{a}
Paro	Malbec	2.58 ^{a}	1.73 ^{a}	1.68 ^{a}	17.82 ^{c}	2.80 ^{a}
Paro	Merlot	1.45 ^{bc}	1.40 ^{bc}	1.42 ^{bcd}	21.70 ^{ab}	1.92 ^{a}
Paro	Petit Manseng	1.05 ^{c}	1.25 ^{c}	1.27 ^{d}	24.12 ^{a}	2.60 ^{a}
Paro	Pinot Noir	1.75 ^{b}	1.40 ^{bc}	1.62 ^{ab}	19.43 ^{bc}	2.12 ^{a}
Paro	Sauvignon Blanc	1.75 ^{b}	1.50 ^{ab}	1.70 ^{a}	18.65 ^{bc}	2.20 ^{a}
Paro	Syrah	1.57 ^{bc}	1.32 ^{bc}	1.30 ^{cd}	20.65 ^{bc}	2.80 ^{a}
	Location (L)	***	0.72	***	***	0.12
	Treatment (TMT)	***	0.7	***	***	0.001
	L X TMT	***	0.2	***	***	0.001

*P<5%, ** P<1%, and ***P<0.1%, ns. not significant

3.2.2 Yield parameters

The two-way ANOVA revealed significant effects of location and variety on cluster number, cluster weight, cluster length, berries per cluster, yield per vine, and yield per acre ($p < 0.05$), while cluster diameter and berry count were not significantly influenced by treatment alone

(Table 5). Strong location x variety interactions was also observed, indicating that yield performance varied considerably across environments.

At Bajo, Pinot Noir and Sauvignon Blanc recorded the highest yields with 4.12 kg/vine and 3.60 kg/vine, respectively, translating to 6.95–6.05 MT/acre (Figure 3). These values notably exceed those reported by Eleonora et al. (2019), who observed only 1.13 kg/vine in Pinot Noir. The lowest yield was observed in Chardonnay with 0.75 kg/vine. These results suggest that the warm mid-altitude environment favours higher productivity in varieties with compact clusters.

At Lingmethang, overall yields were lower compared with those recorded at Paro and Bajo. Among the evaluated varieties, Cabernet Sauvignon (1.04 kg/vine) and Malbec (1.10 kg/vine) exhibited relatively better performance, whereas Pinot Noir was the least productive, yielding only 0.14 kg/vine. The reduced yields at this location are likely due to the coincidence of harvest with the monsoon season, during which late July rainfall promotes berry rot and associated yield losses. This observation is consistent with the findings of Keller (2010), who reported that elevated temperatures combined with high humidity during fruit maturation can adversely affect grapevine productivity in subtropical environments.

At Paro, yields were highest among locations, particularly in Sauvignon Blanc (6.85 kg/vine; 11.53 MT/acre) and Malbec (5.45 kg/vine; 9.17 MT/acre). Cabernet Sauvignon and Cabernet Franc also produced over 5 kg/vine, demonstrating strong adaptation to cool, high-altitude conditions. The mid-September harvest at this site, coinciding with the end of the monsoon, likely contributed to favourable conditions for cluster development and yield stability (Jones et al., 2005; Leeuwen et al., 2004). In all the locations, the major diseases in all the varieties were powdery mildew and downy mildew, while the main pests were wasp attack and bird damage on the berries.

Overall, the results confirm that site-specific adaptability is crucial for yield optimization. Bajo, a warm mid-altitude, favoured Pinot Noir, Petit Manseng, and Sauvignon Blanc; Paro, a cool highland, supported the highest yields in Sauvignon Blanc, Malbec, and Cabernet cultivars, while a lowland like Lingmethang was less favourable for most varieties, though Malbec and Cabernet Sauvignon showed relative tolerance.

Since all the evaluated varieties exhibited optimum TSS suitable for winemaking together with promising yield potential, all nine were approved and formally released during the 27th Variety Release Committee (VRC) meeting. A limitation of this study was the absence of on-farm data, as the vines had not yet reached the economic bearing stage, and the varieties cultivated on-farm differed from those maintained on-station. This discrepancy prevented direct comparison between on-farm and on-station performance. Future research should therefore prioritize the evaluation of additional varieties under on-farm conditions, and if they demonstrate consistent performance, propose them for release to expand the range of varietal options available for viticulture in Bhutan.

Table 5. Yield parameter of nine winegrape varieties in three multilocation

Location	**Varieties (Treatment)**	**Cluster (no)**	**Cluster weight (g)**	**Cluster diameter(cm)**	**Cluster length (cm)**	**Berries/cluster (no)**	**Yield/vine (kg)**
Bajo	Cabernet Franc	40.00 ^{ab}	72.20 ^{ab}	4.50 ^{ab}	8.52	49.75	2.88 ^{ab}
Bajo	Cabernet Sauvignon	35.00 ^{abc}	78.35 ^{ab}	4.22 ^{b}	9.2	72.25	2.73 ^{ab}
Bajo	Chardonnay	8.00 ^{c}	91.17 ^{ab}	4.53 ^{ab}	10.1	60.75	0.75 ^{b}
Bajo	Malbec	28.50 ^{abc}	51.5 ^{b}	3.77 ^{b}	8.97	66.5	1.48 ^{ab}
Bajo	Merlot	31.50 ^{abc}	66.1 ^{ab}	4.97 ^{ab}	8.67	61.75	2.08 ^{ab}
Bajo	Petit Manseng	38.25 ^{ab}	82.3 ^{ab}	4.12 ^{b}	8.32	64.5	3.17 ^{ab}
Bajo	Pinot Noir	37.00 ^{ab}	112.2 ^{a}	5.88 ^{a}	10.47	85.25	4.12 ^{a}
Bajo	Sauvignon Blanc	53.00 ^{a}	67.90 ^{ab}	4.72 ^{ab}	9.52	59.25	3.6 ^{a}
Bajo	Syrah	17.00 ^{bc}	92.60 ^{ab}	5.07 ^{ab}	9.72	61.5	1.52 ^{ab}
Lingmethang	Cabernet Franc	7.25 ^{bcd}	82.57 ^{b}	6.07	12.89 ^{ab}	54.08 ^{ab}	0.55 ^{ab}
Lingmethang	Cabernet Sauvignon	18.75 ^{a}	77.53 ^{b}	6.72	11.82 ^{abc}	55.95 ^{ab}	1.04 ^{a}
Lingmethang	Chardonnay	12.00 ^{abcd}	73.25 ^{b}	7.25	11.75 ^{abc}	62.50 ^{ab}	0.85 ^{ab}
Lingmethang	Malbec	12.75 ^{abcd}	147.51 ^{a}	7.2	13.78 ^{a}	73.72 ^{a}	1.10 ^{a}
Lingmethang	Merlot	14.50 ^{abc}	74.61 ^{b}	6.15	10.90 ^{abc}	58.95 ^{ab}	0.86 ^{ab}
Lingmethang	Petit Manseng	16.25 ^{ab}	71.97 ^{b}	6.7	9.85 ^{bcd}	79.75 ^{a}	0.82 ^{ab}
Lingmethang	Pinot Noir	2.75 ^{d}	42.76 ^{b}	5.2	6.67 ^{d}	23.43 ^{b}	0.14 ^{b}
Lingmethang	Sauvignon Blanc	12.00 ^{abcd}	76.90 ^{b}	6.57	8.47 ^{cd}	47.10 ^{ab}	10.62 ^{ab}
Lingmethang	Syrah	3.50 ^{cd}	97.21 ^{ab}	6.78	10.71 ^{abc}	45.60 ^{ab}	0.36 ^{ab}
Paro	Cabernet Franc	24.00 ^{ab}	145.00 ^{b}	7.75	13.57 ^{ab}	113.8	5.15 ^{abc}
Paro	Cabernet Sauvignon	38.00 ^{a}	138.50 ^{b}	8.12	15.05 ^{a}	113.8	5.05 ^{abc}
Paro	Chardonnay	16.25 ^{b}	217.75 ^{ab}	7.92	14.60 ^{a}	105	3.67 ^{bc}
Paro	Malbec	25.75 ^{ab}	271.00 ^{a}	10.53	14.47 ^{ab}	120.3	5.45 ^{ab}
Paro	Merlot	30.00 ^{ab}	134.75 ^{b}	7.05	15.93 ^{a}	109	3.05 ^{bc}
Paro	Petit Manseng	27.75 ^{ab}	106.50 ^{b}	8.6	12.15 ^{ab}	133	2.75 ^{c}
Paro	Pinot Noir	30.50 ^{ab}	131. 50 ^{b}	6.78	10.72 ^{b}	94	3.33 ^{bc}
Paro	Sauvignon Blanc	32.50 ^{a}	192.75 ^{ab}	7.73	12.68 ^{ab}	102	6.85 ^{a}
Paro	Syrah	24.25 ^{ab}	155.00 ^{ab}	7.15	14.05 ^{ab}	128.8	3.35 ^{bc}
	Location (L)	***	***	***	***	***	***

Treatment (TMT)	***	***	0.51	***	0.2	***
L X TMT	***	***	0.02	***	0.26	***

**P<5%, ** P<1%, and ***P<0.1%, ns. not significant*

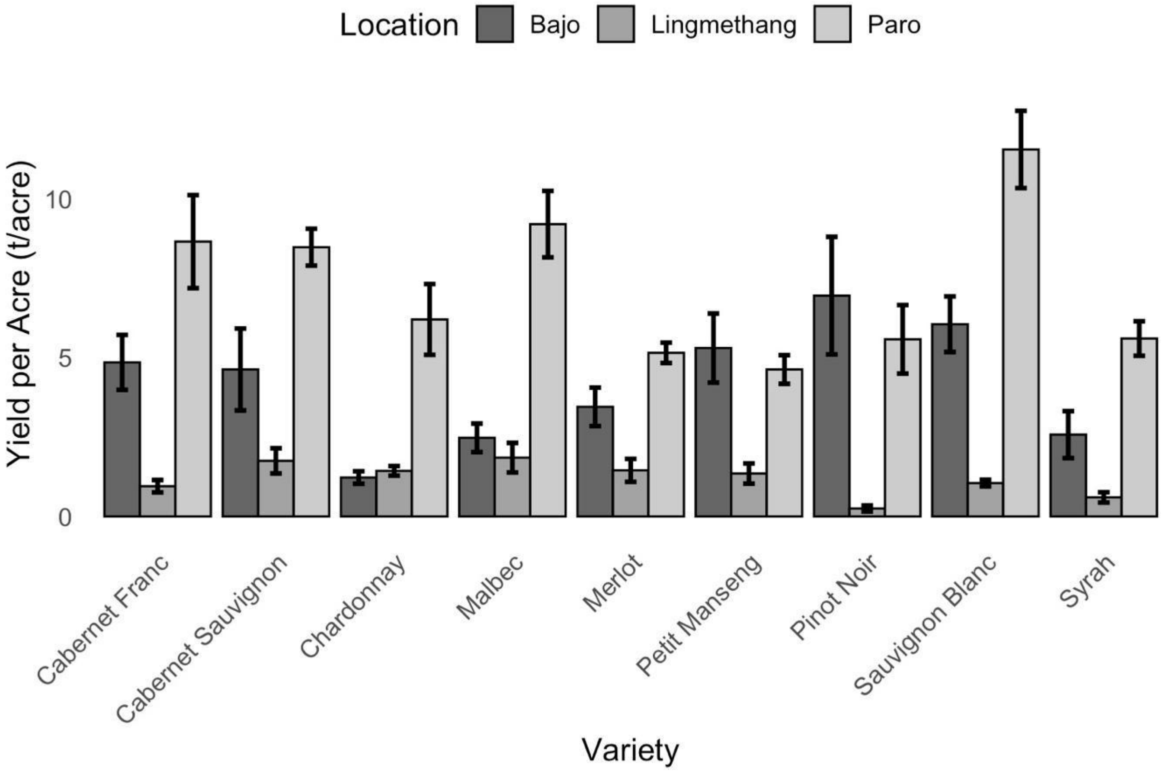


Figure 3. Yield per acre of nine winegrape varieties in three locations

4 Conclusion

Overall, all nine grape varieties exhibited TSS levels within the optimum range for winemaking, coupled with promising yield potential for commercial production in Bhutan. Nevertheless, varietal performance was strongly influenced by location-specific growing conditions, reflecting clear genotype × environment interactions. At Bajo, Pinot Noir, Petit Manseng, and Sauvignon Blanc achieved superior yields, whereas at Paro, Sauvignon Blanc, Malbec, and both Cabernet cultivars (Franc and Sauvignon) recorded the highest productivity. In contrast, Lingmethang appeared less suitable for most varieties, although Malbec and Cabernet Sauvignon demonstrated comparatively better adaptability. These findings underscore the importance of site-specific varietal recommendations, with cool highland regions favouring higher yield potential, mid-altitudes supporting balanced productivity, and lowland environments requiring more careful varietal selection to optimize performance. Future studies should focus on integrating and comparing on-station and on-farm performance data to further validate varietal suitability and support wider adoption by growers.

5 Acknowledgement

We would like to thank the Bhutan Wine Company for providing us with the seedlings and technical guidance for evaluation. A sincere gratitude goes to the Program Director of ARDCs and NSC Paro for the constant guidance and support, and to all the ESPs involved in setting up this trial. We would like to thank all the researchers who were involved in this study.

6 Authors' contribution statement

Pema Yangdon was involved in the implementation of research, data collection, and manuscript preparation. Kinzang Wangmo, Mandira Acharja, Pema Dechen, Kinley Gyeltshen, Thinley Wangdi, and Choki Wangchuk were involved in implementation and data collection, while Thinley Gyeltshen and Kinzang Thinley were involved in data analysis and interpretation of the results.

7 References

Almeida, L. W. (2017). *Planting density for Chardonnay grapevines in the south of Minas Gerais* [Master's thesis, Universidade Federal de Lavras].

Cosme, F., Filipe-Ribeiro, L., & Nunes, F. M. (2024). Introductory chapter: Impact of climate change on grapes and grape products. In *Global warming and the wine industry: Challenges, innovations and future prospects*. IntechOpen. https://doi.org/10.5772/intechopen.1005092

Dry, P. R., Longbottom, M. L., McLoughlin, S., Johnson, T. E., & Collins, C. (2010). Classification of reproductive performance of ten winegrape varieties. *Australian Journal of Grape and Wine Research, 16*(S1), 47–55. https://doi.org/10.1111/j.1755-0238.2009.00085.x

Eleonora, N., Alina, D., Dobrei, A., & Ciorica, G. (2019). Studies on growth and yield components in Merlot, Pinot noir and Syrah varieties. *Journal of Horticulture, Forestry and Biotechnology*, *23*(1), 44-50.

Fataliyev, H., Lezgiyev, Y., Gadimova, N., Ismayilov, M., & Hajiyev, M. G. (2025). Study of the influence of the number of clusters retained in the vine on the mechanical composition of grapes, the productivity and the quality of the product. *International Journal of Innovative Research and Scientific Studies, 8*(4), 676–689. https://doi.org/10.53894/ijirss.v8i4.7915

Ghule, V. S., Ranpise, S. A., Somkuwar, R. G., Kulkarni, S. S., Wagh, R. S., Naik, R. M., & Nimbalkar, C. A. (2021). Effect of rootstocks on growth parameters of Red Globe grapevines (*Vitis vinifera* L.). *International Journal of Chemical Studies, 9*(1), 3483–3487. https://doi.org/10.22271/chemi.2021.v9.i1aw.11773

International Organisation of Vine and Wine. (2025). *State of the world vine and wine sector in 2024*.

Jones, G. V., White, M. A., Cooper, O. R., & Storchmann, K. (2005). Climate change and global wine quality. *Climatic Change, 73*(3), 319–343. https://doi.org/10.1007/s10584-005-4704-2

Jones, J. E., Kerslake, F. L., Close, D. C., & Dambergs, R. G. (2014). Viticulture for sparkling wine production: A review. *American Journal of Enology and Viticulture, 65*(4), 407–416. https://doi.org/10.5344/ajev.2014.13099

Joshi, V. (2022). Evaluation of yield attributes of wine varieties of grape under Telangana conditions. *Environment and Ecology, 28*(8A), 281–290. https://doi.org/10.53550/EEC.2022.v28i08s.042

Keller, M. (2010). Managing grapevines to optimise fruit development in a challenging environment: A climate change primer for viticulturists. *Australian Journal of Grape and Wine Research, 16*(S1), 56–69. https://doi.org/10.1111/j.1755-0238.2009.00077.x

Moccio, L., Cladis, D., & Chang, B. (2023). *In pursuit of dry Petit Manseng: Understanding Petit Manseng acid chemistry*. Binghamton University, State University of New York.

National Center for Hydrology and Meteorology. (2024). *Weather data* [Dataset]. Royal Government of Bhutan.

National Statistics Bureau. (2025). *Integrated agriculture and livestock census of Bhutan 2025*. Royal Government of Bhutan.

Rousserie, P., Lacampagne, S., Vanbrabant, S., Rabot, A., & Geny-Denis, L. (2020). Wine tannins: Where are they coming from? A method to assess the importance of berry part on wine tannins content. *MethodsX, 7*, Article 100961. https://doi.org/10.1016/j.mex.2020.100961

Shikhamany, S., & Srinivasulu, B. (2014). *Wine grape cultivation, wine making and improvement of wine quality*. Andhra Pradesh Horticultural University.

Ting, J. H., Surratt, A. A., Moccio, L. E., Sandbrook, A. M., Chang, E. A., & Cladis, D. P. (2025). Ripening kinetics and grape chemistry of Virginia Petit Manseng. *Beverages, 11*(4), Article 108. https://doi.org/10.3390/beverages11040108

U.S. Department of Agriculture. (2025). *Production: Table grapes*. https://www.fas.usda.gov/data/production/commodity/0575100 van Leeuwen, C., Friant, P., Choné, X., Tregoat, O., Koundouras, S., & Dubourdieu, D. (2004). Influence of climate, soil, and cultivar on terroir. *American Journal of Enology and Viticulture, 55*(3), 207–217. https://doi.org/10.5344/ajev.2004.55.3.207

Venkitasamy, C., Zhao, L., Zhang, R., & Pan, Z. (2019). Grapes. In Z. Pan, R. Zhang, & S. Zicari (Eds.), *Integrated processing technologies for food and agricultural by-products* (pp. 133–163). Academic Press. https://doi.org/10.1016/B978-0-12-814138-0.00006-X

Yuyuen, P., Boonkerd, N., & Wanapu, C. (2015). Effect of grape berry quality on wine quality. *Suranaree Journal of Science and Technology*, *22*(4), 349-356.

https://doi.org/10.55925/btagr.26.9102

Domestication, Evaluation of Yield and Substrates of Wild Enoki Mushroom (*Flammulina velutipes*)

Sabitra Pradhan[1], Tshering Choki[1], Chencho Dukpa[1] and Tshering Wangmo[1]

Abstract

Domestication and cultivation of a local Enoki mushroom (Flammulina velutipes) isolates were conducted to assess its viability for commercial production and its suitability on different substrates. The experiments were carried out in three stages. The first trial examined whether the wild strain could be successfully domesticated on sawdust substrates. The results demonstrated successful domestication, with the first flush yielding significantly more than subsequent flushes ($p < 0.001$), indicating nutrient depletion and reduced productivity in later harvests. In the second phase, the local strain was compared with four commercial enoki strains. Based on a single first harvest, the local strain yielded significantly less than the commercial hybrid strains ($p < 0.001$). However, the local strain produced taller fruiting bodies and exhibited less variability in stipe length than three of the commercial strains. In the third phase, three sawdust substrates namely oak, alder, and poplar were tested for their effects on the colonisation rate and yield of the local strain. There was a highly significant difference ($p < 0.001$) in the number of days to first harvest: 63 days on alder, 65 days on poplar, and 70 days on oak. However, there was no significant difference in yield at the first harvest across substrates. Overall, the productivity of the local strain was comparatively lower than that of the commercial strains. Nevertheless, the native strain demonstrated strong adaptability, a high level of stability in productivity, and desirable fruiting characteristics, indicating its potential as a resilient strain for cultivation.

Keywords: *Bhutanese wild enoki; Domestication; Enoki Mushroom; Yield comparison; Substrates*

Corresponding author. sabitrap@moal.gov.bt

[1] National Mushroom Centre, Department of Agriculture, Ministry of Agriculture and Livestock

1 Introduction

Mushrooms have gradually been recognised as a nutritious and functional food, rivalling both plant and animal foodstuffs. They contain high levels of protein (up to 35%), crude fibre (19%), vitamins, and minerals, with minimal fat and calories and no starch, thereby qualifying them as a healthy food for diabetic, hypertensive, and cardiovascular patients (Hamza et al., 2024). In addition to their nutritional importance, mushrooms possess significant pharmacological properties, including antioxidant, immunomodulatory, and anticancer activities (Chang & Miles, 2004).

Global mushroom production has increased dramatically over the last three decades, rising by up to 13.8-fold from 1990 to 2020 (FAOSTAT, 2022). Similarly, Singh et al. (2020) estimated global mushroom production for 2018–2019 at approximately 43 million tonnes. The majority of production is concentrated in Asia (82.8%), with smaller contributions from Europe (12%), the Americas (4.5%), Oceania (0.4%), and Africa (0.3%) (FAOSTAT data, as reported by Market.US & Market.biz, 2026). Major cultivated species include shiitake (26%), wood ear mushroom (21%), oyster mushroom (16%), button mushroom (11%), enoki (7%), king oyster (5%), straw mushroom (1%), and other minor species (13%).

Among these, *Flammulina velutipes*, commonly known as enoki, enokitake, or needle mushroom, is valued for its delicate flavour, crisp texture, and medicinal properties. Belonging to the family Physalacriaceae, it is widely cultivated in East Asia, particularly in China, Japan, and Taiwan. Enoki cultivation is typically carried out on lignocellulosic substrates such as straw or sawdust, where both substrate type and fungal strain significantly influence yield and quality (Chang & Miles, 2004).

Nutritionally, *F. velutipes* comprises approximately 58% carbohydrates, 27.5% protein, 7% fat, and 7.4% ash (Ko et al., 2007, as cited in Cai et al., 2013). The mushroom is rich in polysaccharides and exhibits strong antioxidant activity (up to 99.7%), making it a suitable candidate for nutraceutical and functional food applications (Yeh et al., 2014). Enoki polysaccharides have also been reported to possess antioxidant and anticancer properties in *in vitro* studies (Ko et al., 2007). These attributes make it a promising functional food and nutraceutical ingredient.

Domestication and cultivation of *F. velutipes* have been extensively studied in East Asia, where enoki is one of the most commercially important edible fungi. China alone produces an estimated 2.1–2.4 million metric tonnes of enoki mushrooms annually, making it the world's largest producer, followed by Japan and South Korea at comparatively lower production levels (Industry data, 2025). Yield and quality are significantly influenced by environmental factors such as substrate composition, temperature, humidity, photoperiod, and carbon dioxide concentration (Rezaeian & Pourianfar, 2017; Sakamoto, 2018).

In Bhutan, mushroom cultivation has predominantly focused on shiitake (*Lentinula edodes*) and oyster mushrooms (*Pleurotus* spp.), with recent diversification into nameko (*Pholiota nameko*) and *Ganoderma* species. Although Bhutan possesses rich mushroom diversity and favourable agro-climatic conditions, the potential for enoki cultivation remains largely unexplored due to its stringent environmental requirements (Sakamoto, 2018). Wild strains, however, may offer better adaptation to local conditions and provide opportunities for diversification. Therefore, investigating their domestication is both necessary and timely to meet the increasing demand for enoki mushrooms, particularly in high-end hotels driven by the growing popularity of hotpot and noodle-based cuisines.

Bhutan harbours a rich diversity of wild mushrooms, including the valuable edible species *F. velutipes*. There is considerable potential for the domestication, cultivation, and breeding of this species to develop indigenous Bhutanese strains. To date, limited work has been undertaken on the domestication and cultivation of wild edible mushrooms in the country, primarily due to a lack of facilities and technical expertise.

In response, the National Mushroom Centre (NMC) has initiated efforts to collect and domesticate selected wild edible mushroom species. This initiative aligns with the broader objective of promoting the sustainable use of wild mushrooms while reducing dependence on wild harvesting. To alleviate pressure on natural populations and ensure long-term sustainability, one of the most effective strategies is the domestication and cultivation of saprophytic mushrooms using locally available resources.

This study was therefore conducted to assess the yield potential of a local enoki strain, followed by an evaluation of its growth, yield, and morphological characteristics in comparison with commercial strains, and finally to determine its performance on three different common sawdust substrates (oak, alder, and poplar). The specific objectives were to:

1. Domesticate the local strain of wild enoki.
2. Assess the production of local and commercial strains.

3. Assess the yield and performance of the local enoki strain using three different types of sawdust substrates.

2 Materials and Methods

2.1 General

This study comprised three different independent trials designed to achieve the objectives outlined above. All three trials were carried out at the National Mushroom Centre (NMC), Wangchutaba, Thimphu, Bhutan. The local enoki strain utilized in the study was cultured from a specimen maintained in the NMC Fungarium identified with collection number as BNF01350. This specimen had originally been collected from the Royal Botanical Garden in Serbithang, Thimphu. For the second phase of the study, commercial enoki strains obtained from Thailand and India were used to compare with the local strain.

For all three phases of the trial, the container bottles made of polypropylene were used as it can withstand high temperature and pressure of autoclaving. Each bottle had a capacity of 800 ml and was filled with 430 g of substrate prior to sterilisation.

As enoki mushrooms require temperatures below 10°C during the pre-fruiting stage, the trials were conducted during the cooler months (November to March). All three phases of the study were conducted using a Completely Randomised Block Design (CRBD).

The first phase of the trial (domestication) was carried during the winter of 2023, using 20 bottles containing oak sawdust medium were supplemented with 40% rice bran. The second phase, conducted in December 2024, utilized a substrate composed of 60% oak sawdust enriched with 20% rice bran and 20% wheat bran. Each of the five treatments had 50 replicates (sample bottles), resulting in total of 250 sample bottles.

In the third phase of the trial, conducted from November 2024 to March 2025, the local enoki strain was cultivated on three different sawdust substrates: oak (*Quercus griffithii*), alder (*Alnus nepalensis*), and poplar (*Populus wuana*). Each substrate (62%) was mixed with nutrient supplements consisting of rice bran (20%), wheat bran (6%), and corn cob (12%), and each treatment was replicated 40 times.

2.2 Substrate sterilization, inoculation and incubation

The moisture content of the substrates prior to sterilisation was maintained at 67%. The substrates were sterilised at 121°C for one hour at 15 psi using a Coslab-manufactured vertical high-pressure top-loading sterilisation unit, and were cooled overnight in a sterile room before inoculation.

Inoculation of the substrates was carried out using a UR Bio-coction-manufactured laminar airflow cabinet equipped with HEPA (High Efficiency Particulate Air) filters, a powder-coated body, and UV lighting to maintain a sterile environment.

The inoculated sample bottles were incubated in a dark room, where the temperature was maintained between 16°C and 22°C and relative humidity between 70% and 80%, until mycelial colonisation was complete. The fully colonised bottles were then transferred to sprouting (fruiting) chambers.

2.3 Initiating sprouting

The caps of the substrate bottles was removed, and approximately 3 - 5 cm of the hardened top layer of the colonized medium were manually scraped off in order to promote the initiation of pin heads. A room temperature of 13°C - 16°C and humidity of 90 - 95% were maintained to induce the sprouting of mushroom pinheads.

2.4 Fruiting

Pinheads emerged within 12–15 days, after which the room temperature was lowered to 5°C –6°C and the relative humidity was adjusted to 80–90% to regulate growth and maintain mushroom quality. When the fruiting bodies extended 2–3 cm above the bottle mouth, cylindrical plastic collars or sleeves were fitted to encourage the development of long, slender stipes.

This method creates a microenvironment with elevated CO_2 levels around the fruiting bodies, promoting elongation as the mushrooms grow towards fresh air (Ikeda et al., 2021), resulting in the development of long, slender stipes and compact caps, characteristic of high-quality enoki mushrooms. Harvesting was carried out after about one week, or when the stipe exceeded 12 cm in length and the caps had not fully expanded.

2.5 Data collection and statistical analysis

Data were recorded for parameters including days to first harvest, interval between flushes, fruit body length and fresh yield weight. The data were analysed using Stata version 15.1 (StataCorp LLC). One-way ANOVA was applied assess significant differences among treatment means, followed by post-hoc pair wise comparisons using Bonferroni-correction.

3 Results and Discussion

3.1 First phase – Domestication Trial

The first phase of the study evaluated the ability of the wild enoki strain to produce fruiting bodies on an artificial sawdust substrate under controlled conditions. Additional observations included morphological traits, yield performance, the number of subsequent flushes, and sensory qualities after cooking.

3.1.1 Morphological description

As reported in the NMC Annual Report 2020-2021, the wild enoki specimen collected from the Royal Botanical Garden in 2019 was characterized as follows: Cap**:** 1–6 cm in diameter, convex at the early stage and becoming broadly convex to flat at maturity. The surface is sticky when fresh. The cap colour ranges from dark orange-brown to orangish brown or yellowish brown; often paler toward the margin, fading with age, with the margin becoming straited. Stipe**:** 2–5 cm in length and 3–10 mm in thickness, uniform or slightly enlarged toward the base. The texture is firm, and the colour varies from whitish to pale to yellowish brown or orange-brown when young. The stipe is covered from the base upwards with a dark brown to blackish velvety layer.

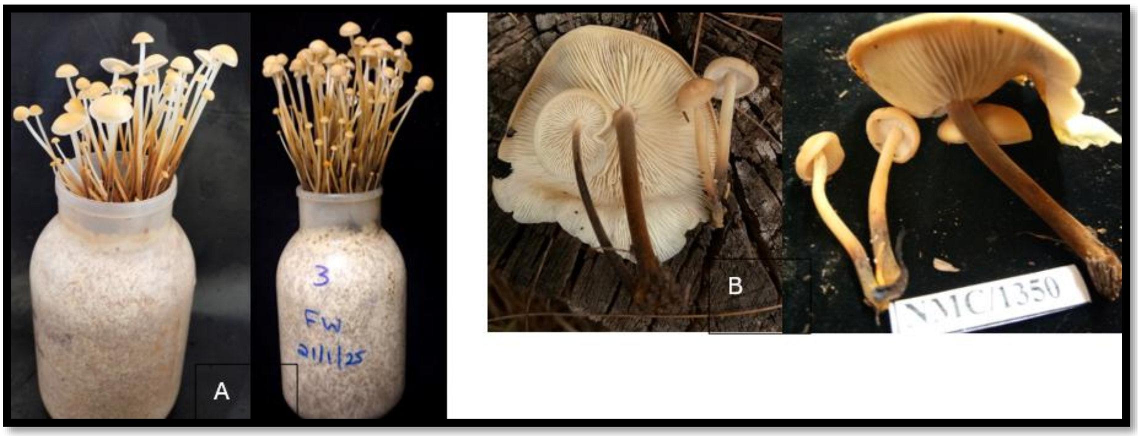


Figure 1. Enoki domesticated (A) from the wild specimen (B)

Figure 1 present images of the domesticated enoki (A) alongside the wild specimen (B) from which it was derived using the tissue culture techniques. In this trial, the domesticated enoki exhibited few minor differences from its parent source. The cap size reduced to 1.3–2.3 cm in diameter, while the stipe length increased significantly to 12–14 cm and the cap colour paler. This result indicates controlling factors such as light duration, CO_2 concentration (through the use of cylindrical plastic collar), and temperature below 10 °C effectively induced the desired traits of cultivated enoki, mainly smaller caps and elongated stipes.

3.1.2 Yield

The local enoki strain performed well, producing an average yield of 31 g per bottle during the first flush, followed by three additional flushes (Figure 2).

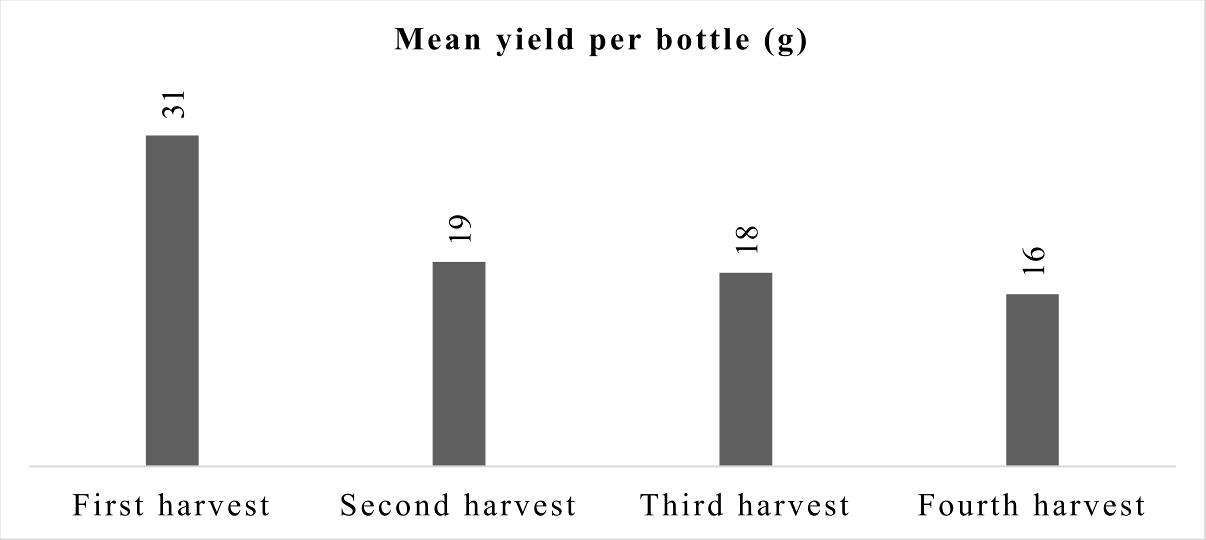


Figure 2. Mean yield of four flushes (g/bottle)

Although variations among the four flushed were visually apparent in Figure 2, a one-way ANOVA indicated highly significant differences (Table 1). Post-hoc pairwise t-tests with Bonferroni correction showed that the first flush produced significantly higher yields than the subsequent flushes (*p*<0.001), while no significant differences were observed among the second, third and fourth flushes.

Table 1. Summary of one-way ANOVA of fresh weight of local enoki among different flushes

Source	SS	df	MS	F	Prob > F
Between groups	2275.06	3	758.35	23.05	0.0000
Within groups	1842.66	56	32.90		

This analysis was crucial in determining the economic feasibility of continuing production up to the fourth harvest. Yield declined progressively, decreasing by 38% in the second flush, 41% in the third, and 48% in the fourth compared to the first flush. This trend aligns with the finding from previous studies on edible mushrooms such as *Pleurotus ostreatus*, where the first flush accounts for the majority of the total yield due to rapid utilization of available nutrients (Kim et al., 2020; Zhang et al., 2018).

The average interval between the first and second flushes was 22 days, and between the second and third flushes was 24 days. These results suggest that, for commercial production, it may not be economically efficient to retain the substrate in the fruiting chamber beyond the first flush, considering the additional energy and labour required to maintain optimal conditions. However, for household or hobby cultivation, the results demonstrate that the local enoki strain is capable of producing multiple flushes. Growers may continue production up to fourth harvest, provided that adequate moisture, temperature, and ventilation are maintained.

3.1.3 Evaluation of taste

A preliminary sensory evaluation was conducted using mushrooms harvested from the trial. The fruiting bodies were prepared as curry and hotpot soup. The local enoki strain cultivated on oak sawdust substrate exhibited a pleasant flavour and a firm, crisp texture. Compared to commercial strains, the relatively larger caps provided a desirable bite size. Additionally, the caps developed a slightly slimy texture when cooked, which was particularly suitable for soup-based dishes.

3.2 Second phase of the trial

3.2.1 Yield Comparison of Local Enoki with commercial strains

Yield comparison was conducted using only the first flush (Table 2) because although the Thai Golden strain also yielded additional flushes, whereas the other commercial strains did not produce any further flushes. However, it is important to note that the local strain produced satisfactory yield in subsequent flushes.

Table 2. Summary of one-way ANOVA on first flush yield of five enoki strains.

Source	SS	df	MS	F	Prob > F
Between groups	84243.61	4	21060.90	35.25	0.0000
Within groups	152375.20	255	597.54		

Table 3 shows that the local strain yielded the least, with highly significant differences compared to the commercial strains.

Table 3. Mean yield of the first flush of five enoki strains

Strain	Mean Weight (g) ± SD	SE
Local Enoki	30.39 ± (10.48)	---

Enoki Thai Golden	64.68 ± (14.08) ^{a}	4.22
Enoki Indian	72.38 ± (27.91) ^{a}	4.41
Enoki 1	83.58 ± (26.39) ^{b}	4.29
Enoki Thai	71.14 ± (31.42) ^{ac}	4.46

Note: Means with the same superscript letters are not significantly different.

Among the four commercial strains, Thai Golden recorded the lowest mean yield at 64.68 g per bottle. The yield of the local enoki strain's yield was more than half lower than that of Thai Golden. Nevertheless, in terms of multiple fruiting ability, the local strain produced up to four flushes (Figure 1), Thai Golden up to two flushes, while the other three strains fruited only once. Commercial strains are typically selected for high yields in the first flush, as growers are generally less interested in subsequent flushes due to declining profit margins.

3.2.2 Comparison of height among the strains

Height of the enoki biomass is key quality parameter. For most commercial applications, a minimum height of 10 cm is preferred, while premium grade products typically reach around 15 cm. Figure 3 illustrates the height of the fruiting bodies of the local and commercial strains after the removal of the plastic collars. In addition to height, other morphological traits, such as fruit body colour, are also presented in Figure 3. Commercially cultivated *F. velutipes* (enoki mushroom) is characterized by elongated stems and small caps, features that enhance visual appeal and are generally associated with a tender texture that are highly in the fresh mushroom market (Lee et al., 2025; Zhang et al., 2025).

Figure 3. From left to right - Thai Golden, Local and the other three commercial strains which are predominantly white

However, the experimental results differed markedly from expectations. With the exception of Thai Golden, the other three commercial strains exhibited shorter fruiting body heights than the local enoki strain. The local strain also showed greater uniformity in height, whereas the commercial strains displayed wider variation, including occasional outliers (Figure 4). Comparable findings have been reported in domestication studies of Shiitake (Chang & Miles, 2004) and oyster mushrooms (Rezaeian & Pourianfar, 2017), where wild strains in some cases outperformed commercial strains in specific morphological traits.

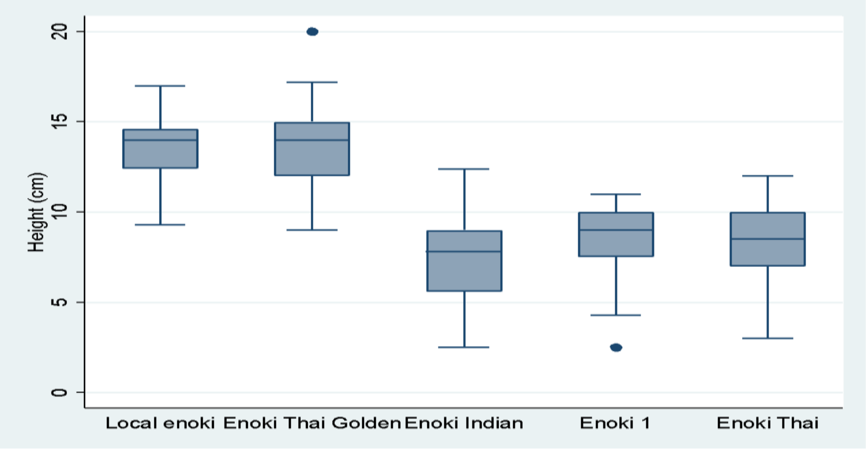


Figure 4. Boxplot showing data spread on height of the 5 strains

3.3 Third phase of the trial: Comparison of different sawdust on local strain

3.3.1 Rate of mycelial colonization and days to first harvest

The third phase of the trial evaluated the performance of the local enoki strain across three different types of sawdust substrates. Initial assessment focused on the rate of mycelial colonization. The time required for the local enoki mycelium to colonize the substrates varied notably among treatments, being fastest on alder (2) (mean ≈ 48 days), moderate on poplar (3) (mean ≈ 50 days) and the slowest on oak (1) (mean ≈ 53 days). Figure 5 illustrates the progression of colonization across three different substrates overtime.

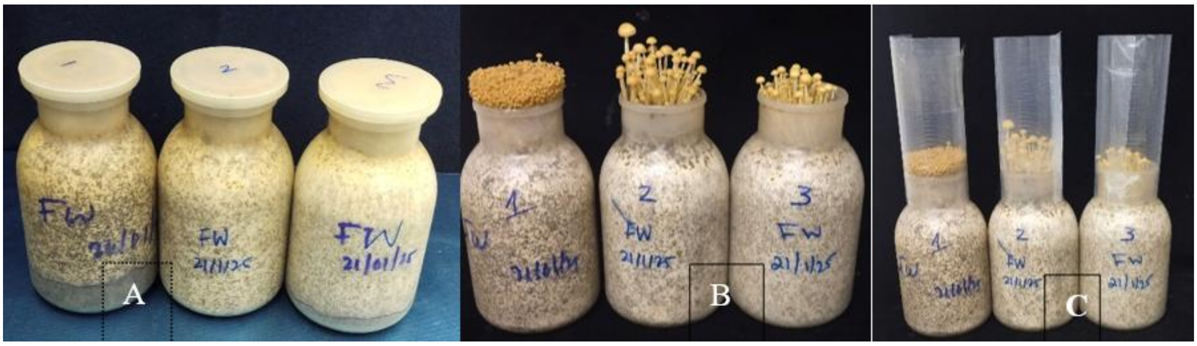


Figure 5. Difference of mycelium colonization and fruiting on different substrate; A=41 days, B and C=59 days with sleeves and without sleeves

Secondly, the time required to reach the first harvest was evaluated, a parameter closely associated with the rate of mycelial colonization. Days to first harvest is a critical factor in commercial production, as shorter production cycles are generally linked to enhanced profitability. One-way ANOVA indicated a highly significant difference among the three substrates (F (2,110) = 21.04F (2,110) = 21.04, p=0.000p=0.000). The shortest time to harvest was observed on alder (62.84), followed by poplar (65 days), while oak required the longest duration (69.88 days). The significant difference was found between oak and the other two substrates, whereas no significant difference was observed between alder and poplar.

Alder, being a fast-growing deciduous species, typically has a less compact lignocellulosic structure and lower phenolic content compared to oak. This facilitates more efficient enzymatic activity and substrate degradation by the mycelium, resulting in faster growth on such substrates (Park et al., 2014). The results of this study therefore suggest that alder and poplar are suitable alternatives for enoki cultivation, as they are more readily available and considerably less expensive than oak.

3.3.2 Yield of enoki mushroom

The yield performance of the enoki mushrooms was subsequently evaluated across the three types of substrates. Unlike the commercial strains, the local enoki produced fruit bodies over multiple flushes, although the yields dropped sharply from the first to the second and more so by the third harvest. For the first harvest, mean yields were 31.60 g/bottle on oak, 30.77 g/bottle on alder, and 30.60 g/bottle on poplar, with one-way ANOVA indicating no significant difference (F (2,110) = 0.30, p = 0.74). In the second harvest, yields were 20.26 g/bottle for oak, 22.94 g/bottle for alder, and 21.35 g/bottle for poplar, again showing no significant difference (F (2,98) = 1.10, p = 0.33). When the first and second harvests were combined, total yields reached 47.93 g/bottle for oak, 51.36 g/bottle for alder, and 51.39 g/bottle for poplar, with no significant differences observed (F (2,110) = 1.56, p = 0.21). Data from the third harvest were excluded due to contamination in many samples, which rendered them unreliable for analysis. From a commercial perspective, growers are also unlikely to wait for a third harvest due to diminishing returns. Nevertheless, it was noteworthy that some samples, particularly from alder and poplar substrates remained uncontaminated and sufficiently vigorous to produce a third flush.

Substrate composition plays a critical role in determining mushroom productivity, as the physical and chemical properties of the medium directly influence mycelial growth, fruiting and overall yield. Hardwood substrates are widely regarded as suitable for enoki cultivation due to their balanced lignocellulosic composition and structural stability. This suitability is often attributed to the gradual and consistent nutrient release and superior water-holding capacity of hardwoods such as oak, which provide a stable condition for mycelial colonisation and fruit body development (Chang & Miles, 2004; Rezaeian & Pourianfar, 2017). Previous studies have similarly reported that substrates with moderate density and lignin content tend to produce consistent yields in enoki cultivation (Kang et al., 2016).

However, the findings of this study indicate that alder and poplar substrates perform comparably to oak in terms of yield. Moreover, when considering the shorter time required to reach the first harvest, alder and poplar demonstrated a clear advantage over oak. Similar trends have been reported in studies conducted in China and Korea (Han et al., 2025).

4 Conclusion

This study demonstrates that the wild Enoki strain from Bhutanese forest can be successfully cultured and domesticated under controlled environment. Manipulation of the micro-environment can be proved effective in inducing desired morphological characteristics, including reduced cap size and elongated stipes. The cultivated fruit bodies exhibited acceptable height, morphology and texture. Yields were also satisfactory especially when multiple flushes were taken in account. However, in comparison to the commercial strains, typically selected for a single, high yielding flush, the first flush yield of the local strain was approximately half or less.

With regard to substrate selection, the result indicate that Alder and Poplar sawdust perform comparably to Oak in terms of yield, while offering additional advantages such as faster mycelial colonisation and quicker time to harvest. These characteristics suggest that Alder and Poplar sawdust may be more practical substrate options. Further research should focus on optimizing environmental conditions and substrate supplantation to enhance yield consistency, biological efficiency, and fruit body morphology across different cultivation systems.

5 Acknowledgement

The authors would like to thank the Research Program of the National Mushroom Centre for facilitating the conduct of the trials. Special thanks go to the Spawn Laboratory Program for producing the mother and cultivation spawn required for the trials without fail. The team also would like to thank all the colleagues of NMC in different programs for assisting us whenever there was need of extra hands during the experiments and data collection. Lastly but not the least, the team would also like to thank the Department of Agriculture for always pushing us to do research on mushrooms.

6 Authors' contribution statement

Sabitra Pradhan designed the study concept, conducted the trials and wrote the result and over all finalization of the paper, Tshering Chuki, helped during the trial and data collection as while as wrote the materials and method. Chencho Dukpa, constantly guided during the experiments and did the data analysis and review of the technical part of the paper and Tshering Wangmo was involved during the experiments as while as during the data collection and wrote the introduction part of the paper.

7 References

Cai, M., Lin, Y., Luo, Y., Liang, H., Sun, P., & Chen, Y. (2013). Nutritional value of *Flammulina velutipes* and its potential for application. *Journal of Food Science, 78*(2), R128–R134. https://doi.org/10.1111/1750-3841.12024

Chang, S. T., & Miles, P. G. (2004). *Mushrooms: Cultivation, nutritional value, medicinal effect, and environmental impact* (2nd ed.). CRC Press.

Food and Agriculture (FAO). (2022). *FAOSTAT Statistical Database*. Rome:FAO. http://www.fao.org/faostat/en/

Hamza, A., Mylarapu, A., Krishna, K. V. R., & Kumar, D. S. (2024). An insight into the nutritional and medicinal value of edible mushrooms: A natural treasury for human health. *Journal of Biotechnology, 381*, 86–99. https://doi.org/10.1016/j.jbiotec.2023.12.014

Han, M.-L., Zhao, Y.-L., Liu, T.-Y., Bian, L.-S., Li, Z.-H., Li, M.-X., Li, J., Yang, & An, Q. (2025). The effect of alkali lignin on laccase activity and mycelial biomass of *Flammulina velutipes*. *BioResources, 20*(1), 2243–2258. https://doi.org/10.15376/biores.20.1.2243-2258

Ikeda, S., Yamouchi, M., & Watari, T. (2021). Development of enokitake (*Flammulina velutipes*) mushroom cultivation technology using spent mushroom substrate under anaerobic digestion residue. *Environmental Technology & Innovation, 24*, 102046. https://doi.org/10.1016/j.eti.2021.102046

Kang, H., Park, J., & Kim, S. (2016). Influence of hardwood sawdust composition on yield consistency of *Flammulina velutipes*. *Journal of Mushroom Science, 40*(2), 85–93.

Kim, J. H., Lee, Y. S., & Park, S. H. (2020). Yield dynamics and patterns of nutrient depletion in multi-flush cultivation of *Pleurotus ostreatus*. *Mycobiology, 48*(1), 29–37. https://doi.org/10.1080/12298093.2020.1714233

Ko, J., Park, H., Kim, J., & Kim, S. (2007). Composition of nutrients in enoki mushrooms. *Food Chemistry, 103*(4), 1345–1352. https://doi.org/10.1016/j.foodchem.2006.10.042

Lee, K.-W., Park, C.-H., Lee, S.-C., Shin, J.-H., & Park, Y.-J. (2025). High carbon dioxide concentration inhibits pileus growth of *Flammulina velutipes* by downregulating cyclin gene expression. *Journal of Fungi, 11*(8), 551. https://doi.org/10.3390/jof11080551

Market.biz. (2026). *Mushroom production by region statistics*. http://market.biz/mushroom-statistics/

Park, J., Lee, K., & Cho, H. (2014). Substrate chemical composition and enzymatic degradation by *Flammulina velutipes*. *Journal of Agricultural and Food Mycology, 12*(3), 97–106. https://doi.org/10.1371/journal.pone.0093560

Rezaeian, S., & Pourianfar, H. R. (2017). Effect of substrates and environmental parameters on growth of *Flammulina velutipes*. *Mycology, 8*(2), 90–98. https://doi.org/10.1080/21501203.2017.1329496

Sakamoto, Y. (2018). Mushroom breeding and cultivation in the genomic era. *Breeding Science, 68*(1), 25–38. https://doi.org/10.1270/jsbbs.17087

Singh, M., Kamal, S., & Sharma, V. P. (2020). Status and trends in world mushroom production—III: World production of different mushroom species in the 21st century. *Mushroom Research, 29*(2), 75–111. https://doi.org/10.36036/MR.29.2.2020.113703

Wu, M., Luo, X., Xu, X., Wei, W., Yu, M., Jiang, N., Ye, L., Yang, Z., & Fei, X. (2014). Antioxidant and immunomodulatory activities of a polysaccharide from *Flammulina velutipes*. *Journal of Traditional Chinese Medicine, 34*(6), 733–740. https://doi.org/10.1016/S0254-6272(15)30088-5

Yeh, J., Chen, W., & Liu, C. (2014). Bioactive compounds and health benefits of *Flammulina velutipes*. *Food Research International, 62*, 352–359. https://doi.org/10.1016/j.foodres.2014.03.056

Zhang, L., Sun, X., & Li, D. (2018). Nutrient utilization patterns in sequential flushes of *Flammulina velutipes*. *Bioresource Technology, 256*, 456–462. https://doi.org/10.1016/j.biortech.2018.02.051

Zhang, Y., Chen, R., Long, Y., Li, X., & Jiang, Y. (2025). Pan-genome analysis reveals genomic variations during enoki mushroom domestication with emphasis on genetic signatures of cap colour and stipe length. *Journal of Advanced Research, 75*, 199–212. https://doi.org/10.1016/j.jare.2024.11.005

https://doi.org/10.55925/btagr.26.9103

Evaluation of the Summer Queen Passion Fruit in Bhutan

Pema Yangdon[1], Mandira Acharja[1], Kinzang Wangmo[1], Thinley Gyeltshen[1], Tshering Penjor[1], Ngawang Yeshi[1], Nangsel Tshomo[2] and Chandra Kumar Monger[3]

Abstract

Passion fruit (Passiflora edulis Sims) is valued for its rich nutritional profile and increasing global demand for both fresh consumption and juice processing. In Bhutan, only the local purple variety has been released for commercial cultivation; however, its small fruit size, low pulp, and limited juice content constrain its consumption as a table fruit and industrial utility. The establishment of Bhutan Agro Industries Limited in Lingmethang has further intensified the demand for passion fruit underscoring the need for improved varieties. To address this gap, the hybrid cultivar 'Summer Queen', a cross between purple and yellow passion fruit introduced from Japan, was evaluated across three research centres (Agriculture Research and Development Centre, Wengkhar, Bajo, and Samtenling) representing diverse agroecological zones. A trial established in 2022 used a Completely Randomized Design with three replications to compare Summer Queen with the local variety. Data on fruit and yield traits, including fruit weight, dimensions, total soluble solids (TSS), pulp content, juice content, number of fruits per vine, and yield per vine, were analysed using ANOVA and Tukey's test in R software. Results consistently demonstrated the superiority of Summer Queen, which produced significantly larger fruits, higher pulp and juice content, and greater yields per vine across locations. On average, Summer Queen yielded 10.7 kg per vine, with 16.3% TSS and 16.8 ml juice per fruit, representing a five-fold increase in yield compared to the local variety. Although agroecological factors influenced specific traits such as fruit size, pulp content, TSS, and juice recovery, Summer Queen outperformed the local variety under both low and mid-elevation conditions, highlighting its adaptability and commercial potential for both fresh consumption and processing. In recognition of its superior performance, Summer Queen was officially released for commercial cultivation during the 27th Variety Release Committee meeting.

Keywords: *Summer Queen; TSS; Yield; Juice content; Processing*

Corresponding author: *pyangdon@moal.gov.bt*

[1] Agriculture Research and Development Centre, Wengkhar, Department of Agriculture, Ministry of Agriculture and Livestock

[2] Agriculture Research and Development Centre, Bajo, Department of Agriculture, Ministry of Agriculture and Livestock

[3] Agriculture Research and Development Centre, Samtenling, Department of Agriculture, Ministry of Agriculture and Livestock

1 Introduction

Passion fruit (*Passiflora edulis* Sims) is a subtropical perennial vine crop native to South America, widely cultivated for its nutritional and economic importance. The species comprises three main types: the purple passion fruit (*P. edulis Sims f. edulis*), the yellow passion fruit (*P. edulis f. flavicarpa*), and their hybrids. The purple type is generally smaller, sweeter, and richer in flavor, making it suitable for fresh consumption as a table fruit, whereas the yellow type is larger, more acidic, and predominantly used in juice and concentrate production (Ortiz et al., 2012; Viera et al., 2020). Hybrid varieties have been developed to combine desirable traits of both forms. Among them, the Summer Queen passion fruit is a notable hybrid between *P. edulis* and *P. edulis f. flavicarpa.* It was originally developed/hybridized at the Agriculture Experiment Station for Kyushu and Okinawa District. Following its development, it was introduced and widely cultivated in Kagoshima, Japan (Macha et al., 2006). This cultivar is distinguished by its high sucrose and malic acid content, significant levels of α- and β-carotene, and richness in vitamin C (Viera et al., 2025). Furthermore, it thrives in acidic soils and consistently produces large, high-quality fruits (Niwayama & Higuchi, 2019). Beyond its nutritional appeal, passion fruit is valued for its bioactive compounds, which exhibit medicinal properties such as anticancer, anti-inflammatory, and anxiolytic activities (Arabzai et al., 2025).

In Bhutan, passion fruit cultivation remains limited, with a total production of 58.57 MT in 2025, representing only 0.13% of the country's fruit production (National Statistics Bureau, 2025). Until now, only the local purple variety has been officially released for commercial cultivation. Although popular for fresh consumption, this variety is characterized by small fruit size, low pulp, and reduced juice yield, thereby limiting its suitability for large-scale processing. On the other hand, the establishment of Bhutan Agro Industries Limited (BAIL) in Lingmethang, Mongar, has significantly increased the demand for passion fruit for juice processing. The company requires approximately 30 metric tonnes (MT) of fruits annually; however, in 2025, it was able to procure only 7 MT from Zhemgang Dzongkhag due to limited production and a lack of supply from eastern Bhutan.

To address this production gap and provide farmers with a higher-value alternative, the Summer Queen passion fruit hybrid was introduced from Japan in 2019. Compared to the local variety, it produces larger fruits with higher pulp and juice content, while maintaining a rich flavor, attributes that make it well-suited for both processing and fresh consumption.

Recognizing this potential, the Agriculture Research and Development Center (ARDC) Wengkhar initiated multilocation evaluation trials of Summer Queen in 2022 and simultaneously promoted the variety in farmers' fields. At present, five farmers are cultivating Summer Queen passion fruit across 6.31 acres of land in the eastern region under contractual arrangements with BAIL, ensuring assured markets. In addition, trellis systems for passion fruit production have been promoted with funding support from the CARLEP project, as trellis support is essential for optimal vine growth, yield, and fruit quality.

Given the increasing demand for passion fruit, the limitations of the local variety, and the promising attributes of the Summer Queen hybrid, a systematic evaluation of its agronomic and qualitative performance under Bhutanese agroecological conditions was lacking. Therefore, the main objective of the study was to assess the performance of the Summer Queen variety across multi locations, focusing on yield potential, fruit quality parameters, and its suitability for both fresh consumption and industrial processing.

2 Materials and methods

2.1 Study area

The evaluation trial was initiated in 2022 across three locations: ARDC Wengkhar (Latitude- 27.272307, Longitude- 91.270944, Altitude- 1591.8masl) in the eastern region, ARDC Bajo (Latitude- 27.492538, Longitude- 89.9016385, Altitude-1237 masl) in the western region and ARDC Samtenling (Latitude- 26.9084684, Longitude- 90.4321884, Altitude- 375 masl) in the southern region to assess the performance and adaptability of Summer Queen passion fruit under Bhutan's diverse agroecological zones. The experiment was carried out over three consecutive years from 2022 to 2025. Yield and fruit quality parameter data from all three years were included in the analysis. Passion fruit plants typically reach the economic bearing stage between 18 months and three years after planting.

Over the last three years, Wengkhar received an average annual rainfall of 776 mm, with mean maximum and minimum temperatures of 22.4°C and 13.7°C, respectively. Samtenling recorded comparatively higher rainfall, averaging 5080 mm, with mean maximum and minimum temperatures of 29.1°C and 20.7°C (Figure 1). In Bajo, the average annual rainfall was 485.4 mm, accompanied by higher temperatures, with mean maximum and minimum values of 26.6 °C and 15 °C, respectively (National Center for Hydrology and Meteorology [NCHM], 2025).

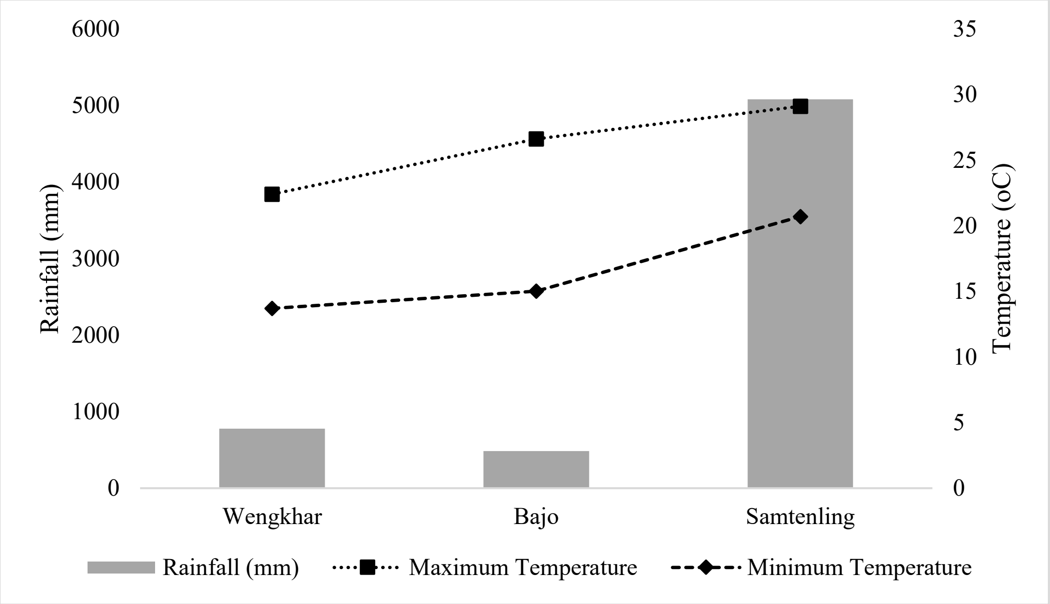


Figure 5. Average rainfall, maximum temperature and minimum temperature of three locations over the last three years (2022-2024)

2.2 Study Design

The experiment was conducted using the Summer Queen and local passion fruit varieties in a Completely Randomized Design (CRD) with three replications across all locations. Seedlings of both varieties were propagated through cuttings. The trial was established using a mound planting system at a spacing of 6 m × 3 m between rows and plants, comprising a total of 36 vines. Each replication consisted of six vines per variety, resulting in 18 vines per variety and 36 vines in total. Planting pits of 40 cm depth were prepared and filled with a mixture of topsoil and farmyard manure (FYM), after which they were raised into mounds 20–30 cm above ground level. Seedlings were planted at the center of each mound. An annual application of 10–20 kg FYM per vine was carried out, and irrigation was provided through a drip irrigation system. To support vine growth and ensure uniform management, a Kniffin trellis system was established across all locations. Under this system, two primary leaders were trained on either side of the main stem, while the lateral shoots arising from these leaders were allowed to hang freely. Pruning was conducted annually between January and February, immediately after the completion of fruit harvest.

Data collection included both fruit quality and yield attributes. Fruit quality parameters included fruit weight, fruit length, fruit diameter, total soluble solids (TSS), pulp content, juice content, and number of seeds per fruit. Yield parameters comprised the number of fruits per vine and total yield per vine. Yield data were recorded from all vines, while fruit quality parameters were assessed using samples of 10 randomly selected fruits per harvest. A total of seven to eight harvests were conducted between September and February. To minimize variation, a larger sample size was used for both fruit quality and yield assessment by recording observations from 10 fruits in each harvest. For every harvest, the total number of fruits and cumulative fruit weight were also recorded. Fruit weight was measured using a digital weighing balance, fruit dimensions were measured using a vernier caliper, and TSS was determined using a refractometer.

2.3 Data analysis

The average fruit and yield parameters of the Summer Queen passion fruit in three locations were compared using one-way ANOVA in R software at a 95% confidence interval. Similarly, two-way ANOVA was used to assess the interactions between variety and location. For all ANOVA models, the assumptions of normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) were verified. Where a significant F-test ($p < 0.05$) was found, Tukey's Honest Significant Difference (HSD) post-hoc test was applied for mean separation.

3 Results and Discussion

3.1 Descriptive statistics

3.1.1 Fruit parameters and yield parameters

Overall, the hybrid cultivar Summer Queen outperformed the local variety in both fruit and yield parameters (Table 1). Summer Queen produced larger fruits, with a mean fruit weight of 64.3 g (range: 48.9-82.9 g), compared with 47.7 g (range: 30.1-76.0 g) in the local variety. These results are consistent with the findings of Macha et al. (2006), who reported average fruit weights of 60-100 g for Summer Queen in Japan. In terms of fruit dimensions, Summer Queen recorded mean values of 6.7 cm in height and 5.8 cm in diameter, whereas the local variety produced comparatively smaller fruits, averaging 5.4 cm in height and 4.9 cm in diameter.

For TSS, Summer Queen showed a mean of 16.3% (range: 14.5-17.7%), while the local variety recorded a slightly lower mean TSS of 15.2% (range: 11.9-18.5%). The mean juice content was also higher in Summer Queen with 16.8 ml than in the local variety with 12.4 ml. Similarly, the mean pulp weight of Summer Queen was 31.5 g, which is greater than that of the local variety (21.1 g). Yield components followed a similar trend, with Summer Queen outperforming the local variety. The average number of fruits per vine was 168.6 for Summer Queen, compared with only 53 fruits per vine for the local variety. Consequently, the mean yield per vine of Summer Queen was substantially higher at 10.7 kg whereas the local variety yielded only 2.4 kg per vine (Figure 2).

Table 1. Descriptive summary of Summer Queen and Local passion fruit

Variety	**Descriptive statistics**	**Fruit weight (g)**	**Fruit height(cm)**	**Diameter (cm)**	**TSS (%)**	**Juice content (ml)**	**Pulp weight (g)**	**Fruits/vine (no)**	**Yield/vine (kg)**
Summer Queen	Mean	64.3	6.7	5.8	16.3	16.8	31.5	168.6	10.7
	Median	59.6	6.8	5.9	16.5	16.8	30.7	168.0	10.2
	Minimum	48.9	5.6	4.8	14.5	13.6	21.4	109.0	6.4
	Maximum	82.9	9.5	6.4	17.7	20.3	47.8	262.0	16.1
	Standard Deviation	11.5	0.7	0.5	1.0	2.1	6.2	33.4	2.7
local	Mean	47.7	5.4	4.9	15.2	12.4	21.1	53.0	2.4
	Median	44.3	5.3	4.7	15.9	11.1	18.9	48.5	2.5
	Minimum	30.1	4.8	4.4	11.9	6.8	13.1	21.0	0.8
	Maximum	76	6.6	6	18.5	25.0	41.0	110.0	4.6
	Standard Deviation	13.6	0.5	0.5	1.9	4.3	7.5	24.8	0.9

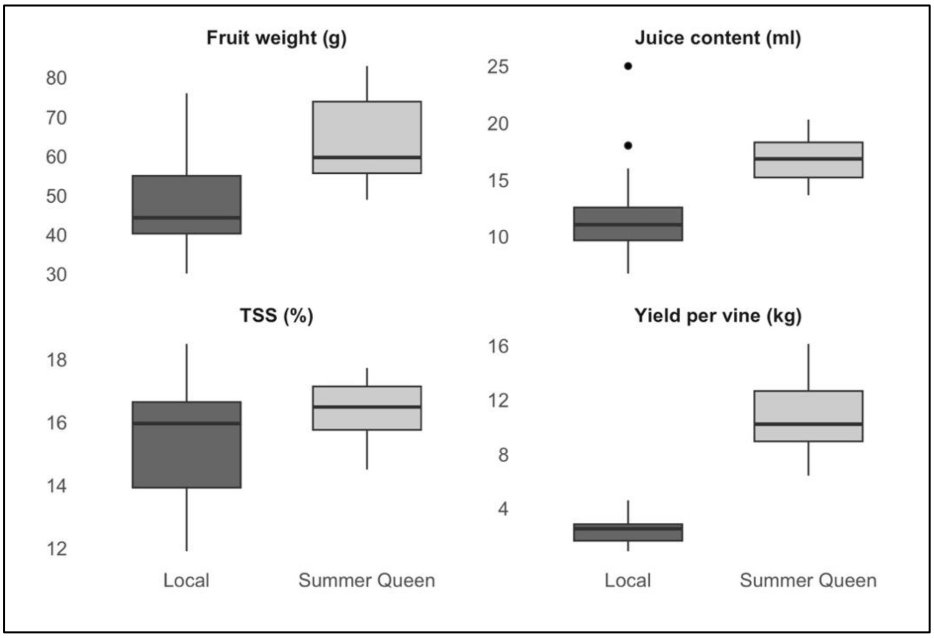


Figure 6. Comparison of key fruit quality and yield traits between Summer Queen and local passion fruit varieties

Overall, the findings demonstrate that Summer Queen consistently outperforms the local variety in fruit size, pulp and juice content, and yield, underscoring its potential as a promising cultivar for both fresh consumption and industrial processing.

3.2 Interactions between locations and treatments

3.2.1 Performance of Summer Queen across three locations

To assess the performance of Summer Queen across three locations, the one-way ANOVA was used. The one-way ANOVA revealed significant differences ($p < 0.05$) among locations for most fruit quality and yield parameters of Summer Queen, except for the number of fruits per vine (Table 2).

At Wengkhar (1591.8 masl), vines produced significantly heavier fruits (78.8 g), along with the highest pulp weight (37.8 g/fruit) and yield per vine (12.7 kg/vine). However, TSS and juice content were comparatively lower, recorded at 15.4% and 15.4 ml/fruit, respectively. The higher yield observed at Wengkhar appears to be associated with the larger fruit size, which supports the findings of Joseph et al. (2021), who reported a significant positive correlation between fruit weight and yield per vine in passion fruit grown in Kerala.

At Samtenling (375 masl), fruits recorded the highest TSS (17.3%) and fruit height (7.2 cm), indicating superior sweetness and flavor intensity. However, this location showed comparatively lower pulp weight (25.7 g/fruit) and yield (9.6 kg/vine). In contrast, Bajo (1237 masl) showed intermediate performance, with moderate fruit size, pulp weight (31.0 g/fruit), and yield (9.8 kg/ vine), while recording the highest juice content per fruit (18.4 ml). The TSS value at Bajo (16.1%) was also higher than that recorded at Wengkhar. The tendency of fruits grown at lower elevations to accumulate higher TSS may be attributed to higher temperatures that enhance sugar accumulation and reduce acidity, compared to fruits grown under cooler and higher elevation conditions as the optimum temperature for Summer Queen is between 18^0C-25^0C (Macha et al., 2006).

The variation in fruit size, physical characteristics, and yield across locations may be attributed to differences in climatic conditions, soil properties, and crop management practices. In addition, fruit set, pulp content, and juice yield are strongly influenced by pollination efficiency. Since passion fruit pollen is sticky, wind pollination is ineffective, and successful pollination largely depends on pollinators such as bumble bees or manual pollination (Fischer et al., 2018). In terms of fruit color, Summer Queen produced fully developed, vibrant purple fruits at maturity in Bajo and Samtenling, whereas fruits grown at Wengkhar exhibited a slightly tainted purple hue. This observation suggests that the variety may be better adapted to lower elevations for optimal fruit color development. Furthermore, the fruits exhibited a distinct sweet aroma in all the locations, indicating strong potential for processing into juice, jams, desserts, and fermented products (Zheng et al., 2024).

While the data unequivocally demonstrate the superior yield and physical fruit traits of Summer Queen, a critical examination reveals important considerations for its commercial rollout. The significant location-based variation presents not just statistical effects, but practical trade-offs for growers. The high-yielding environment of Wengkhar came at the cost of reduced Brix, potentially requiring processors to blend for sweetness. Conversely, the superior TSS at Samtenling aligns with premium fresh market demands, albeit with lower yield. Therefore, the optimal production zone may depend on the target market: mid-elevations for bulk processing contracts, and lower elevations for direct fresh sales where sweetness commands a price premium.

Table 2. Fruit and yield parameters of Summer Queen among three locations

Location	**Fruit weight (g)**	**Fruit height (cm)**	**Diameter (cm)**	**TSS (%)**	**Juice content (ml)**	**Pulp weight (g)**	**Fruits/vine (no)**	**Yield/vine (kg)**
Bajo	55.7^{b}	6.0^{b}	5.1^{c}	16.1^{b}	18.4^{a}	31.0^{b}	174.0^{a}	9.8^{b}
Samtenling	58.7^{b}	7.2^{a}	6.3^{a}	17.3^{a}	16.7^{ab}	25.7^{c}	165.0^{a}	9.6^{b}
Wengkhar	78.8a	6.8^{a}	5.9^{b}	15.4^{b}	15.4^{b}	37.8^{a}	167.0^{a}	12.6^{a}
p-Value	***	***	***	***	***	***	NS	*
SD	11.5	0.7	0.5	0.9	2.1	6.2	33.8	2.7

** Means within a column followed by different superscript letters are significantly different according to Tukey's Honestly Significant Difference (HSD) test at *p* < 0.05. NS: not significant; SD: standard deviation. Significance of main effects is indicated as: ***p* < 0.001, **p* < 0.01, *p* < 0.05.*

3.2.2 Interaction analysis of Summer Queen and local passion fruit across two locations

To compare the performance of Summer Queen against the local variety and to test for genotype-by-environment interaction, a two-way ANOVA was employed. This model included the fixed effects of Variety (Summer Queen, Local) and Location, as well as their interaction. Due to insufficient fruiting and yield data for the local variety at the Samtenling site, this analysis was restricted to data from the Bajo and Wengkhar locations only, ensuring a valid and balanced comparison. The local passion fruit at Samtenling had a fewer fruiting as the vines could not grow well which is likely due to excessive heat and rainfall.

The two-way ANOVA revealed significant varietal differences for most fruit quality and yield parameters, except for TSS, which showed no significant difference between varieties ($p = 0.07$). Location had a significant effect on TSS and juice content, while the interaction between variety and location was significant for most fruit physical and yield parameters, indicating differential varietal response across agro-ecological conditions (Table 3).

Across both locations, Summer Queen consistently outperformed the local variety in terms of fruit size, pulp weight, and yield per vine. Although fruit weight was comparable between varieties at Bajo, the substantially higher pulp recovery and fruit number per vine in Summer Queen contributed to its superior yield performance. At Wengkhar, Summer Queen produced significantly larger fruits (78.8 g), higher pulp weight (37.8 g per fruit), and substantially higher yield (12.6 kg per vine) compared to the local variety. Although TSS did not differ significantly between varieties, location had a strong influence on sugar development. Fruits grown at Bajo recorded higher TSS compared to Wengkhar, which may be attributed to higher temperature conditions at lower elevations that promote sugar accumulation and reduce acidity. Juice content was significantly influenced by location and variety. At Bajo, Summer Queen recorded the highest juice content (18.4 ml/fruit), whereas both varieties showed reduced juice content at Wengkhar, possibly due to cooler temperature conditions influencing juice accumulation and fruit water content.

The significant variety × location interaction observed for most fruit quality and yield parameters indicates that varietal performance is strongly influenced by environmental conditions. Summer Queen showed better adaptability across both locations, while the local variety exhibited comparatively lower productivity and fruit quality, particularly at higher elevation. From a utilization perspective, Summer Queen demonstrated strong potential for both fresh consumption and processing while the local variety showed limited commercial processing potential due to lower yield and pulp recovery across both locations.

Overall, the results demonstrate the superior performance and wider adaptability of the Summer Queen variety compared to the local variety across mid to high elevation production environments. Thus, the Summer Queen variety has been released for commercial production in the 27th Variety Release Committee (VRC) Meeting. One limitation of the present study was the absence of data from farmers' fields for comparison with the on-station results. Future studies should incorporate on-farm data and undertake comparative analyses with on-station findings to ensure greater robustness and external validity of the conclusions.

Table 3. Two-way ANOVA of Summer Queen and Local passion fruit between two locations

Variety	**Location**	**Fruit weight (g)**	**Fruit height (cm)**	**Diameter (cm)**	**TSS (%)**	**Juice content (ml)**	**Pulp weight (g)**	**Fruits/ vine (no)**	**Yield/vine (kg)**
Summer Queen	Bajo	55.7^b	6.0^b	5.1^a	16.1^b	18.4^a	31.0^b	174.0^a	9.8^b
Summer Queen	Wengkhar	78.8^a	6.9^a	5.9^b	15.4^b	15.4^b	37.8^a	167.0^a	12.6^a
Local	Bajo	54.6^a	5.8^a	5.1^a	16.8^a	14.9^a	24.2^a	33.9^b	1.8^b
Local	Wengkhar	40.8^b	5.0^b	4.6^b	13.7^b	9.9^b	18.0^b	72.1^a	2.9^a
	Variety	***	***	***	0.07	***	***	***	***
	Location	NS	NS	NS	***	***	NS	NS	0.002
	Variety x location	***	***	***	***	NS	0.002	0.02	NS

** Means within a column followed by different superscript letters are significantly different according to Tukey's Honestly Significant Difference (HSD) test at *p* < 0.05.NS: not significant; SD: standard deviation. Significance of main effects is indicated as: ***p* < 0.001, **p* < 0.01, *p* < 0.05.*

4 Conclusion

This study demonstrated significant variation in fruit quality and yield performance of the Summer Queen passion fruit across different agro-ecological locations. The consistently superior performance of Summer Queen compared to the local variety across locations confirms its higher productivity potential and wider adaptability under mid- to high-elevation growing conditions.

At Wengkhar, Summer Queen produced significantly larger fruits with higher yield per vine but lower TSS and juice content, indicating strong suitability for juice processing at mid evelation. In contrast, Bajo and Samtenling recorded relatively higher TSS and juice content, indicating better suitability for both fresh consumption and juice processing at lower elevation.

Overall, the findings demonstrate that Summer Queen possesses strong commercial potential for both fresh consumption and processing industries and can be recommended for cultivation across mid- to high-elevation production environments.

5 Acknowledgement

We would like to thank Dr. Tshering Penjor of ARDC Wengkhar for introducing the Summer Queen passion fruit seedlings from Japan. A heartfelt gratitude goes to all the ESPs involved in setting up this trial and to the Program Directors of the three research centers for your constant guidance and support. Finally, we would like to thank all the researchers involved in this study.

6 Authors' contribution statement

Pema Yangdon was involved in writing the manuscript, trial setup, data collection, and analysis. Mandira Acharja, Kinzang Wangmo, Ngawang Yeshi, Chandra Kumar Monger, and Nangsel Tshomo were involved in data collection and management of the trial block while Thinley Gyeltshen was involved in data analysis and Tshering Penjor was involved in overall guidance for trial set up and data collection.

7 References

Arabzai, M. G., Mohammadi, N. K., Olunuga, O. A., Ullah, H., Qin, Y., & Wang, L. (2025). Nutritional values and current research on passion fruit (*Passiflora edulis*). *Novel Techniques in Nutrition and Food Science, 8*(2). https://doi.org/10.31031/NTNF.2025.08.000683

Fischer, G., Melgarejo, L. M., & Cutler, J. (2018). Pre-harvest factors that influence the quality of passion fruit: A review. *Agronomía Colombiana, 36*(3), 217–226. https://doi.org/10.15446/agron.colomb.v36n3.71751

Joseph, A. V., Sobhana, A., Joseph, J., Bhaskar, J., Vikram, H. C., & Sankar, S. J. (2021). Performance evaluation of passion fruit (*Passiflora edulis* Sims.) genotypes. *Journal of Tropical Agriculture, 59*(2). https://jtropag.kau.in/index.php/ojs2/article/view/933

Macha, M. M., Chowdhury, A. K., Nomura, K., Ide, M., & Yonemoto, Y. (2006). Effect of temperature regime and soil moisture level on fruit quality of 'Summer Queen' passionfruit (*Passiflora edulis* × *P. edulis* f. *flavicarpa*). *Japanese Journal of Tropical Agriculture, 50*(2), 70–75.

National Center for Hydrology and Meteorology. (2024). *Weather data* [Data set]. Royal Government of Bhutan.

National Statistics Bureau. (2025). *Integrated agriculture and livestock census of Bhutan 2025* (p. 95). Royal Government of Bhutan. https://www.nsb.gov.bt

Niwayama, S., & Higuchi, H. (2019). Passion fruit quality under acidic soil conditions. *The Horticulture Journal, 88*(1), 1–7. https://doi.org/10.2503/hortj.OKD-169

Ortiz, D. C., Bohórquez, A., Duque, M. C., Tohme, J., Cuéllar, D., & Mosquera Vásquez, T. (2012). Evaluating purple passion fruit (*Passiflora edulis* Sims f. *edulis*) genetic variability in individuals from commercial plantations in Colombia. *Genetic Resources and Crop Evolution, 59*(6), 1089–1099.

Shimada, A., Kuramoto, K., Park, B.-J., Hashimoto, F., & Yamamoto, M. (2020). Relationship between temperature and fruit quality during ripening period in passion fruit. *Journal of the Japanese Society for Tropical Agriculture, 64*(2), 47–53. https://doi.org/10.11248/jsta.64.47

Thokchom, R., & Mandal, G. (2017). Production preference and importance of passion fruit (*Passiflora edulis*): A review. *International Journal of Agricultural Science and Research, 4*(1), 27–30.

Viera, W., Brito, B., Zambrano, E., Ron, L., Merino, J., Campaña, D., & Álvarez, H. (2020). Genotype × environment interaction in the yield and fruit quality of passion fruit germplasm grown in the Ecuadorian Littoral. *International Journal of Fruit Science, 20*(Suppl. 3), S1829–S1844. https://doi.org/10.1080/15538362.2020.1778971

Viera, W., Shinohara, T., Iyooka, C., Terada, N., Sanada, A., & Koshio, K. (2025). Physical and chemical characterization of passion fruit, focusing on the differences in juice carotenoids and sugars. *The Horticulture Journal, 94*(2), 190–199. https://doi.org/10.2503/hortj.QH-150

Zheng, L., Wang, S., Yang, Y., Zheng, X., Xiao, D., Ai, B., & Sheng, Z. (2024). Volatile aroma compounds of passion fruit seed oils: HS-GC-IMS analysis and interpretation. *Food Chemistry: X, 21*, 101212. https://doi.org/10.1016/j.fochx.2024.101212

https://doi.org/10.55925/btagr.26.9104

Impact of Arecanut Plantation on Farming System and Livelihood: A Case Study of Chhuzanggang Gewog, Sarpang

Ugyen Gyeltshen[1] and Tenzin Wangchuk[2]

Abstract

Farming systems are dynamic and context-specific, shaped by interactions between crops, livestock, labour and natural resources. In Bhutan, a growing shift from subsistence mixed farming to market-oriented cash crop cultivation has become prominent in the subtropical regions. This study assessed the impact of increasing arecanut plantations on the performance of the farming system and the livelihoods of the farmers of Chhuzanggang Gewog, Sarpang Dzongkhag. A cross-sectional multi-stage sampling method was employed, combining a household survey (n = 117) with field observations and secondary data. Farms were categorized into three groups based on arecanut land coverage: Low Arecanut Coverage (LAC) (<25%), Medium Arecanut Coverage (LAC) (25-75%), and High Arecanut Coverage (LAC) (>75%). Four farm indicators, i.e., area allocation for crop cultivation, food self-sufficiency, income per capita, and crop diversity, were compared among three farm categories. The results showed higher farms under MAC (n=62, 53%), followed by LAC (n=44, 38%), and HAC (n=11, 9%). The HAC farms showed the lowest percent household calorific fulfilment of 0.00% compared to MAC (14.7%) and LAC (92.20%). However, HC farms were found to have the highest per capita income of 5.52$PPP per day per person. Crop diversity was also observed to decrease with increasing arecanut plantations; an SDI score of 0.16 was recorded in HAC, indicating low diversity. These findings highlight a trade-off between household food self-sufficiency and income generation, suggesting that rapid arecanut expansion may undermine the rich agro-biodiversity and household food self-sufficiency in the long term. Therefore, there is a need for balanced land use polices promoting sustainable arecanut production integrated with food crops to enhance the livelihood of farming communities.

Keywords: *Arecanut; farming system; impact; livelihood*

Corresponding email: ugyengyeltshen7@gmail.com *or* ugyeltshen@moal.gov.bt

[1] National Soil Services Centre, Department of Agriculture, Ministry of Agriculture and Livestock

[2] Department of Agriculture, College of Natural Resources, Royal University of Bhutan

1 Introduction

Farming systems represent the complex interactions between crops, livestock, labour, and natural resources within specific agro-ecological and socio-economic settings. They form the backbone of rural economies, with almost half of the world's households connected to agri-food systems and employing around 1.23 billion people globally (FAO, 2023). These systems have continuously evolved as a result of demand for food and fibre, adapting to local ecological conditions and cultural contexts (Reijntjes *et al*., 1992). A prominent trend has been the gradual shift from diverse, subsistence-based farming to specialised, market-oriented cash crop production, driven by population growth, market demand, climate variability, and technological change (Thompson & Scoones, 2009; Reardon et al., 2018; Kuchimanchi et al., 2022).

Understanding the changing characteristics of farming systems is critical for enhancing technology adoption, guiding policy decisions, and reducing risks for rural communities (Valbuena et al., 2015). Farming households are sensitive to external shocks, such as market variations and input price volatility, which can destabilise livelihoods. For example, the removal of fertilizer subsidies in Mali during the COVID-19 outbreak drastically reduced cotton production, forcing farmers to shift toward lower-input crops and indicating the importance of affordable inputs in sustaining cash crop production (Dissa et al., 2024).

In South Asia, farming is typically integrated, combining agriculture, livestock, forestry, and fisheries, with 65% of the population residing in rural areas. (FAO & IFAD, 2019). Despite this reliance, 14.9% of the population remains undernourished, and 216 million live below the international poverty line of $ 1.90 per day (Shrestha et al., 2021). Land use changes, including the expansion of commercial crops, have increasingly displaced native crops, reducing dietary diversity and threatening long-term food and nutrition security (ICIMOD, 2018).

Bhutan reflects many of these regional dynamics, but with unique challenges due to its geography. Only 2.93% of land is arable, much of it situated on steep slopes, and the average household landholding is just 2.16 acres (Ministry of Agriculture and Forests [MoAF], 2011; National Statistics Bureau [NSB], 2018). Farming systems are primarily subsistence-oriented, integrated, and self-sufficient, with households cultivating diverse crops and rearing livestock to meet food needs (Katwal, 2013). However, Bhutan remains dependent on imports for essential food items, with cereal self-sufficiency estimated at 77.3% and rice at only 47.1% (Dukpa et al., 2021).

Cash crop cultivation has emerged as a major livelihood source, reducing poverty and improving rural infrastructure (Shangdiar, 2021). Additionally, exports create employment opportunities and promote economic growth. Conversely, the shift towards cash crop farming disrupts ecosystem services and promotes monoculture (Zhang et al., 2012). In Bhutan, the gradual replacement of indigenous crop varieties by cash crops poses a significant risk of permanent displacement and on-farm extinction (Wangda et al., 2019).

Arecanut cultivation has become a major cash crop in Bhutan's subtropical region, with cultivation now extending to ten districts. The production has risen by 69.3% in recent years, (NSB, 2021), driven by the rapid expansion of cultivated area. Consequently, it adds income advantages. Yet, this shift towards cash crop cultivation raises concerns about reduced food self-sufficiency at the farm level, poor dietary diversity, and the displacement of indigenous crop varieties (Behera et al., 2016). Despite its growing importance, the implications of expanding arecanut cultivation for farming systems and community livelihoods have not been studied. Understanding this transition is essential for evidence-based agricultural planning and policy decision-making. Therefore, this study assessed the impact of the increasing arecanut plantations on the farming system and livelihoods. This study was conducted in Chhuzanggang Gewog, where arecanut cultivation predominates in dryland areas. A cross-sectional mixed-method research design was employed, incorporating both quantitative and qualitative data collection approaches.

2 Materials and method

2.1 Study area

This study was conducted in five Chiwogs of Chhuzanggang Gewog under Sarpang Dzongkhag (Figure 1) in December 2022. Geographically, it is located on the eastern side of Sarpang (26°52'04.76'' N and 90°31'32.25'' E) and 56 km away from the Dzongkhag headquarters. It has a land area of 53.20 square kilometres and an altitude ranging from 195-300 masl. The cropping system is centred on paddy as the primary cereal crop, with arecanut, ginger, and winter vegetables produced as major cash crops. Cattle, poultry, and goats are the most commonly raised livestock. Chhuzagang was selected as the study site since it is one of the highest arecanut-producing gewogs in the district, with farmers increasingly allocating more dryland to arecanut plantations each year.

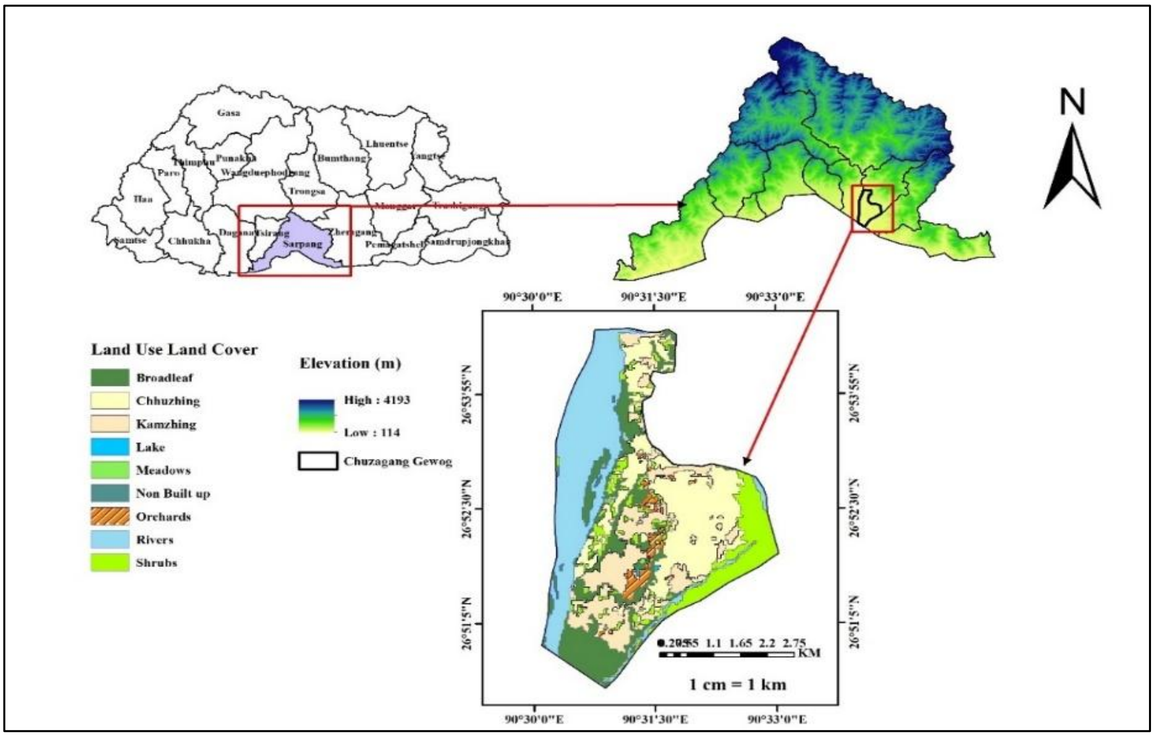


Figure 1: Map of Bhutan showing the location of the study area, Chhuzanggang Gewog in Sarpang Dzongkhag

2.2 Sampling method and data collection

A cross-sectional, mixed-methods research design was employed, integrating both quantitative and qualitative data collection approaches. Primary data were obtained from selected farmers using a semi-structured questionnaire. Chhuzagang comprises 389 households, of which 30% (*n* = 117) were included in the study (Table 1). Given the similarity of farming systems across the five Chiwogs in Chhuzagang, a 30% sampling intensity was considered sufficient to represent the population. Moreover, Hair et al., (2009) suggest that a minimum sample size of 100 observations is generally adequate for inferential analyses. After determining the sample size, a three-stage sampling procedure was employed; purposive sampling in the first stage, followed by proportionate sampling, and finally, simple random sampling.

Purposive sampling was employed to select Gewogs aligned with the study objectives, followed by proportionate sampling to ensure that Chiwogs with larger household numbers and more extensive arecanut cultivation contributed proportionally to the sample. Simple random sampling was then conducted within each Chiwog, providing all households an equal probability of selection.

Table 1: Proportionate sample from each Chiwog with geo-coordinates

Chiwog	**Total household (*N*)**	**Sample size (30% of *N*)**	**Geo-coordinates**
Barthang	102	31	26.85ºN, 90.52ºE
Yueling	103	31	26.87ºN, 90.52ºE
Shawapang	61	18	26.90ºN, 90.52ºE
Pangzor	69	21	26.91ºN, 90.52ºE
Chaskhar	54	16	26.88ºN, 90.52ºE
Total	**389**	**117**	

The questionnaire was developed in "My Survey Solution" web-based application, and the data collection was carried out using the mobile application "Interviewer" through face-to-face interviews. The survey questionnaires captured information on multiple variables, such as household information, land holding and utilization, crop and livestock production, household income from the crop, and area allocation for crop production. In addition, field observations, literature reviews, and secondary data maintained by the Renewable Natural Resources Extension Centre of Chhuzanggang and the NSB were also used.

2.3 Farm categorization

Most of the farmers in Bhutan are small-holders, and their ways of producing crops vary based on the availability of resources. Thus, this study included three farm categories to examine the impact of increased arecanut plantations on the farming system and livelihood. The farm categorization was carried out similarly to the study conducted by (Anderman et al., 2014), where it was based on a percentage of land allocated to cash crops; Farm Category 1: Low Arecanut Coverage (LAC); defined as land having <25% of arecanut plantations, Farm Category 2: Medium Arecanut Coverage (MAC); defined as land having 25% to 75% of arecanut plantations, and Farm Category 3: High Arecanut Coverage (HAC); defined as land having >75% of arecanut plantations.

2.4 Parameter used for assessing the impact

To determine the impact of increased arecanut plantation, the study considered four indicators: 1) area allocation for the crop cultivation, 2) food self-sufficiency, 3) Income per capita, and 4) diversity of crops.

2.4.1 Area allocation (ac)

The cultivated area refers to the land utilized by individual farms for growing crops. It was determined by adding the areas dedicated to cereal, vegetables, spices, fruit, and arecanut cultivation. The survey inquired about farming practices, including recording the household's total cultivated land and crop-wise area allocation. These measurements were based on estimates provided by the farmers for one year.

2.4.2 Food self-sufficiency (FSS%)

Food self-sufficiency was calculated based on the fulfilment of the household calorific need by on-farm production of calories. The total calories produced on a farm were estimated from the household's annual crop production, and the calorie requirements were derived based on the gender and age composition of household members. Food self-sufficiency was then expressed as the percentage of total caloric needs met through on-farm production, as shown in the equation (Eq.1).

$$\mathrm{FSS}(\%) = \frac{\text{Calorie produced on farm}}{\text{HH calorie requirement}} * 100 \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \;(1)$$

In this study, only cereal crops, *i.e.*, paddy, maize and millet, were considered as food crops supplying calories, as they are the most commonly cultivated and consumed in Bhutan. Since food self-sufficiency focuses on self-produced food, food purchased outside the farm was not included in the calculation. The caloric content of each cereal was determined according to standards set by the FAO (2001), and the total on-farm calorie production was calculated using the equation (Eq. 2):

$$\text{Calorie produced on farm} = \textstyle\sum_{c}(\text{calorie content}_{c} * \text{prod}_{c}) \;\ldots\ldots\ldots\ldots \;(2)$$

Where:

$\text{Calorie content}_{c}$: the crop-specific calorie content (kcal kg^{-1})

$prod_{c}$: production of the crop (kg)

The total household caloric requirement was estimated using Equation (Eq.3), accounting for differences in gender (male and female) and generations (children and adults). As the study was conducted within a farming community, an active lifestyle was assumed. Caloric requirements for each gender and generation were derived following Britten et al. (2006).

$$\text{HH calorie requirement} = 365 * \textstyle\sum(a + b + c + d) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \;(3)$$

a = Reqcal * TMC

b = Reqcal * TMA

c = Reqcal * TFC
d = Reqcal *TFA

Where:

Reqcal: Calorie requirement as per the gender and generation day (kcal day^{-1})
TMC= Total number of male children
TMA= Total number of male adults
TFC= Total number of female children
TFA= Total number of female adults
a: calorie requirement for the total number of male children per day (kcal day^{-1})
b: calorie requirement for the total number of male adults per day (kcal day^{-1})
c: calorie requirement for the total number of female children per day (kcal day^{-1})
d: calorie requirement for the total number of female adults per day (kcal day^{-1})
HH calorie requirement: Total household calorie requirement per year (kcal $year^{-1}$)

2.4.3 Income per capita

Per capita income was calculated using the equation (Eq. 4), taking into account the revenue generated from crop sales and the number of household members present in a year. Per capita income was expressed in US dollars purchasing power parity ($PPP) to compare with the international poverty line of 2.15 $PPP day^{-1} person (World Bank, 2022). Local currency income (Bhutanese Ngultrum, BTN) was converted to USD PPP using the PPP conversion factor of 19.37 BTN per USD PPP for 2022, as reported by CEIC (2023).

$$\text{Income per capita (\$ppp/day)}=\frac{\text{Net crop income}}{\text{HH members*365}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(4)}$$

2.4.4 Diversity of crops

Crop diversity was calculated using Simpson's diversity index (SDI) because it considers relative crop abundance; additionally, it is the most commonly used indicator in many studies (Lourme-Ruiz et al., 2021). The proportional abundance of each crop type was determined by dividing the area of each crop type by the total area of all crops. The crop diversity was calculated using the equation (Eq. 5), and the resulting Simpson Diversity Index (SDI) scores were interpreted according to the classification proposed by Guajardo (2015).

$$Simpson\ Diversity\ Index\ (SDI) = 1 - \sum(pi)^2 \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(5)}$$

Where:

pi = Proportional abundance of each crop type

2.5 Data Analysis

The statistical analysis was performed using R (version 4.5.1) and Microsoft Excel (version 2021) to assess the impact of arecanut plantations. The normality of the data was initially checked using Q-Q plots and histograms, but the assumptions required for parametric tests were not met. Therefore, the Kruskal-Wallis H test was used to examine the effects of the three farm types on area allocation for crop cultivation, food self-sufficiency, per capita income and crop diversity. When significant differences were observed among groups with more than two levels, post-hoc pairwise comparisons were conducted using Dunn's test with Bonferroni correction at a significance level of $p < 0.05$. Additionally, descriptive statistics, including mean, standard deviation, minimum, maximum, and quartiles, were computed for each farm category.

3 Results and Discussion

3.1 Demographic characteristics of the respondents

Table 2 presents the profiles of the study respondents, showing that the majority were female (55.60%). Male respondents accounted for a smaller proportion (44.40%), resulting in a male-female ratio of 1.24. The mean age of the respondents was recorded as 52.78 ±14.66 years, ranging from 23 to 80 years of age, and the majority (39.32%) of them were illiterate. Among the literate participants, those who had received non-formal education constituted the highest proportion (29.06%), while participants with tertiary education represented the lowest proportion (1.7%).

Table 2: Demographic profile of the respondents (n=117)

Characteristics	Categories	Percentage (%)
Gender	Male	44.40
	Female	55.60
Age	60 years and below	67.50
	61 years and above	32.50
Literacy level	Illiterate	39.32
	NFE	29.06
	Primary school	18.80
	High school	11.11
	Tertiary education	1.71

3.2 Impact of arecanut plantation

The majority of households in Chhuzanggang fall under the medium arecanut coverage farm category, followed by low and high (shown in Figure 2). The statistical analysis revealed a significant difference in food self-sufficiency, income per capita, crop diversity, and area allocation for cereal among the three categories ($p < .05$). However, the results did not show a significant difference in the area allocation of vegetables, spices, and fruit ($p > .05$).

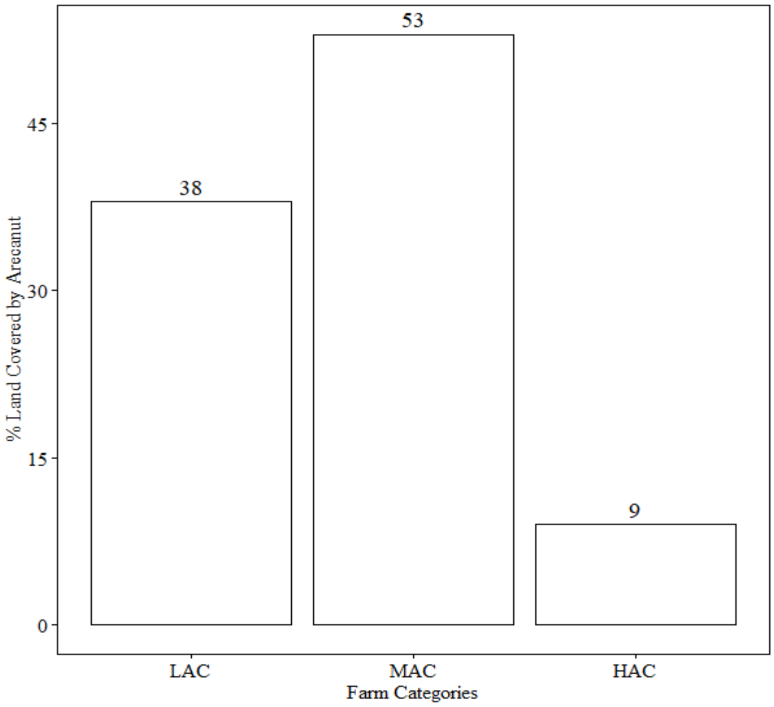


Figure 2: Number of households under each farm category

3.2.1 Area allocation for the cultivation of crops:

The study results revealed a significant difference in land allocation for cereal cultivation among farm categories, $H(2) = 20.41$, $p < 0.001$ (Table 3). Post-hoc pairwise comparisons using Dunn's test with Bonferroni adjustment indicated that households with Low Arecanut Coverage allocated significantly more land than those with Medium Arecanut Coverage ($Z = 3.17$, $p = 0.005$) and High Arecanut Coverage ($Z = 4.11$, $p < 0.001$). No statistically significant difference was observed between Medium and High Arecanut Coverage groups ($Z = 2.33$, $p = 0.060$).

Table 3: Descriptive statistics of the area (ac) allocated for crop (Mean ± SD)

Farm categories	***n* =*117***	**Cereal (ac)**	**Vegetable (ac)**	**Spices (ac)**	**Fruit (ac)**
LAC	44	2.09 ± 1.65^{a}	0.08 ± 0.17^{a}	0.03 ± 0.09^{a}	0.08 ± 0.05^{a}
MAC	62	1.13 ± 1.35^{b}	0.06 ± 0.09^{a}	0.04 ± 0.11^{a}	0.09 ± 0.05^{a}

HAC	11	0.17 ± 0.42^b	0.04 ± 0.02^a	0.05 ± 0.08^a	0.10 ± 0.04^a

* Values with different superscripts within rows are significantly different [$p < .05$] at a 95% confidence interval. Significance values have been adjusted by the Bonferroni correction for multiple tests.

No significant differences were found in the allocation of land for fruit, vegetable, and spices cultivation among the three farm categories ($p > 0.05$). This lack of difference may be attributed to the widespread practice of intercropping among farmers, with 93.20% cultivating arecanut alongside vegetables, fruits, and spices, which results in similar land allocation patterns across farm types. The feasibility of intercropping other crops with arecanut was supported by previous studies. For example, Sujatha et al. (2006) recommended utilizing the space available between arecanut trees for crop cultivation, as arecanut occupies only 35% of the available area for growth and survival, leaving the remaining 65% underutilized. In contrast, cereal crops were not intercropped with arecanut due to their higher sunlight requirements (Dufour et al., 2013), which resulted in observable differences in area allocation for cereals among the three farm categories.

3.2.2 Food self-sufficiency

According to the FAO (1999), “The concept of food self-sufficiency is generally taken to mean the extent to which a country can satisfy its food needs from its domestic production.” This study considers the concept of food self-sufficiency at the household level. The study found a significant relationship between household food self-sufficiency and the allocation of land to arecanut plantations. The test results (Figure 3) showed a significant difference in food self-sufficiency among households with different levels of arecanut coverage ($H(2) = 14.64$, $p < 0.001$). Post-hoc pairwise comparisons indicated that households with Low Arecanut Coverage were significantly more self-sufficient than those with Medium ($Z = 2.56$, $p = 0.032$) and High Coverage ($Z = 3.55$, $p = 0.001$). However, no significant difference was observed between Medium and High coverage groups ($Z = 2.12$, $p = 0.102$).

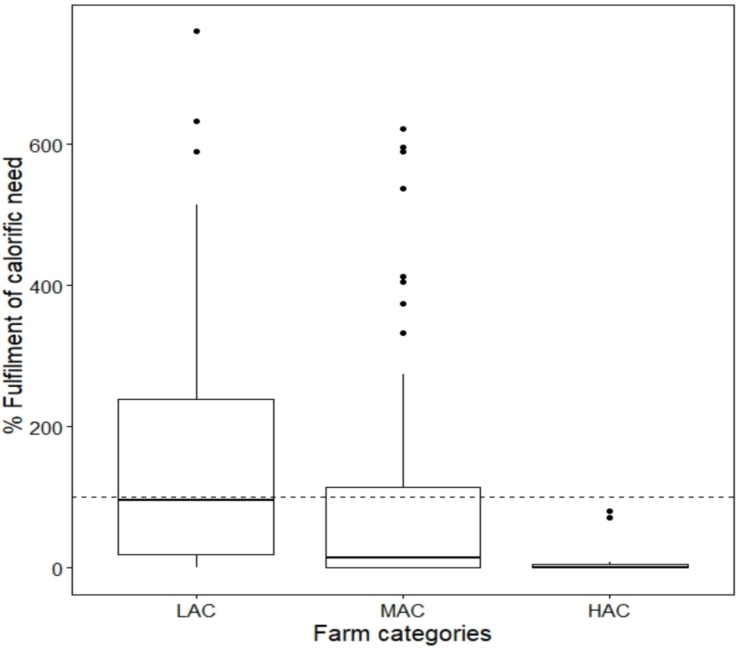


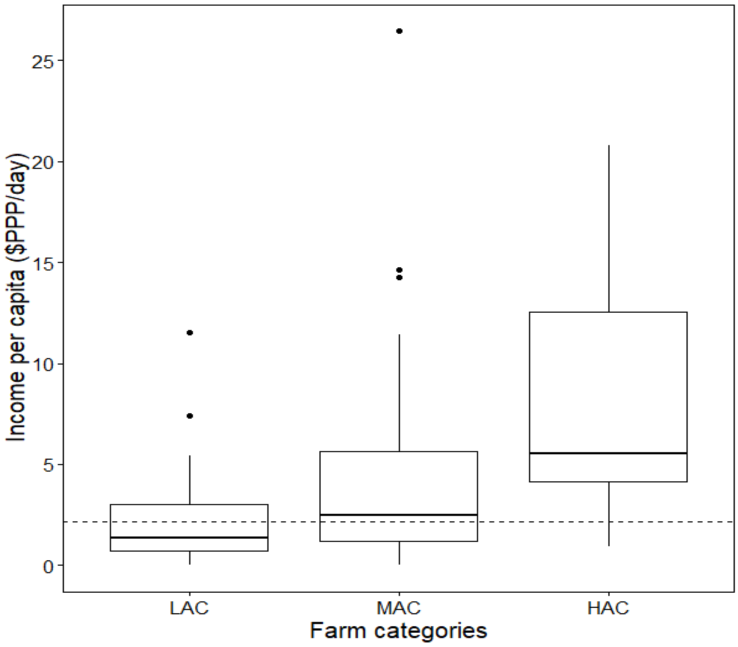
Figure 3: Percentage of calorific fulfilment of the three farm categories. The horizontal black dashed line shows the 100% benchmark for calorie self-sufficiency

The median percentage of caloric fulfilment was highest in Low Arecanut Coverage households (96.2 [19.0-239.0]), followed by Medium (14.7 [0-115.0]), and lowest in High Coverage households (0 [0-4.32]). These results suggest that increasing land allocation to arecanut may reduce household food self-sufficiency. Similar observations have been reported by (Anderman et al., 2014), who found that farmers dedicating more land to cash crops faced greater challenges in meeting their own food needs. Furthermore, households with greater emphasis on cash crop production were also found to have poorer dietary habits.

3.2.3 Income per capita

The income per capita varied significantly across the three farm categories (Figure 4), $H(2) = 17.60$, $p < 0.001$. The household with Low Arecanut Coverage had significantly lower income than those with Medium Arecanut Coverage ($Z = -2.69$, $p = 0.022$) and High Arecanut Coverage ($Z = -3.95$, $p < 0.001$). Additionally, the household with Medium Arecanut Coverage had significantly lower income than those with High Arecanut Coverage ($Z = -2.45$, p = 0.043). Median income per capita ($PPP day^{-1} person^{-1}) was lowest among households with Low Arecanut Coverage (1.36 [0.71-3.01]), followed by Medium Arecanut Coverage (2.50 [1.20-5.63]), and was highest among households with High Arecanut Coverage (5.52 [4.12-12.50]).

The observed income differences across arecanut coverage categories suggest a positive association between arecanut cultivation intensity and household income, consistent with previous findings that cash crop expansion can enhance farm income ((Meng et al., 2020; Masanjala., 2006). These results are based on univariate group comparisons and observed differences in income across farm categories; variation in farm size, household size, education, and labour availability may influence the results, which were not considered in the analysis.



.

Figure 4: Income per capita of a household of three farm categories. The horizontal black dashed line represents the benchmark for the international poverty line of 2.15 $PPP day-^{1} person

3.2.4 Crop diversity

The study documented 37 crop types, with individual households cultivating between 3 and 29 types. Arecanut was cultivated by all households, and its cultivation significantly affected household-level crop diversity. A statistically significant difference in crop diversity was observed among households with different Arecanut Coverage, H (2) = 17.68, $p < 0.001$. Post hoc pairwise tests showed that households with Low Arecanut Coverage did not differ significantly from those with Medium Arecanut Coverage ($Z = -0.08$, $p = 1.000$). However, households with Low Arecanut Coverage had significantly higher diversification than those with High Arecanut Coverage ($Z = 3.92$, $p < 0.001$), and households with Medium Arecanut Coverage also had significantly higher diversification than those with High Arecanut Coverage ($Z = 4.09$, $p < 0.001$).

Households with low and medium arecanut coverage exhibited moderate levels of crop diversity, whereas households with high arecanut coverage showed comparatively low crop diversity (Table 4). This pattern is consistent with the findings of Abebe (2013), who reported that increased allocation of farm resources to cash crops is associated with reduced agrobiodiversity, a decline in staple crop cultivation, and a shift toward monoculture practices. At the national level, poverty alleviation and food self-sufficiency remain key development priorities, with crop diversification identified as a central approach to achieve these objectives (MoF, 2004; MoAF, 2020). However, the expansion of arecanut plantations in Chhuzanggang appears to constrain crop diversification within the study area.

Table 4: Descriptive statistics of crop diversity index of farm categories (Mean ± SD)

Farm categories	**Household (%)**	**Simpson score**	**Degree of diversity**
LAC	38	0.44 ± 0.14^a	Moderate diversity
MAC	53	0.42 ± 0.18^a	Moderate diversity
HAC	9	0.16 ± 0.13^b	Low diversity

* Values with different superscripts within rows are significantly different [$p > .05$] at a 95% confidence interval. Significance values have been adjusted by the Bonferroni correction for multiple tests.

4 Conclusions

The findings of this study demonstrate that the expansion of arecanut plantations has a significant influence on food self-sufficiency, income generation, and crop diversity of the farm. Farms with higher proportions of land under arecanut cultivation achieved higher income per capita, indicating the economic benefits of arecanut as a cash crop. However, this economic gain was accompanied by a marked decline in household food self-sufficiency. The reduction in cereal cultivation area among farms with high arecanut coverage underscores the trade-off between commercial crop production and household food self-sufficiency. These findings suggest that increased dependence on arecanut may enhance short-term income but poses long-term risks to household nutritional security and agricultural resilience. Furthermore, as more land was allocated to arecanut plantations, crop diversity on the farm decreased significantly. Therefore, there is a need for a balanced agricultural development approach that integrates both cash crop production and food crop cultivation to sustain rural livelihoods. Policymakers and extension agencies should promote diversified farming systems and intercropping models that enable farmers to maintain food production while benefiting economically from arecanut.

5 Acknowledgments

We would like to express our sincere gratitude to the College of Natural Resources for its invaluable support in conducting this research. Additionally, we are deeply thankful to all the government officials involved, particularly Mr Tashi Dawa, the Agriculture Extension Supervisor of Chhuzanggang Gewog.

6 Author's contribution statement

Ugyen Gyeltshen contributed as the corresponding author, conceptualization, writing-original draft, review and editing, developing the survey questionnaire, coordinating the survey, data curation, and formal analysis. Tenzin Wangchuk contributed to supervision, conceptualisation, writing, review, and editing, and formal analysis.

7 References

Abebe, T. (2013). Determinants of crop diversity and composition in enset-coffee agroforestry homegardens of southern Ethiopia. *Journal of Agriculture and Rural Development in the Tropics and Subtropics, 114*(1), 29–38.

Achterbosch, T. J., van Berkum, S., & Meijerink, G. W. (2014). *Cash crops and food security: Cash crops, household food security and nutrition*. LEI Wageningen UR.

Anderman, T. L., Remans, R., Wood, S. A., DeRosa, K., & DeFries, R. S. (2014). Synergies and trade-offs between cash crop production and food security: A case study in rural Ghana. *Springer*, 541–554. https://doi.org/10.1007/s12571-014-0360-6

Behera, R. N., Nayak, D. K., Andersen, P., & Måren, I. E. (2016). From jhum to broom: Agricultural land-use change and food security implications on the Meghalaya Plateau, India. *Ambio, 45*(1), 63–77. https://doi.org/10.1007/s13280-015-0691-3

Britten, P., Marcoe, K., Yamini, S., & Davis, C. (2006). Development of food intake patterns for the MyPyramid food guidance system. *Journal of Nutrition Education and Behavior*. https://doi.org/10.1016/j.jneb.2006.08.007

CEIC. (2023). Bhutan BT: PPP conversion factor: GDP [Database]. CEIC Data. https://www.ceicdata.com/en/bhutan/gross-domestic-product-purchasing-power-parity/bt-ppp-conversion-factor-gdp

Dissa, A., Slingerland, M., Giller, K. E., & Descheemaeker, K. (2024). Effects of the COVID-19-induced cotton crisis on agricultural production and livelihoods of smallholders in southern Mali. *Frontiers in Sustainable Food Systems, 7*, 1269355. https://doi.org/10.3389/fsufs.2023.1269355

Dufour, L., Metay, A., Talbot, G., & Dupraz, C. (2013). Assessing light competition for cereal production in temperate agroforestry systems using experimentation and crop modelling. *Journal of Agronomy and Crop Science, 199*(3), 217–227. https://doi.org/10.1111/jac.12008

Dukpa, W., Bajgai, Y., Phuntsho, L., Ghimiray, M., Lakey, L., Yangzom, T., Subba, M., Tobgay, T., & Om, K. (2021). Self-sufficiency and dietary energy supply of food crops in Bhutan: A status report (Issue October).

FAO. (1999). *Implications of economic policy for food security: A training manual*. Food and Agriculture Organization.

FAO. (2001). *Food balance sheets: A handbook*. Food and Agriculture Organization. https://www.fao.org/4/x9892e/x9892e00.pdf

FAO. (2023, April 3). Almost half the world's population lives in households linked to agrifood systems. Food and Agriculture Organization. https://www.fao.org/newsroom/detail/almost-half-the-world-s-population-lives-in-households-linked-to-agrifood-systems/en

FAO, & IFAD. (2019). United Nations Decade of Family Farming 2019-2028. Global Action Plan. Food and Agriculture Organization of the United Nations (FAO); International Fund for Agricultural Development (IFAD).

https://openknowledge.fao.org/server/api/core/bitstreams/5479e317-17b9-428b-9963-ba88e681ff16/content

Garbach, K., Milder, J. C., DeClerck, F. A. J., Montenegro de Wit, M., Driscoll, L., & Gemmill-Herren, B. (2017). Examining multi-functionality for crop yield and ecosystem services in five systems of agroecological intensification. *International Journal of Agricultural Sustainability, 15*(1), 11–28. https://doi.org/10.1080/14735903.2016.1174810

Guajardo, S. (2015). Measuring diversity in police agencies. *Journal of Ethnicity in Criminal Justice, 13*, 1–15. https://doi.org/10.1080/15377938.2014.893220

Hair, J. F., Black, W. C., Babin, B. J., & Anderson, R. E. (2009). *Multivariate data analysis* (7th ed.). Pearson Education.

ICIMOD. (2018). *Commercialization of agriculture in developing countries: Boon or bane?* https://www.icimod.org/article/commercialization-of-agriculture-in-developing-countries-boon-or-bane/

Kuchimanchi, B. R., Bosch, R. R., De Boer, I. J. M., & Oosting, S. J. (2022). Understanding farming systems and their economic performance in Telangana, India: Not all that glitters is gold. *Current Research in Environmental Sustainability, 4*, 100120. https://doi.org/10.1016/j.crsust.2021.100120

Kumari, A., & Choudhary, M. (2011). Annual intercrops: An alternative pathway for sustainable agriculture. *Australian Journal of Crop Science, 5*(4), 396–410. https://doi.org/10.53550/EEC.2022.v28i08s.037

Lourme-Ruiz, A., Dury, S., & Martin-Prével, Y. (2021). Linkages between dietary diversity and indicators of agricultural biodiversity in Burkina Faso. *Food Security, 13*(2), 329–349. https://doi.org/10.1007/s12571-020-01137-5

Masanjala, W. H. (2006). Cash crop liberalization and poverty alleviation in Africa: Evidence from Malawi. *Agricultural Economics, 35*(2), 231–240. https://doi.org/10.1111/j.1574-0862.2006.00156.x

Meng, L., Gan, C., Ma, W., & Jiang, W. (2020). Impact of cash crop cultivation on household income and migration decisions: Evidence from low-income regions in China. *Journal of Integrative Agriculture, 19*(10), 2571–2581. https://doi.org/10.1016/S2095-3119(20)63161-6

Ministry of Agriculture and Forests. (2011). *Bhutan land cover assessment 2010: Technical report*. Royal Government of Bhutan.

Ministry of Finance. (2004). *Bhutan poverty reduction strategy* (Issue 04). Royal Government of Bhutan.

Ministry of Agriculture and Forests. (2020). *RNR strategy 2040*. Royal Government of Bhutan.

National Statistics Bureau. (2021). *Statistical yearbook of Bhutan 2021*. Royal Government of Bhutan.

Reardon, T., Echeverria, R., Berdegué, J., Minten, B., Liverpool-Tasie, S., Tschirley, D., & Zilberman, D. (2018). Rapid transformation of food systems in developing regions: Highlighting the role of agricultural research and innovations. *Agricultural Systems, 172*, 47–59. https://doi.org/10.1016/j.agsy.2018.01.022

Reijntjes, C., Haverkort, B., & Waters-Bayer, A. (1992). *Farming for the future: An introduction to low-external-input and sustainable agriculture*. Macmillan.

Shangdiar, O. (2021). Marketing: Farmers' promotion of cash crops as a strategy to support livelihoods and improve living standards. *Journal of Social Entrepreneurship Theory and Practice, 1*(2), 1–13. https://doi.org/10.31098/jsetp.v1i2.570

Shrestha, R. B., Ferrand, P., Penunia, M. E., Dave, M., & Ali, Y. (2021). *United Nations Decade of Family Farming 2019–2028: Regional action plan to implement the UNDFF and achieve the SDGs in South Asia*. SAC, FAO, AFA, and ICA-AP. https://doi.org/10.4060/cb5030en

Sujatha, S., Balasimha, R. B. D., & Kannan, C. (2006). Crop diversification in arecanut plantation through intercropping of medicinal and aromatic plants. *Journal of Plantation Crops*, 34(2), 318–322.

World Bank. (2022). *Fact sheet: An adjustment to global poverty lines*. https://www.worldbank.org/en/news/factsheet/2022/05/02/fact-sheet-an-adjustment-to-global-poverty-lines

Thompson, J., & Scoones, I. (2009). Addressing the dynamics of agri-food systems: An emerging agenda for social science research. *Environmental Science & Policy, 12*(4), 386–397. https://doi.org/10.1016/j.envsci.2009.03.001

Valbuena, D., Groot, J. C. J., Mukalama, J., Gérard, B., & Tittonell, P. (2015). Improving rural livelihoods as a "moving target": Trajectories of change in smallholder farming systems of Western Kenya. *Regional Environmental Change, 15*(7), 1395–1407. https://doi.org/10.1007/s10113-014-0702-0

Wangchuk, T. (2021). COVID-19-induced changes in farm performance: A case study.

Wangda, D., Lakey, L., & Chophel, S. (2019). Agricultural research and development: Policy and program priorities in Bhutan. In *Agricultural policy & program framework: Priority areas for research & development in South Asia* (pp. 72–99). SAARC Agriculture Center.

Zhang, Y., Tian, C., Jiang, L., Li, Y., Xiao, Z., & Li, J. (2012). Advantages of perennial crop on conservation of agroecological environment. *Advanced Materials Research, 518–523*, 5213–5216. https://doi.org/10.4028/www.scientific.net/AMR.518-523.5213

https://doi.org/10.55925/btagr.26.9105

Evaluating Systemic Challenges and the Future Viability of Bhutan's Organic Agriculture Movement

Tshetrim La[1] and Kesang Tshomo[1]

Abstract

Bhutan's organic agriculture movement, anchored in the nation's Gross National Happiness (GNH) framework, represents a unique effort to harmonise agricultural livelihoods with ecological conservation. Since the formalisation of the National Organic Programme in 2006, Bhutan has sought to transition from awareness-driven initiatives to commercially viable organic production. This study synthesises secondary data from government reports, monitoring datasets, and international databases spanning 2010-2023 to evaluate achievements, challenges, and strategic lessons for Bhutan's organic sector. The analysis focuses on four thematic pillars: certified area, input production, commodity value chains and market development. Findings reveal notable progress, including the establishment of a dual-track certification system (LOAS and third-party certification), the development of 17 organic input production units, and targeted capacity-building programmes. However, systemic constraints persist, including weak market linkages, fragmented smallholder value chains, low adoption of organic fertilisers, and vulnerability to external shocks, as exemplified by the COVID-19 pandemic. The study identifies four key challenges: conceptual narrowness, institutional and structural deficits, economic disincentives, and cross-sectoral missed opportunities. To ensure the future viability of Bhutan's organic sector, strategic interventions are recommended, including the creation of a national organic production and market database, performance-linked incentives, and diversification into high-value, climate-resilient crops and non-wood forest products. Collectively, these measures can consolidate Bhutan's organic sector, enhancing both ecological sustainability and economic resilience while providing evidence-based guidance for policy and investment in emerging organic economies.

Keywords: *Organic Agriculture; Certification; Value Chains; Market Development; Sustainability*

Corresponding author: tshetrimla@moal.gov.bt

[1] Department of Agriculture, Ministry of Agriculture and Livestock

1 Introduction

The global organic agriculture movement has evolved from a niche practice to a significant component of sustainable food systems, driven by consumer demand for safe food and growing concerns over environmental degradation (Willer et al., 2023). In this context, Bhutan's development paradigm, anchored in the principle of Gross National Happiness (GNH), offers a distinctive framework. GNH pillars of environmental conservation and sustainable development make organic agriculture a natural strategic fit (Neuhoff et al., 2014).

Formalised by the National Framework for Organic Farming in 2006, Bhutan's organic journey aimed to harmonise agricultural livelihoods with its pristine ecological heritage (Department of Agriculture [DoA], 2006). Initial efforts under the National Organic Programme (NOP) focused on awareness and capacity building. However, they were hampered by a lack of commercial focus, limited infrastructure, and insufficient investment in value chains, resulting in a persistent gap between policy ambition and on-the-ground implementation (La & Tshomo, 2023).

To address these systemic gaps, the Royal Government of Bhutan (RGoB) launched the National Organic Flagship Programme (NOFP) during the 12th Five-Year Plan (2019–2023). The NOFP marked a strategic pivot from advocacy to commercialisation, targeting priority commodities, strengthening domestic and export certification systems, and scaling up organic input production (La & Tshomo, 2023).

The national policy impetus for organic agriculture in Bhutan has been systematically embedded within successive Five-Year Plans, reflecting its strategic importance in achieving environmentally sustainable and socially inclusive agricultural development (Ministry of Agriculture and Livestock [MoAL], 2025). Organic agriculture initiatives have been implemented through a multi-agency approach, coordinated primarily by the Department of Agriculture (DoA) under the Ministry of Agriculture and Livestock (MoAL), with support from regional research and extension centres.

A significant institutional milestone was the establishment of the National Centre for Organic Agriculture (NCOA) under the DoA, which is mandated to lead research, technology development, and the coordination of organic farming activities nationwide (National

Organic Agriculture Centre [NCOA], 2025). This development marked a critical step toward institutionalising organic agriculture within Bhutan's broader agricultural research and innovation system.

However, despite these institutional advancements, progress in terms of area-wide adoption, production scale, and measurable economic returns has remained modest. Organic agriculture continues to face systemic constraints, including limited commercialisation, weak market linkages, and inadequate private sector engagement (Neuhoff et al., 2014; Willer et al., 2023). Consequently, while policy integration and institutional frameworks have advanced, translating policy intent into tangible economic outcomes remains a persistent challenge for Bhutan's organic agriculture movement (Paull, 2023).

Although several official reports have documented the achievements and incremental progress of Bhutan's organic agriculture initiatives, there has been limited breakthrough success in realising transformative outcomes (McCrae-Hokenson, 2014). Moreover, a comprehensive and critical synthesis of the sector's cumulative achievements, persistent challenges, and strategic lessons remains largely absent from the existing literature. This study addresses that gap by examining two key questions: (1) What are the principal achievements and challenges of Bhutan's organic agriculture sector? and (2) What policy lessons can be drawn from past interventions to guide future organic pathways?

By engaging with these questions, this paper provides an evidence-based evaluation of Bhutan's organic agriculture movement, aiming to support the strategic transition from fragmented, project-based initiatives to a fully integrated, economically viable, and ecologically resilient national organic system.

2 Materials and Method

This study employed a systematic qualitative synthesis of secondary data, complemented by a comparative policy analysis and targeted quantitative assessments. Multiple verified data sources were used to ensure triangulation and strengthen analytical validity. Primary programmatic data were obtained from progress reports, monitoring datasets, and evaluation summaries of the National Organic Programme (NOP) and the Ministry of Agriculture and Livestock (MoAL) covering the period from 2010 to 2023. Additional secondary sources included the Annual Agriculture Statistics and certification reports from the Bhutan Food and Drug Authority (BFDA).

Marketing data were sourced from Bio Bhutan Pvt. Ltd., a leading private-sector exporter of organic products in Bhutan. Export revenue data were aggregated by commodity and year and analysed using time-series techniques to assess market trends, revenue volatility, and export concentration by product. Due to the limited availability of systematically published data on organic agriculture, data from the World of Organic Agriculture series published by FiBL were also analysed. The FiBL data on certified organic areas, originally reported in hectares, were converted to acres using a conversion factor of 1 hectare = 2.471 acres to ensure consistency across datasets.

The analysis was conducted in three sequential phases. First, a descriptive mapping of organic agriculture activities was undertaken across four thematic pillars: certified area, input production, commodity value chains, and market development, through which key performance indicators were identified. Second, thematic analysis was applied to assess achievements and challenges, to interpret quantitative outputs, and to link findings to relevant literature on organic transitions. Finally, strategic integration aligned emergent themes with Bhutan’s future organic pathway, examined the transferability of institutional mechanisms, and identified potential synergies and risks.

2.1 Conceptual Framework

To analyse the systemic challenges and future viability of Bhutan's organic agriculture movement, this study employs a conceptual framework adapted from the Multi-Level Perspective (MLP) on socio-technical transitions (Geels, 2011). The MLP is particularly appropriate, as it enables analysis of complex transitions by examining interactions among three analytical levels: the socio-technical landscape, the socio-technical regime, and technological niches.

In this framework (Figure 1), the Landscape denotes the broad, exogenous context that exerts pressure on the existing regime but is difficult to change. The regime is the dominant, incumbent system, in this case, Bhutan's conventional agricultural system and its associated policies, practices, and markets. The niche is the protected space where innovations emerge.

Here, it represents the organic agriculture movement, including certified farmers, the LOAS system, and organic input producers.

The transition to a viable organic sector is conceptualised as a process where landscape pressures create windows of opportunity for niches to grow and challenge the dominant regime. However, this transition is hindered by systemic barriers, which are multifaceted impediments that prevent the niche from scaling and replacing the regime.

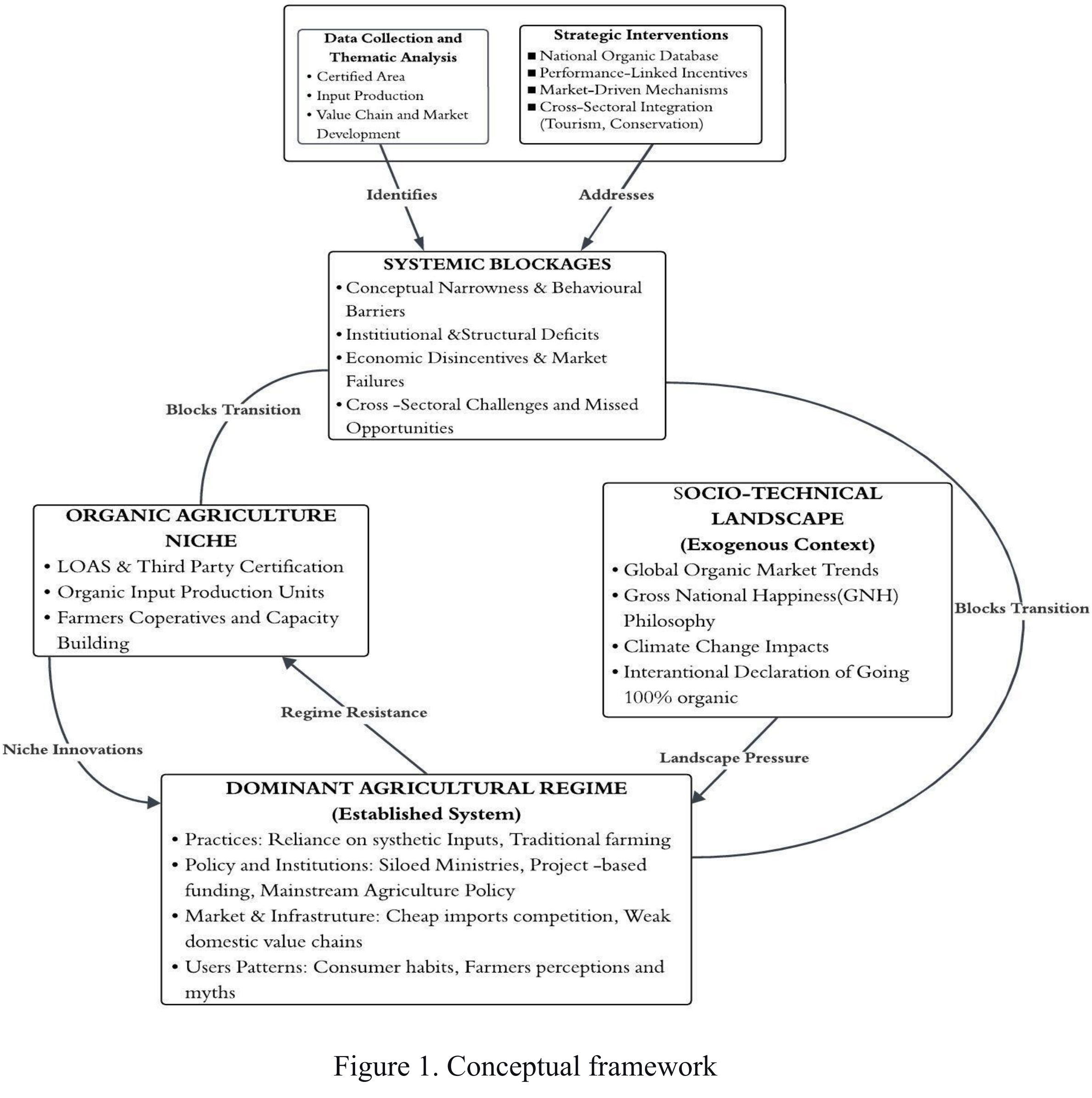


Figure 1. Conceptual framework

This study's analysis is structured to identify these blockages. It synthesises data across four thematic pillars, certified area, input production, value chains, and market development, to diagnose the specific failures at the regime level and weaknesses within the niche that are preventing a successful transition. The ultimate output is a set of targeted strategic interventions designed to overcome these blockages, thereby aligning the regime with the niche and landscape pressures to create a more resilient and viable organic sector.

3 Results and Discussion

The following analysis evaluates the progress of Bhutan's organic sector through the lens of the Multi-Level Perspective (MLP). The data reveal that the niche innovation, the organic movement, is struggling to disrupt a deeply entrenched socio-technical regime of conventional agriculture. The primary impediments to this transition are not a lack of technical solutions, but persistent regime-level blockages in markets, infrastructures, and policy, which are exacerbated by specific niche-level weaknesses in economic resilience and farmer organisation. This section diagnoses these blockages across the core thematic pillars.

3.1 Area under Organic Certification

The expansion of organically certified agricultural areas in Bhutan demonstrates a complex trajectory over the period 2011-2023 (Table 1, Figure 3). The certified agricultural area showed a marked decline after 2012, while the certified non-wood forest product (NWFP) area fluctuated significantly (Figure 2). These trends suggest volatility driven by project-based funding cycles and shifting certification priorities, rather than sustained, market-driven growth. This pattern of "boom and bust" in the certified area is not unique to Bhutan and is often observed in contexts in which policy-driven initiatives are not firmly anchored in stable market demand (Luttikholt, 2007; Smith & Marsden, 2004; Willer et al., 2023).

Table 1. The certified organic areas under agriculture and non-wood forest products reported under FiBL

Year	Certified agriculture area (ac)	Certified NWFP area (ac)	Total area (ac)	Total agriculture producers (No)	Area per producers (ac)	NWFP dependency (%)
2011	51878.65	38559.96	90438.60	0	NA	43
2012	15211.48	38559.96	53771.43	0	NA	72
2013	16619.95	38559.96	55179.90	0	NA	70
2014	16874.46	15604.37	32478.82	2680	6.296	48
2015	17173.45	15604.37	32777.82	2680	6.408	48
2016	16387.67	39009.68	55397.35	4293	3.817	70
2017	16387.67	39009.68	55397.35	4295	3.816	70
2018	16387.67	19140.37	35528.04	4354	3.764	54
2019	16387.67	19140.37	35528.04	4354	3.764	54
2020	10118.75	5493.03	15611.78	1265	7.999	35

2021	13857.37	5493.03	19350.40	1998	6.936	28
2022	13857.37	5493.03	19350.40	1998	6.936	28
2023	9152.58	5493.03	14645.62	2760	3.316	38

A key institutional achievement was the introduction of a dual-track certification system: the Local Organic Assurance System (LOAS) for domestic markets and third-party certification for exports (La & Tshomo, 2023). This innovative model is a critical success factor, balancing inclusivity for smallholders with the stringency required for international trade. Such Participatory Guarantee Systems (PGS), such as LOAS, have been shown to reduce barriers to entry and foster local market development in other countries (IFOAM, 2025).

However, a critical analysis reveals inherent challenges. The dual-track system risks creating a perceptual hierarchy in which LOAS-certified produce is viewed as of lower quality by both domestic consumers and farmers, potentially limiting its market value and undermining farmers' motivation to pursue more stringent export certification. Furthermore, the long-term integrity of PGS systems depends heavily on robust social capital and continuous community engagement, which can be eroded by fluctuating farmer interest and external shocks (Luttikholt, 2007; Smith & Marsden, 2004). The sharp contraction in certified producers during the COVID-19 pandemic underscores this vulnerability, indicating that the LOAS niche lacked the institutional and market depth to withstand landscape-level pressures.

The core vulnerability is the number of certified organic producers, which decreased to 1,265 in 2020 from 4,354 in 2019. This sharp contraction indicates the sector's severe vulnerability to external shocks like the COVID-19 pandemic and underscores a lack of structural resilience, a common issue in nascent organic sectors that lack diversified market channels (Nemes, 2009; Reganold & Wachter, 2016; Wynen Els, 2002). Furthermore, the certified organic area constitutes only 1.39% of Bhutan's total cultivated land (Paull, 2023), a stark contrast to the national ambition. The high NWFP dependency (ranging from 28% to 72% of total certified area) highlights the ecological integration of Bhutan's systems but also a potential vulnerability to changes in forest management and climate impacts on forest ecosystems (Rijal et al., 2011).

The central challenge illuminated by this data is the weak market linkage. LOAS-certified products often lack reliable price premiums, providing insufficient economic motivation for farmers to sustain certification amid higher labour inputs and perceived risks. This finding underscores a critical lesson for Bhutan and similar contexts: certification infrastructure alone is insufficient; parallel mechanisms to ensure guaranteed market access and premium prices are required, without which participant attrition is inevitable (Crowder & Reganold, 2015; Nemes, 2009). The economic rationale for farmers is paramount; studies consistently show that secure offtake agreements and visible price differentials are the strongest predictors of sustained organic adoption (Wilson et al., 2021).

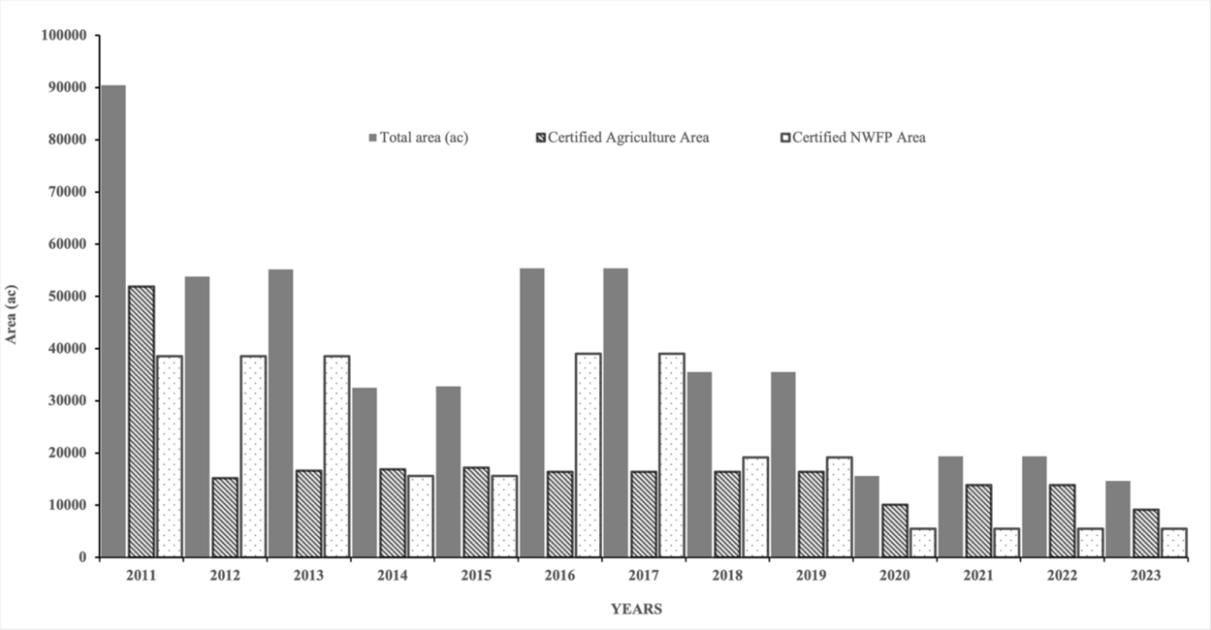


Figure 2. Structural composition of certified organic area by agriculture and non-wood forest products (NWFPs), illustrating persistent dependence on forest-based certification

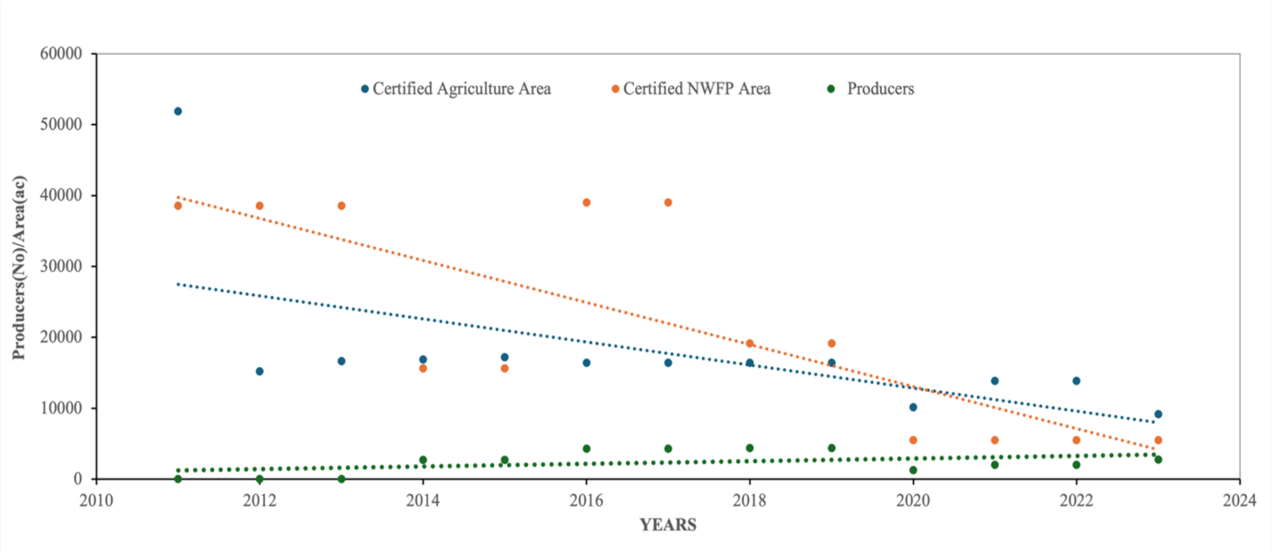


Figure 3. Long-term trends in certified agricultural area, NWFP area, and producers (2011–2023), highlighting sectoral volatility and sensitivity to external shocks

3.2 Organic Fertiliser Production

The commercial production of organic fertilisers in Bhutan has emerged as a critical achievement, marking a significant shift from exclusive reliance on household-level farmyard manure. Since 2015, substantial progress has been made in establishing a domestic bio-input industry, with 17 production units currently operational (Table 2). As illustrated in Figure 4, Chicken manure and vermicompost dominated until FY 2021-22; since then, compost production has surged, attributed to compost plants established under the National Organic Flagship Program. Collectively, these enterprises have created an annual production capacity of 4,827 metric tons, supplemented by Effective Microorganisms (EM) produced and distributed by the Agricultural Research and Development Centres (ARDCs) (La & Tshomo, 2023). This expansion of production infrastructure demonstrates both technical feasibility and growing commercial interest in supplying local organic inputs.

Table 2. The organic fertiliser producers and their production capacity in the country

Organic fertilizers	Number of producers	Production capacity/annum (MT)
Compost	8	4016
Chicken manure compost	1	500
Vermicompost	7	309
Biofertilisers	1	2

However, despite increased availability, farmer adoption rates remain critically low. The disconnect between production capacity and on-farm utilisation points to significant structural and economic barriers. Organic fertilisers face practical challenges, including bulkiness, slow nutrient release, and higher costs relative to conventional chemical alternatives. As Scialabba & Hattam (2002) note, these characteristics often lead farmers to prefer readily available synthetic fertilisers, despite awareness of their long-term ecological benefits. The experience of successful organic transitions in countries like Austria and Denmark underscores that production capacity alone is insufficient without parallel mechanisms to address affordability, distribution, and farmer education (Läpple & Rensburg, 2011).

For Bhutan's organic sector to advance, a more integrated strategy is essential. This should include targeted subsidies to reduce cost disparities, enhanced extension services to demonstrate effective application techniques, and regulatory measures that gradually restrict the use of the most harmful chemical inputs. Without such comprehensive support, the nascent organic input sector risks operating below capacity, undermining both its commercial viability and Bhutan's broader organic agricultural ambitions.

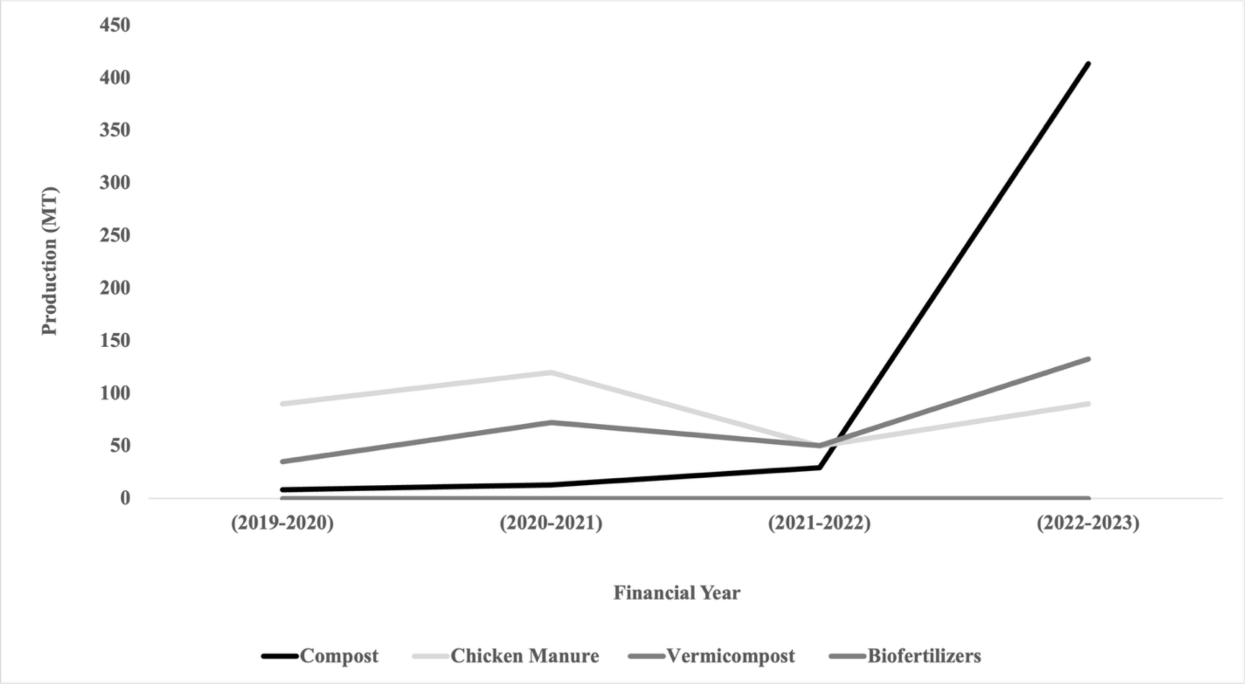


Figure 4. The trend in organic fertiliser production from 2019 to 2023

3.3 Organic Plant Protection

The use of organic plant protection measures in Bhutan has remained very limited over the past decade, with adoption rates showing little improvement and, in some cases, even declining (Figure 5). This slow uptake is attributed to factors such as limited technical capacity, lack of locally produced organic inputs, and heavy reliance on intermittent government or donor funding rather than sustained institutional or market support (National Plant Protection Centre [NPPC], 2022). During the fiscal year 2021–2022, imports of organic plant-protection materials temporarily increased, largely due to funding support provided under the National Organic Flagship Programme (NOFP). However, this improvement was short-lived, underscoring that the adoption and promotion of organic plant protection in Bhutan are closely tied to the availability of external financial assistance (CARLEP, 2022).

Currently, the use of organic plant-protection products remains insignificant, with only approximately 5 metric tonnes (MT) of organic inputs used, compared with approximately 44

MT of synthetic agrochemicals (National Statistics Bureau [NSB], 2025). Furthermore, the diversity of organic options is limited, with farmers primarily relying on pheromones, sticky traps, and neem-based products (Wangmo et al., 2023). This narrow range of available solutions highlights the need for sustained investment in research, development, and extension services to diversify organic pest management options and promote long-term adoption beyond short-term funding cycles (Paull, 2023).

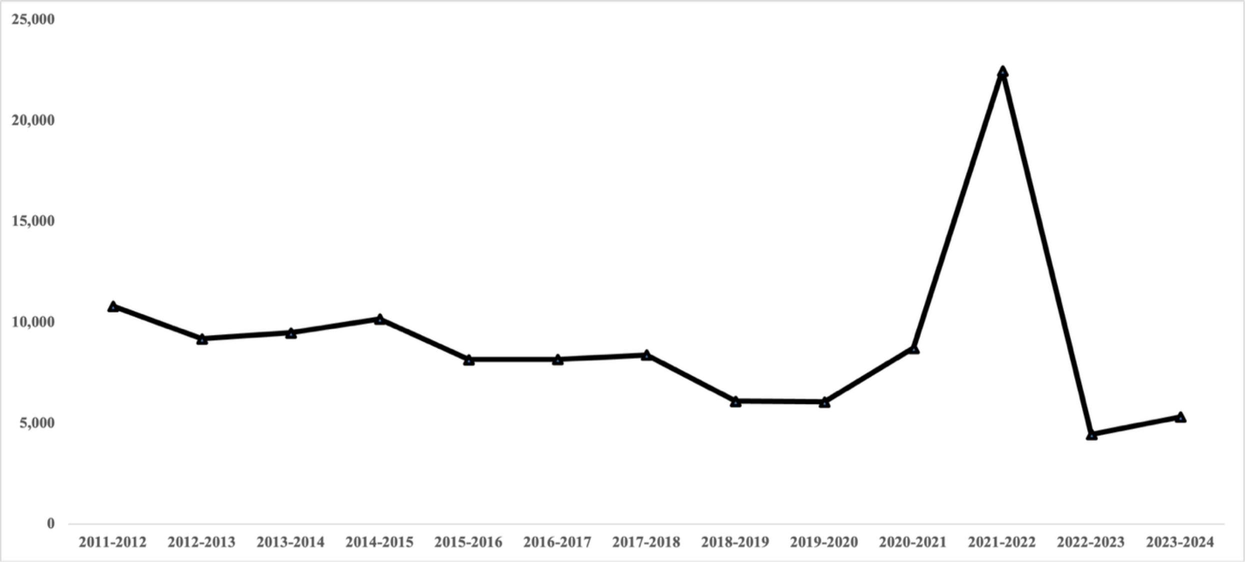


Figure 5. The organic plant protection supply trend in the country from 2011-2024

3.4 Commodity Value Chains: From Production to Market

A persistent and structurally significant constraint on Bhutan's organic agriculture movement is the absence of robust, transparent, and disaggregated market data. This condition reflects the marginalised status of the organic niche within the dominant agri-food regime. National agricultural statistics do not systematically distinguish organic from conventional production, obscuring the sector's true economic footprint and constraining evidence-based planning and investment. As Nemes 2009 argues, the inability to quantify organic sector performance is a fundamental barrier to mobilising sustained policy and private-sector support.

The limited export data available from Bio Bhutan Pvt. Ltd. nevertheless provides important insights into the structural weaknesses of Bhutan's organic value chains. As illustrated in Figure 7, organic export revenues between 2015 and 2024 are highly volatile and heavily concentrated in a single commodity (lemongrass essential oil). Other certified products, including ginger, honey, turmeric, mustard, and rhododendron, contribute only marginal and irregular export values. The stacked composition of exports (Figure 6) reinforces this picture, showing that lemongrass accounts for roughly four-fifths of cumulative organic export revenue over the period, with no other commodity achieving a stable or meaningful market share.

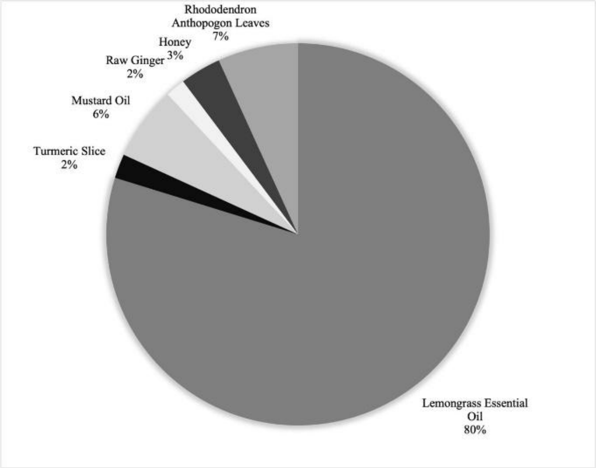


Figure 6. Cumulative composition of organic export revenues by commodity (2015–2024)

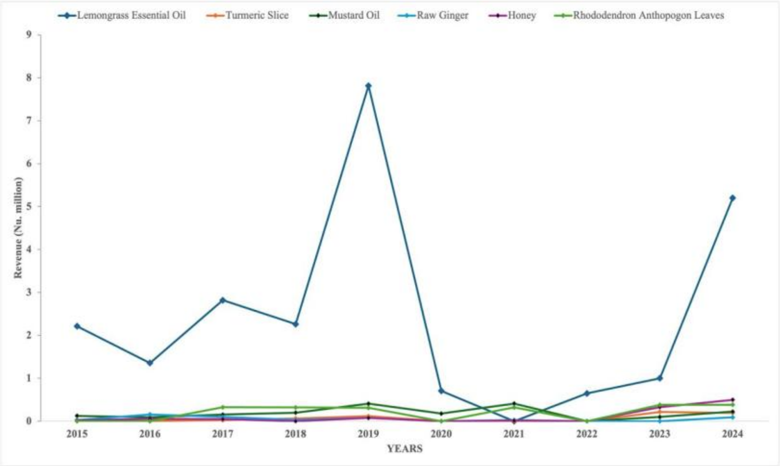


Figure 7. Time series trends in organic export values (2015–2024)

From a multi-level perspective, this extreme concentration suggests a niche that has not yet developed diversified and resilient market pathways capable of challenging the dominant regime. Rather than reflecting comparative advantage alone, the dominance of a single export commodity points to a deeper regime-level blockage: the absence of dense, reliable market linkages. Organic production remains fragmented among geographically dispersed smallholders, resulting in volumes that are costly and inefficient to aggregate (McCrae-Hokenson, 2014; Neuhoff et al., 2014). Without effective aggregation mechanisms, exporters and processors tend to engage only with commodities that offer low transaction costs and predictable volumes, thereby reinforcing path dependency around a narrow product base.

The pronounced year-to-year fluctuations in export values, especially the sharp expansion in 2019 followed by contraction during the COVID-19 period, indicate that organic production in Bhutan is largely reactive to short-lived market opportunities rather than anchored in stable demand or contractual arrangements. The core constraint, therefore, is not simply the absence of price premiums but the lack of secure offtake mechanisms. Without guaranteed buyers, forward contracts, or structured procurement platforms, farmers carry a disproportionate share of market risk, including the possibility of unsold surplus. This risk undermines farmer confidence and makes the higher labour requirements and transition costs of organic farming economically unattractive, a dynamic widely observed in early-stage organic sectors that lack coordinated value chains (Bijaman et al., 2010; Nemes, 2009).

The business case for organic production is further weakened by competition from cheaper, often subsidised conventional imports, a regime-level economic barrier that the organic niche cannot overcome without targeted policy support. As Scialabba and Hattam (2002) note, the failure to internalise the environmental and social externalities of conventional agriculture systematically disadvantages organic systems in price-based competition. In Bhutan, this imbalance reinforces farmers' dependence on conventional markets, despite strong policy rhetoric in favour of organic agriculture.

Taken together, the export data and value-chain analysis show that Bhutan's organic sector is constrained less by technical capacity or certification systems than by systemic market failures at the regime level. Addressing these barriers will require deliberate institutional innovation, including the development of farmer cooperatives, specialised intermediaries, and coordinated aggregation and marketing platforms that can achieve economies of scale and stabilise organic supply chains. Without such interventions, the organic niche is likely to remain confined to a narrow range of opportunistic export products, limiting its ability to evolve from a policy-driven initiative into a commercially viable and resilient agricultural system.

3.5 Synthesis of Systemic Challenges and Strategic Imperatives

The analysis of past interventions reveals that, while foundational pillars were established, multifaceted systemic challenges impede the development of organic agriculture. These can be synthesised into four interconnected categories:

3.5.1 Limited Understanding and Conceptual Narrowness

A narrow understanding of organic farming as mere input substitution, rather than a holistic ecosystem management approach, prevails. This is compounded by persistent myths about yield reduction, creating psychological barriers to adoption that are unsupported by local evidence. Meta-analyses show that while yields can be lower for certain staples, they are often comparable for many crops, especially when assessed over longer timeframes, and the profitability can be higher due to premiums (Crowder & Reganold, 2015; Ponisio et al., 2015).

3.5.2 Institutional and Structural Deficits

The organic sector suffers from fragmented implementation due to fiscal decentralisation, weak inter-ministerial coordination, and critical shortages in the commercial input supply chain, thereby perpetuating dependence on conventional inputs. The siloed approach to agricultural policy, in which organic goals are not integrated into mainstream agricultural and rural development plans, is a common pitfall identified in the policy integration literature (Candel & Biesbroek, 2016).

3.5.3 Economic Disincentives and Market Failures

The absence of reliable price premiums and clear market differentiation for certified organic produce constitutes a fundamental market failure. The business case for the transition to organic production is further undermined by competition from lower-cost imports and variable product quality. The work of Scialabba & Hattam (2002) emphasises that internalising environmental costs into conventional production is key to levelling the economic playing field for organic systems.

3.5.4 Cross-Sectoral Challenges and Missed Opportunities

Beyond immediate agricultural concerns, cross-cutting issues present additional barriers to organic scaling. Chronic rural labour shortages encourage herbicide use, while human-wildlife conflicts disproportionately affect organic farmers (Wilson et al., 2021). Potential synergies with tourism (e.g., agro-eco-tourism, organic homestays) and biodiversity conservation remain largely untapped, representing a significant missed opportunity for value addition and brand storytelling, as successfully demonstrated in regions like Tuscany, Italy (Sidali et al., 2019).

Addressing these challenges requires a coordinated, systemic response centred on creating a virtuous cycle of market demand and farmer profitability. This necessitates reframing extension services to provide localised evidence, securing input supply chains through strategic investment, engineering market demand through credible labelling and tourism partnerships, and enhancing institutional coordination via a cross-sectoral apex body with the authority to align policy and budgets, a model proven effective in complex multi-stakeholder initiatives (Bryson et al., 2015).

4 Conclusion

Bhutan's organic agriculture movement demonstrates a remarkable alignment between national development philosophy and sustainable agricultural practice. Anchored in the principles of Gross National Happiness (GNH), the country has made meaningful strides in building the foundational pillars of a national organic sector. The country has laid important institutional and technical foundations, including an adaptive certification system, expanding organic input infrastructure, and sustained investments in human capacity. These achievements demonstrate that organic agriculture in Bhutan is not merely aspirational but operationally feasible, institutionally credible, and increasingly visible within national development discourse.

Despite these successes, the sector faces persistent systemic challenges that threaten its long-term viability. Weak market linkages, fragmented smallholder production, and limited adoption of organic fertilisers hinder the economic attractiveness of organic farming, contributing to volatility in certified area and producer numbers. Structural and institutional deficits, such as insufficient inter-ministerial coordination, lack of integration with mainstream agricultural policy, and dependency on project-based funding, further constrain sectoral growth. Cross-sectoral challenges, including labour shortages, human-wildlife conflicts, and underutilised opportunities in agro-tourism and biodiversity-linked value addition, underscore the need for a holistic, multi-dimensional approach to organic agriculture.

The analysis highlights that technical capacity and certification infrastructure alone are insufficient to sustain an economically viable organic sector. A future-oriented strategy must prioritise market-driven mechanisms that ensure reliable price premiums and secure offtake agreements, alongside targeted incentives and regulatory frameworks to reduce dependence on chemical inputs gradually. Building a comprehensive national database on organic production and markets will enhance evidence-based governance, improve monitoring, and attract private-sector investment. Strategic diversification into high-value, climate-resilient crops and premium Non-Wood Forest Products can strengthen income resilience and tap niche domestic and international markets, while integrating organic initiatives with tourism and conservation agendas can create synergistic value chains.

This study is subject to several limitations that should be acknowledged. First, the analysis relies predominantly on secondary data from government reports and international sources;

sinconsistencies, data gaps, and the absence of a comprehensive national organic database limit the precision of economic and value-chain assessments. Second, the lack of primary squalitative data from farmers, traders, and policymakers' limits insight into the behavioural, socio-cultural, and institutional dynamics that shape organic adoption and market participation.

Despite these limitations, this study provides a comprehensive synthesis of Bhutan's trajectory in organic agriculture and identifies critical leverage points for future interventions. At this critical juncture, consolidating past achievements, coupled with targeted policy interventions and systemic market development, can transform the country's organic agriculture from a policy-driven initiative into a self-sustaining, economically viable, and ecologically resilient sector. By addressing structural, economic, and cross-sectoral challenges in a coordinated manner, Bhutan can realise its vision of a fully integrated organic agricultural system, thereby serving as a model for sustainable rural development globally.

5 Acknowledgement

The authors sincerely thank the Ministry of Agriculture and Livestock (MoAL), National Centre for Organic Agriculture-Yusipang, National Plant Protection Centre, and CARLEP for providing access to data and reports, and the Bhutan Food and Drug Authority (BFDA) for their cooperation.

6 Authors' contribution statement

Mrs. Kesang Tshomo provided overall guidance and led the conceptualisation of this research. Mr. Tshetrim La led the development of the conceptual framework and contributed to the research design and writing of the article.

7 References

Bijman, J., Muradian, R., & Cechin, A. (2010). Agricultural cooperatives and value chain coordination. https://www.researchgate.net/publication/254834425_Agricultural_Cooperatives_and_Value_Chain_Coordination

Bryson, J. M., Crosby, B. C., & Stone, M. M. (2015). Designing and implementing cross-sector collaborations: Needed and challenging. *Public Administration Review, 75*(5), 647–663. https://doi.org/10.1111/puar.12432

Candel, J. J. L., & Biesbroek, R. (2016). Toward a processual understanding of policy integration. *Policy Sciences, 49*(3), 211–231. https://doi.org/10.1007/s11077-016-9248-y

Crowder, D. W., & Reganold, J. P. (2015). Financial competitiveness of organic agriculture on a global scale. *Proceedings of the National Academy of Sciences of the United States of America, 112*(24), 7611–7616. https://doi.org/10.1073/pnas.1423674112

Department of Agriculture. (2006). *National framework for organic farming in Bhutan*. Ministry of Agriculture and Livestock, Royal Government of Bhutan.

Geels, F. W. (2011). The multi-level perspective on sustainability transitions: Responses to seven criticisms. *Environmental Innovation and Societal Transitions, 1*(1), 24–40. https://doi.org/10.1016/j.eist.2011.02.002

IFOAM. (2025). Participatory guarantee systems. International Federation of Organic Agriculture Movements. https://www.ifoam.bio/our-work/how/standards-certification/participatory-guarantee-systems

La, T., & Tshomo, K. (2023). *National organic flagship programme report (2019–2023)*. Department of Agriculture, Ministry of Agriculture and Livestock.

Läpple, D., & Van Rensburg, T. (2011). Adoption of organic farming: Are there differences between early and late adoption? *Ecological Economics, 70*(7), 1406–1414. https://doi.org/10.1016/j.ecolecon.2011.03.002

Luttikholt, L. W. M. (2007). Principles of organic agriculture as formulated by the International Federation of Organic Agriculture Movements. *NJAS – Wageningen Journal of Life Sciences, 54*(4), 347–360. https://doi.org/10.1016/S1573-5214(07)80008-X

McCrea-Hokenson, M. (2014). *Organic agriculture in Bhutan: Barriers going to 100%* [Master's thesis, SIT Graduate Institute]. SIT Digital Collections. https://digitalcollections.sit.edu/isp_collection

Ministry of Agriculture and Livestock. (2025). *Bhutan agrifood sector strategy 2034*. Royal Government of Bhutan.

National Plant Protection Centre (2022). *Annual report, FY 2021–2022*. Department of Agriculture, Ministry of Agriculture and Livestock, Royal Government of Bhutan.

National Statistics Bureau. (2025). *Statistical yearbook of Bhutan 2025*. Royal Government of Bhutan. http://www.nsb.gov.bt

National Organic Agriculture Centre. (2025). *Annual report, FY 2024–2025*. Ministry of Agriculture and Livestock, Royal Government of Bhutan.

Nemes, N. (2009). Comparative analysis of organic and non-organic farming systems: A critical assessment of farm profitability. Food and Agriculture Organization of the United Nations.

Neuhoff, D., Tashi, S., Rahmann, G., & Denich, M. (2014). Organic agriculture in Bhutan: Potential and challenges. *Organic Agriculture, 4*(3), 209–221. https://doi.org/10.1007/s13165-014-0075-1

CARLEP. (2022). *Annual progress report FY 2021–2022*. Project for Crop and Livestock Diversification and Marketing. Ministry of Agriculture and Livestock, Royal Government of Bhutan.

Paull, J. (2023). Organic agriculture in Bhutan: The dream of 100% organic is stalled by the reality of 1% organic. *European Journal of Development Studies, 3*(5), 58–61. https://doi.org/10.24018/ejdevelop.2023.3.5.291

Ponisio, L. C., M'gonigle, L. K., Mace, K. C., Palomino, J., De Valpine, P., & Kremen, C. (2015). Diversification practices reduce organic to conventional yield gap. *Proceedings of the Royal Society B: Biological Sciences, 282*(1799). https://doi.org/10.1098/rspb.2014.1396

Reganold, J. P., & Wachter, J. M. (2016). Organic agriculture in the twenty-first century. *Nature Plants, 2*(2), 1–8. https://doi.org/10.1038/nplants.2015.221

Rijal, A., Smith-Hall, C., & Helles, F. (2011). Non-timber forest product dependency in the Central Himalayan foothills. *Environment, Development and Sustainability, 13*(1), 121–140. https://doi.org/10.1007/s10668-010-9252-x

Wangmo, R., Om, K., Choden, P., Acharya, S., Dorji, N., Gyeltshen, K., Islam, M. R., & Hossain, M. B. (2023). Consortium for scaling-up climate-smart agriculture in South Asia (C-SUCSeS) project: Climate-smart agriculture technologies and practices in Bhutan. SAARC Agriculture Centre. http://www.sac.org.bd

Scialabba, N. E. H., & Hattam, C. (Eds.). (2002). *Organic agriculture, environment and food security*. Food and Agriculture Organization of the United Nations.

Sidali, K. L., Spiller, A., & Schulze, B. E. (Eds.). (2019). *Food, agri-culture and tourism: Linking local gastronomy and rural tourism: Interdisciplinary perspectives*. Springer.

Smith, E., & Marsden, T. (2004). Exploring the "limits to growth" in UK organics: Beyond the statistical image. *Journal of Rural Studies, 20*(3), 345–357. https://doi.org/10.1016/S0743-0167(03)00044-5

Willer, H., Schlatter, B., & Trávníček, J. (Eds.). (2023). *The world of organic agriculture: Statistics and emerging trends 2023*. Research Institute of Organic Agriculture (FiBL) & IFOAM – Organics International. https://doi.org/10.5281/zenodo.7572890

Wilson, C., Guivarch, C., Kriegler, E., van Ruijven, B., van Vuuren, D. P., Krey, V., Schwanitz, V. J., & Thompson, E. L. (2021). Evaluating process-based integrated assessment models of climate change mitigation. *Climatic Change, 166*(1), 1–26. https://doi.org/10.1007/s10584-021-03099-9

Wynen, E. (2002). The economics of organic cereal-livestock farming in Australia revisited. Australian Agricultural and Resource Economics Society. https://ageconsearch.umn.edu/record/125991/

https://doi.org/10.55925/btagr.26.9106

Multivariate Analysis of Soil Chemical Properties Associated with TSS/TA Ratio and Betalain Concentration in White fleshed Dragon Fruit (*Selenicereus undatus*)

Nangsel Tshomo[1] and Kuenga Penjor Gyeltshen[1]

Abstract

White fleshed Dragon fruit (Selenicereus undatus) quality is primarily assessed using two key traits: sweetness, estimated by the total soluble solids to titratable acidity (TSS/TA) ratio, and pigmentation, measured as betalain concentration. This study examined the association between soil chemical properties and fruit quality (TSS/TA ratio and betalain concentration) in white fleshed dragon fruit across three production sites representing natural soil variability in Bhutan. Thirty fruits were analyzed for TSS, TA, and TSS/TA ratio, and composite soil samples were analyzed for organic matter (OM), nitrogen (N), phosphorus (P), potassium (K), micronutrients (Fe, Mn, Zn, Cu, B), sulphur (S), electrical conductivity (EC), and pH. Correlation and principal component analyses revealed strong interrelationships among soil parameters, indicating multicollinearity and underlying soil chemical gradients. Sweetness (TSS/TA ratio) was strongly promoted by available phosphorus and potassium but reduced by higher soil EC and pH. Betalain concentration declined with increasing nitrogen and organic matter, while copper showed a positive association. Ridge regression confirmed phosphorus and potassium as the strongest drivers of sweetness, whereas nitrogen and organic matter consistently suppressed both sweetness and pigmentation. Threshold analysis demonstrated nonlinear soil–fruit quality responses, including a decline in predicted betalain concentration at higher soil organic matter levels. Multi-response optimization through joint optimal analysis predicted single point optimum soil condition and their optimal range that balance sweetness and pigmentation, characterized by approximate soil chemical values such as pH of 6.9 (range: 6.7–7.0), organic matter of 0.96% (range: 0.90–1.05%), EC of 1.24 dS/m (range: 1.20–1.30 dS/m), phosphorus of 52 mg/kg (range: 50–60 mg/kg), and potassium of 18 mg/kg (range: 16–21 mg/kg). For sweetness-focused production, higher phosphorus (~86 mg/kg; range: 80–90 mg/kg) and potassium (21 mg/kg; range: 20–22 mg/kg) levels were most favorable. In contrast, pigmentation was favored under lower nitrogen and organic matter (0.84%; range: 0.8–0.9%) and slightly alkaline soil conditions (pH 7.6; range: 7.5–7.7). These findings provide soil-based guidance for precision nutrient management, enabling growers to target either sweetness, pigmentation, or balanced fruit quality under naturally variable field conditions.

Corresponding email: ntshomo@moal.gov.bt

[1] Agriculture Research and Development Centre, Bajo, Department of Agriculture, Ministry of Agriculture and Livestock

Keywords: *Dragon Fruit; TSS/TA Ratio; Betalain, Principal Component Analysis; Ridge Regression; Joint Optimal Analysis*

1 Introduction

White flesh dragon fruit (Selenicereus undatus, formerly Hylocereus undatus), also known as white pitaya or pitahaya, is an exotic tropical fruit valued for its vibrant appearance, rich coloration, and organoleptic, nutritional, and therapeutic properties (Franco et al., 2022). Its commercial appeal and market value are largely determined by two key quality parameters; pigmentation, driven by betalain pigments, and sweetness, determined by the balance of total soluble solids (TSS) and titratable acidity (TA), which together shape coloration and flavor. The TSS/TA ratio serves as a widely used ripening index in fruits, reflecting the balance between sugars (e.g., glucose and fructose) and acids (Majidi et al., 2011).

Soil chemistry and plant nutrition are known to influence fruit biochemical composition and metabolite synthesis (Salam et al., 2023). Abiotic factors such as soil pH, organic matter, and micronutrient availability can alter the accumulation of both primary and secondary metabolites. However, specific soil chemical properties and fruit quality relationships in dragon fruit remain inadequately characterized (Mondal & Alam, 2023). Previous studies have reported associations between soil chemical properties and tissue concentrations of nitrogen, phosphorus, and potassium (Islam et al., 2025) yet findings are often inconsistent across environments, highlighting the role of location-specific soil chemical properties in shaping fruit quality. Therefore, multivariate analytical approaches that consider site-level differences are needed to better understand soil chemical properties and fruit interactions and guide sustainable nutrient management strategies

Optimizing soil nutrients for a single fruit quality trait can produce trade-offs with others. Nutrient regimes that enhance sugar accumulation may suppress pigment synthesis, and vice versa (Srivastava & Malhotra, 2014). Meta-analyses of organic and integrated fertilizer practices further reveal that management strategies promoting yield or sweetness may compromise fruit coloration or antioxidant capacity (Yang et al., 2019). These findings highlight the potential and the importance of simultaneously considering both taste-related attributes (TSS/TA ratio) and pigment-related attributes (betalains) when evaluating influences of soil chemical parameters on dragon fruit quality.

In Bhutan, white flesh dragon fruit cultivation is relatively new but rapidly expanding. According to the Integrated Agriculture and Livestock Census of Bhutan 2025, dragon fruit was cultivated by 3,578 growers, with a total production of 10 MT in 2024, up from 4 MT in 2023 (National Statistical Bureau [NSB], 2025). The census reports dragon fruit production without distinguishing flesh color or cultivar type but the growing number of cultivators is undeniable for dragon fruit overall. Although this study focuses on white flesh dragon fruit as this cultivar seems to be widely grown and found at the study sites, this could be used as a baseline for experienced and emerging dragon fruit growers for precise cultivation and management of dragon fruits tailored to the niche market preferences.

There have been no studies evaluating the association between soils chemistry and dragon fruit quality traits in Bhutan, particularly on TSS/TA ratio and betalain concentration. To address this knowledge gap, the present study was designed to investigate the relationships between selected soil chemical parameters (pH, organic matter, available N, P, K, micronutrients, and EC), betalain concentration in three different locations and the TSS/TA ratio in white flesh dragon fruit. By identifying nutrient thresholds and potential trade-offs between sweetness and colour quality, the study aimed to derive joint optimal soil conditions that support balanced fruit quality. The findings were intended to provide context-specific guidance for optimizing fruit quality and supporting sustainable, white fleshed dragon fruit production in Bhutan.

2 Materials and Method

2.1 Experimental Site and Design

Following the approach used in similar agro-ecological studies (Sun et al., 2022), a comparative observational design was used across three sites to examine relationships between soil chemical properties and fruit quality. The inclusion of multiple sites allowed the study to capture natural variability in soil chemical conditions and evaluate how this variability is associated with the fruit TSS/TA ratio and betalain concentration. Consistent with multi-location spatial soil studies (Lee et al., 2023), production site was treated as a fixed factor to account for location-level environmental variation, while soil chemical properties were examined as continuous explanatory variables. First-harvest white fleshed dragon fruits were collected from three representative sites within the subtropical zone of Wangdue Phodrang and Punakha Dzongkhags, Bhutan (Chhogyel & Kumar, 2018). The site characteristics and anthesis-to-harvest climatic conditions are presented in Table 1.

Table 1. Geographic characteristics and average climatic conditions recorded between

Site	Location	Altitude (masl)	Latitude (°N)	Longitude (°E)	T_{max} (ºC)	T_{min} (°C)	Rainfall (mm)	Relative Humidity (%)
BAJO	Bajo, Wangdue	1,237	27.49	89.9	30.4	22.14	2.57	77.98
FMCL	Kamichu, Wangdue	822	27.28	90	32.13	21.87	3.84	69.65
CRP	Lobesa, Punakha	1,278	27.52	89.87	31.25	20.27	0.69	76.7

anthesis and harvest at the three study sites.

2.2 Data Selection

The first harvest of dragon fruits was selected based on an anthesis-to-fruit-set period of 30–33 days. A total of 10 fruits were collected from each site, with only one fruit harvested per randomly selected plant (n = 30) subsequently soil samples were collected from the basins of the same plants from which the fruit were harvested through all of the three study sites. Using the hand auger, the first 0cm-15cm (topsoil) and the second 15cm-30cm (subsoil) of soil from the active root zone of each plant were collected. From each study site, ten topsoil samples were collected and combined to form a composite sample; similarly, ten subsoil samples were collected and made into a composite sample. This resulted in six composite soil samples from all three sites.

2.3 Data Collection

2.3.1 Soil analysis

A total of six composite soil samples, comprising topsoil and subsoil from each of the three sites, were analyzed. Organic matter, macro-nutrients (Nitrogen [N], Phosphorus [P], and Potassium [K]) and micro-nutrients (Boron [B], Iron [Fe], Zinc [Zn], Sulfur [S], Manganese [Mn], and Copper [Cu]) were determined using the Orlab digital soil testing kit (Orlab Instruments Pvt. Ltd., 2025). For micronutrients such as Zn and Cu, both HCl-extractable and DTPA-extractable forms were measured to assess plant-available concentrations, with DTPA used as it is widely recognised for estimating bioavailable micronutrients in neutral to alkaline soils (Lindsay & Norvell, 1978). Soil pH and electrical conductivity (EC) were measured using a calibrated digital pH meter and EC meter, respectively, with appropriate buffer solutions. Soil pH and electrical conductivity (EC) were measured using a calibrated digital pH meter and EC meter, respectively, with appropriate buffer solutions.

2.3.2 Titratable acidity (Citric acid)

Dragon fruit contains various organic acids that contribute to its characteristic taste, with citric acid being the predominant acid influencing fruit quality (Xie et al., 2022). Citric acid content was determined using a titrimetric method, specifically the acid–base volumetric analysis (Brima & Abbas, 2014). A standardized 0.1 N sodium hydroxide (NaOH) solution was used as the titrant to quantify the citric acid (Alam et al., 2023; Bengaluru & Karnataka, 2022). Fruit pulp was crushed, and 10 mL of juice was extracted and diluted with 90 mL of distilled water to a total volume of 100 mL. The titrant was added from a burette to the analyte containing 2–3 drops of phenolphthalein, with the endpoint indicated by a persistent pale pink color. Each measurement was replicated three times per fruit, and the mean value was calculated to minimize experimental bias. The final citric acid concentration was expressed in grams per liter (g/L) using the appropriate calculation formula (Alam et al., 2023).

$$Titrable\ acid\ (\%) = \frac{\begin{array}{c} ml\ of\ NaoH\ (Final\ reading\ from\ the\ burette) \times \\ Normality\ of\ NaOH\ (Titrant\ concentration) \\ \times\ Dilution\ factor(0.1) \times Equivalnet\ weight\ of\ citric\ acid\ (0.0064\ ) \end{array}}{Volume\ of\ analyte\ (ml)} \ldots(1)$$

2.3.3 Total soluble solutes (TSS) and pH

The presence of total soluble solids determines the sweetness of the fruit extract. TSS includes sugars, vitamins, minerals and other soluble substances, but majorly consists of sugars such as glucose, fructose and sorbitol (Chen et al., 2024). A hand-held digital refractometer was used to measure the TSS in Degree Brix (°B) from the fruit extract from the spulp. Along with that the pH of the extract was also measured using the electronic pH meter to determine the flavour of the fruit right after harvest (Budhathoki et al., 2023).

2.3.4 TSS/TA ratio

The TSS/TA ratio was calculated by dividing the total soluble solids (TSS, °Brix) of the fruit juice by its titratable acidity (TA, g/L) for each sample. This ratio was determined for all fruits using the measured values of TSS and TA to quantify the balance between sweetness and acidity.

2.3.5 Betalain content

Frozen fruit peels were removed from the freezer and allowed to thaw at room temperature. The peels were macerated using a blender, and 10 mL of the extract was dissolved in 50 mL of distilled water, following a modified crude extraction method (Fathordoobady et al., 2016; Pratiwi et al., 2025; Woo et al., 2011), with water as the sole solvent at a 1:5 dilution. The solution was shaken for 45 minutes. Subsequently, 1 mL of the supernatant was mixed with 10 mL of distilled water and analyzed using a UV–Visible spectrophotometer at 538 nm. The final betalain concentration was calculated using the standard formula (GNT International B.V, 2010) given below,

$$Betalian\ Content\ \left(\frac{mg}{L}\right) = \frac{\begin{array}{c}A_{538}(Absorbance\ at\ 538nm)\times D.F(Dilution\ factor, 0.1)\\ \times MW(550g/mol)\times 1000\end{array}}{\epsilon(Coefficent, 60{,}000L/mol)\times l(path\ lenght\ of\ cuvette, 1cm)} \ldots\ldots\ldots\ldots \quad (2)$$

2.4 Data Analysis

2.4.1 Principal Component Analysis

Principal Component Analysis (PCA) was used as an exploratory multivariate tool to reduce data dimensionality and identify patterns in each study site to understand which soil chemical properties are strongly associated and linked to variations in fruit quality (TSS/TA ratio and betalain concentration).

PCA was conducted using the ***prcomp()*** function in R statistical software (version [4.4.3]). Prior to PCA, all parameters were scaled (centered and divided by the standard deviation) using the ***scale()*** function to ensure that variables with larger scales did not disproportionately influence the results.

PCA loading plots were generated to visualize combinations of soil chemical properties that showed strong association to the variation in fruit quality in all three sites, and represented as principal components. A total of eight principal components were generated, of which the first two principal components (PC1 and PC2) were retained for further interpretation as they explained 84% of the total variance.

PCA score plots were subsequently generated while overlaying fruit quality to visualize the relationship between the white flesh dragon fruit samples along with the components of PC1 and PC2. This whole approach allowed us to narrow down specific major soil chemical parameters which were linked to differences in fruit quality.

2.4.2 Ridge Regression

Ridge Regression is a regularization tool used to address multicollinearity among the predictors (independent) variables and quantify the relationship between independent variables (soil chemical properties) with dependent variables (fruit quality: TSS/TA ratio and betalain concentration). Based on exploratory results from principal component analysis (PCA), the soil chemical parameters that showed strong loading on the principal components associated with fruit quality were retained and further subjected to ridge regression analysis. This screening step of PCA reduced model complexity and focused the analysis on soil variables most strongly associated with variation in fruit quality.

Ridge Regression was conducted using the ***glmnet()*** function in R statistical software (version [4.4.3]). Prior to model fitting, all predictor variables were standardized to a mean of zero and a standard deviation of one to ensure the regularization penalty was applied uniformly. Separate ridge regression models were fitted on each dependent variable and the standardized coefficients from the final models were extracted and analysed to assess the direction, magnitude, and relative importance of each soil parameter's influence on the fruit quality. Equation (3) describes the ridge regression mathematical model;

$$\hat{\beta}\, ridge = \arg min_{\beta} \left\{ \sum_{i=1}^{n} \left(y_i - \beta_0 - \sum_{j=1}^{p} x_{ij} \beta_j \right)^2 + \lambda \sum_{j=1}^{p} \beta_j^2 \right\} \text{ …………………… (3)}$$

Where, y_i represents the fruit quality trait (TSS/TA ratio or betalain concentration) for the white flesh dragon fruit sample i. x_{ij} represents the value of soil parameter j measured for i. β_0 is the intercept and β_j are the regression coefficients estimating the association between soil parameters and the fruit quality trait. n is the number of observations made between fruit and soil and p is the number of soil variables included in the model. λ represents the penalty strength introduced to the coefficients, shrinking predictors toward zero to reduce multicollinearity and stabilize model estimates.

2.4.3 Threshold Joint Optimisation Analysis

Ridge regression allowed for the quantification of soil parameters and their type of association to fruit quality. Threshold analysis was then employed to predict how different levels of soil parameters could affect the fruit quality (TSS/TA ratio and betalain concentration). Non-linear response curves were visualized through this analysis which revealed optimal values or plateau points where soil parameters are associated with high fruit quality.

Many of the screened-out soil parameters showed tradeoffs to fruit quality, where one trait was boosted and the other trait diminished. Joint Optimisation Analysis was used to derive balanced soil parameter values that simultaneously optimize both TSS/TA ratio and betalain concentration, multi-response optimization using the desirability function framework was employed. For each parameter, predicted response curves from ridge regression models for betalain and TSS/TA ratio were merged, normalized to a 0–1 scale, and combined using the geometric mean to calculate a composite desirability score as equation (4) follows,

$$D_{combined} = \sqrt{D_{betalain} \times D_{TSS/TA}} \text{............................} \quad (4)$$

The joint optimal value was identified as the ideal soil parameters that represent the best compromise between pigmentation and sweetness optimization for white flesh dragon fruits. Optimal ranges were further derived from the predicted response curves by identifying soil parameter values where the predicted response remained within 95% of the maximum predicted value.

3 Results and Discussion

3.1 Descriptive Statistics

Soil chemical properties presented in Table 2 showed the variation in growing conditions from where the fruit samples were collected. Variation in fruit quality traits was examined in relation to these soil chemical parameters.

Table 2. Field-level estimation of soil chemical properties for the topsoil (Top; 0-15 cm) and subsoil (Sub; 15-30 cm) layers across the three study sites (FMCL, CRP, and BAJO)

Parameters	**Site**					
	FMCL		**CRP**		**BAJO**	
Soil	*Top*	*Sub*	*Top*	*Sub*	*Top*	*Sub*
pH	7.15	6.87	7.63	7.28	7.44	7.61
Soil EC (dS/m)	1.24	1.24	1.68	1.50	1.44	1.30
Organic Matter (%)	1.19	1.11	1.42	1.42	1.06	0.84
Nitrogen (mg/kg)	249.00(M)	222.50(M)	280.00(H)	280.00(H)	208.00(M)	137.00(L)
Phosphorus (mg/kg)	67.65(vH)	86.25(vH)	14.95(M)	17.75(M)	36.95(vH)	21.9(H)
Potassium (mg/kg)	20.75(L)	5.10(L)	6.15(L)	6.80(L)	7.70(L)	6.60(L)
Iron (mg/kg)	0	0	0.33	0	0	0
Manganese (mg/kg)	0.01	0.01	0.01	0.01	0.01	0.01
Zinc (HCL) (mg/kg)	0	1.3	2.4	0.2	1.6	1.1

Zinc (DTPA) (mg/kg)	0	0.6	1.1	0.1	0.7	0.5
Copper (HCL) (mg/kg)	1.55	0	0	1.06	0	0.56
Copper (DTPA) (mg/kg)	0	0	0.29	0	0.15	0.43
Sulphur (mg/kg)	41.17	25.4	31.12	37.66	14.48	31.17
Boron (mg/kg)	0.36	0.29	0.31	0.01	0.19	0.76

Note: Nutrient status in the soil is expressed as: L = Low, M = Medium, H = High, vH = Very High.

Soil chemical properties were significantly associated with the TSS/TA ratio, betalain concentration, total soluble solids (TSS, °Brix), and titratable acidity (TA, % citric acid) of white fleshed dragon fruit ($p < 0.001$ for all traits) as shown in Table 3.

Table 3. Mean (± SD) values of fruit quality traits (TSS/TA ratio, betalain content, TSS, and TA) of white fleshed dragon fruit measured under different soil chemical conditions.

Fruit parameters	**Sites (Mean ± SD)**			**F-value**	**p-value**
	FMCL	**CRP**	**BAJO**		
TSS/TA Ratio	90.2 ± 19.5^{a}	40.5 ± 9.8^{c}	59.8 ± 16.2^{b}	43.21	< 0.001
Betalain (mg/L)	17.0 ± 6.9^{b}	21.2 ± 9.6^{b}	31.7 ± 11.2^{a}	13.41	< 0.001
TSS (°Brix)	13.3 ± 1.1^{a}	10.8 ± 1.0^{b}	10.7 ± 1.3^{b}	35.65	< 0.001
TA (% Citric Acid)	0.16 ± 0.03^{c}	0.28 ± 0.07^{a}	0.19 ± 0.05^{b}	42.61	< 0.001

ANOVA p-values and Tukey's HSD test results ($p < 0.05$) are shown. Values are based on ten fruits sample per site.

Environmental factors such as temperature, water availability, and solar radiation are known to influence fruit development and quality (Sundarrajan et al., 2025; Bacelar et al., 2024). In this study, temperature conditions during the anthesis to harvest period were relatively similar across sites, with mean maximum temperatures ranging from 30.4 to 32.1 °C and mean minimum temperatures from 20.3 to 22.1 °C (Table 1). Some differences in rainfall (0.7–3.8 mm) and relative humidity (69.6–78.0%) were observed between sites (Table 1).

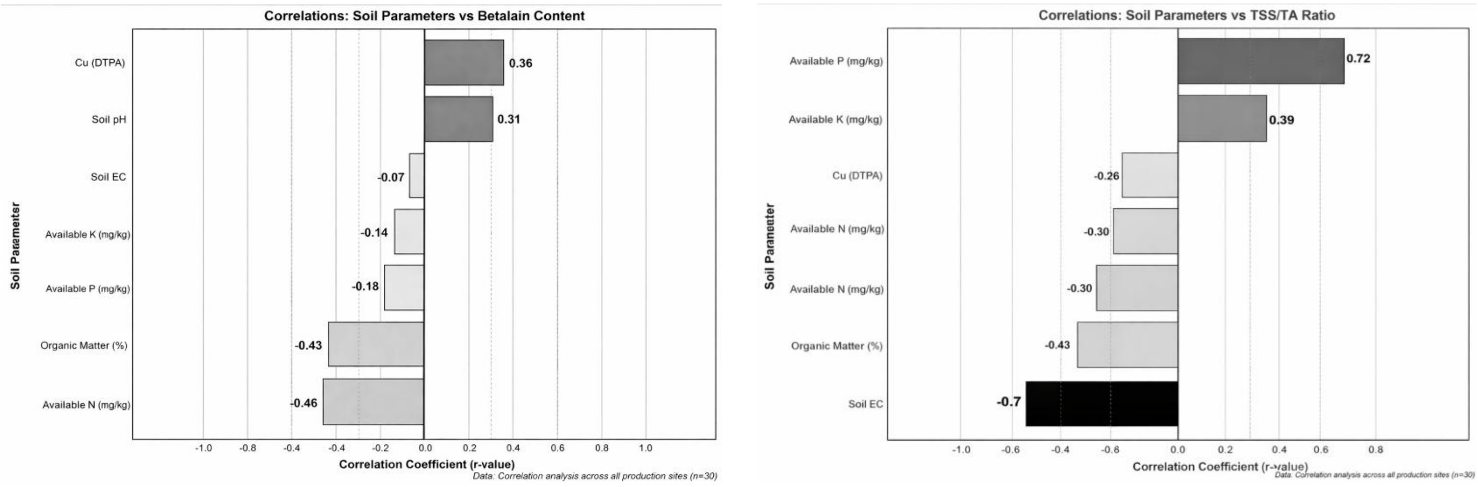
When environmental conditions, particularly temperatures show little variation, variation in fruit quality is often attributed to differences in soil chemical properties and nutrient availability (Papadakis et al., 2005; Nie et al., 2025). This has been demonstrated in fruit crops where trees grown under similar climates but on different soils produced fruit with distinct quality characteristics (Papadakis et al., 2005). For dragon fruit specifically, studies have shown that soil nutrient status, particularly potassium, nitrogen, and phosphorus, plays a key role in determining fruit composition and quality compared to climate (Belbase et al., 2025) better understand the drivers of the observed differences, variation in fruit quality was examined in relation to measured soil chemical parameters.

Higher TSS/TA ratios and TSS values were observed at the FMCL site (TSS/TA: 90.2; TSS: 13.3 °Brix), where soils were characterized by relatively higher phosphorus (67.65–86.25 mg/kg) and potassium availability (topsoil: 20.75 mg/kg). In contrast, fruits from the CRP site, which had comparatively lower phosphorus (14.95–17.75 mg/kg) and potassium (6.15–6.80 mg/kg), exhibited reduced sweetness (TSS/TA: 40.5) and higher acidity (TA: 0.28%). Betalain concentration showed a contrasting trend, with the highest pigment accumulation observed at the BAJO site (31.7 mg/L), where soils exhibited relatively lower nitrogen (subsoil: 137 mg/kg) and organic matter (subsoil: 0.84%). These findings confirm that soil chemical parameters have a strong, measurable influence on dragon fruit quality traits. This is consistent with reports highlighting the role of soil properties in modulating red fleshed dragon fruit composition (Singh et al., 2022), suggesting that similar soil-driven mechanisms could operate in white fleshed varieties.

Sweetness-related traits (TSS/TA ratio and TSS) showed positive associations with soil phosphorus and potassium availability across the study sites. Fruits from the FMCL site, where soils contained relatively higher available phosphorus (67.65–86.25 mg/kg) and potassium (5.10–20.75 mg/kg), exhibited higher TSS/TA ratios (90.2) and TSS (13.3 °Brix). In contrast, the CRP site, characterized by comparatively lower phosphorus (14.95–17.75 mg/kg) and potassium (6.15–6.80 mg/kg), produced fruit with lower TSS/TA ratios (40.5) and elevated titratable acidity (TA: 0.28%). This aligns with previous studies demonstrating that phosphorus and potassium fertilization enhances sugar accumulation and acid balance in dragon fruit (Sahu et al., 2023).

Additionally, betalain concentration exhibited an inverse association with soil nitrogen and organic matter levels across sites. The highest betalain concentration (31.7 mg/L) was recorded at the BAJO site, where soils showed relatively lower nitrogen (137–208 mg/kg) and organic matter (0.84–1.06%). This negative relationship between nitrogen availability and pigmentation reflects the well-documented role of betalain as stress-responsive secondary metabolites, which are often enhanced under low-nitrogen conditions (Puccinelli et al., 2025; Sadowska-Bartosz & Bartosz, 2021)

3.2 Correlation



a

b

Figure 1. Pearson’s correlation coefficients (r-values) between soil chemical parameters and (a) betalain concentration and (b) TSS/TA ratio of white fleshed dragon fruit. Values shown on bars represent correlation coefficients calculated across all samples (n = 30)

As shown in Figure 1a, Pearson’s correlation analysis revealed that betalain concentration was associated with soil nitrogen and organic matter, both showing moderate negative correlations (r = –0.46 and r = –0.43, respectively), while available copper exhibited a positive relationship (r = 0.36). These correlation coefficients could be linked to higher betalain accumulation under lower nitrogen availability and higher copper levels.

In contrast, the TSS/TA ratio (Figure 1b) showed a very strong positive correlation with available phosphorus (r = 0.72), alongside a moderate negative correlation with soil pH (r = −0.52) and a very strong negative correlation with soil electrical conductivity (r = −0.70), as shown by the corresponding r-values in Figure 1b. Phosphorus exhibited only a weak negative correlation with betalain concentration (r = −0.18) as per figure 1a, confirming that its role is largely restricted to sweetness

These correlations provide a mechanistic explanation for variation in fruit quality traits in relation to soil chemical properties, demonstrating that differences in sweetness and pigmentation are driven by underlying soil nutrient availability. The negative relationships between betalain concentration and both nitrogen and organic matter support the plant resource allocation theory, whereby abundant nitrogen promotes vegetative growth at the expense of secondary metabolite production such as betalains (Sadowska-Bartosz & Bartosz, 2021). Also, the positive correlation with copper is scientifically correct, as copper is an essential cofactor for polyphenol oxidases and dioxygenases, key enzymes in betalain biosynthesis (Pratiwi et al., 2025). The strong positive correlation between phosphorus and the TSS/TA ratio reaffirms phosphorus's role in energy transfer (ATP) and sugar metabolism, which are central to fruit sweetness (Sahu et al., 2023). Equally important is the negative effect of soil electrical conductivity, as elevated soluble salts disrupt osmotic balance and water uptake, thereby limiting sugar accumulation and altering acid metabolism. Furthermore, the moderate negative correlation with pH ($r = –0.52$) is consistent with findings in other horticultural crops; for instance, vineyards in alkaline soils showed reduced berry quality, attributed to pH-dependent shifts in nutrient availability that affect sugar and acid metabolism (Li et al., 2024). A similar mechanism may be at play in dragon fruit, where elevated pH could limit the availability of micronutrients essential for these biochemical pathways.

Collectively, these results highlight contrasting nutrient requirements; while high phosphorus supports sweetness (TSS/TA ratio vs. available P, $r = 0.72$; Fig. 1b), it does not enhance betalain (betalain vs. P, $r = −0.18$; Fig. 1a), which instead is favored by low nitrogen (betalain vs. N, $r = −0.46$; Fig. 1a) and higher copper availability (betalain vs. Cu, $r = 0.36$; Fig. 1a). This creates a fundamental trade-off between maximizing sweetness and visual quality in dragon fruit.

3.3 Principal Component Analysis

In this study, Principal Component Analysis (PCA) was used as an exploratory tool to understand how multiple soil chemical parameters varied together and how these components associate together with the fruit quality. The objective of this tool was to identify which soil parameters that most strongly contributed to the variation in sweetness (TSS/TA ratio) and pigmentation (betalain concentration) under natural field conditions. Because soil variables such as pH, nutrients, and EC are highly interrelated, analysing them individually can hide their combined effects.

The first two principal components (PCs) explained 84.0% of the total variance in soil parameters (PC1: 49.6%; PC2: 34.4; Figure 2), indicating that most soil variability relevant to fruit quality could be summarized along two independent axes. The loadings plot (Figure 2) showed that PC1 was mainly associated with soil available phosphorus and electrical conductivity, while PC2 was associated with soil pH and available nitrogen. Together, these components represent the dominant soil chemical patterns influencing fruit quality.

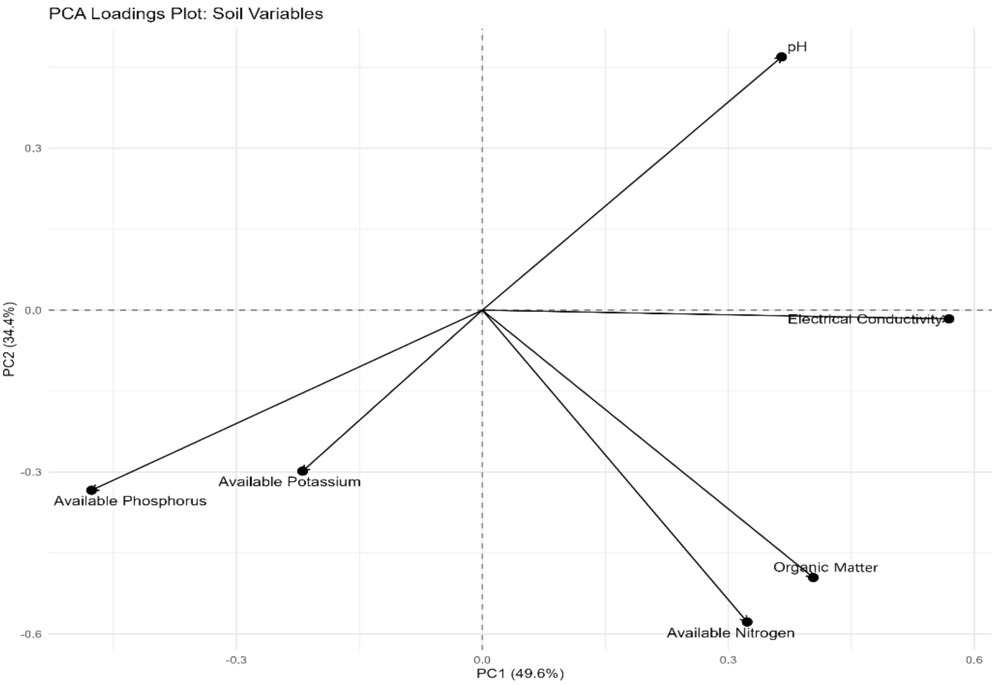
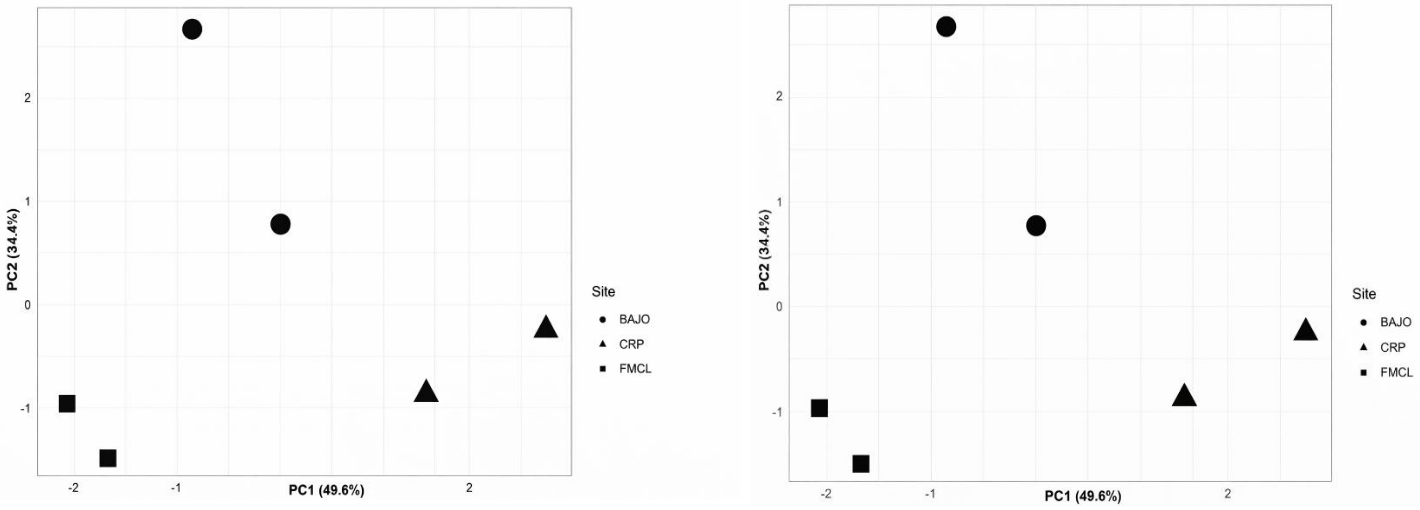


Figure 2. PCA loadings plot of soil parameters. The direction and length of the vectors indicate the contribution and influence of each soil variable on Principal Components 1 and 2.



a b

Figure 3. PCA scores plots showing how samples are distributed along PC1 and PC2 based on soil chemical properties. Different symbols represent the production sites used to capture

natural soil variability. Graph (a) shows the distribution of samples in relation to betalain concentration, and graph (b) shows the distribution in relation to the TSS/TA ratio

The PCA scores plot (Figure 3) visualized how white fleshed dragon fruit samples clustered along these gradients and how this clustering related to fruit quality traits. For betalain concentration (Graph 3a), separation was mostly present along PC2, with BAJO samples clearly distinct from CRP and FMCL. This indicates that lower nitrogen availability and favorable soil pH, the primary contributors to PC2, were the main drivers of differences in fruit pigmentation. In contrast; as per graph 3b. separation based on the TSS/TA ratio (sweetness) occurred mostly along PC1, with FMCL and BAJO positioned on the high-phosphorus, low EC end of the axis and CRP on the opposite end. This demonstrates that phosphorus availability and electrical conductivity were the principal soil factors associated with variation in fruit sweetness.

Consistent with its established use in agronomy (Jolliffe & Cadima, 2016), PCA in this study enhanced the results by revealing a clear soil-based trade-off between sweetness and pigmentation. Soil conditions favouring high betalain concentration were associated with the nitrogen pH gradient (PC2), whereas conditions promoting a high TSS/TA ratio aligned with the phosphorus EC gradient (PC1). This separation indicates that the observed differences in fruit quality across production sites corresponded closely to the underlying soil chemical properties

3.4 Ridge Regression

Several soil chemical properties were found to be moderately to strongly correlated to each other through correlation and PCA that was done earlier which suggested multicollinearity. Specifically, there was a strong covariation between soil nitrogen and organic matter and a positive correlation between phosphorus and potassium. The pH and electrical conductivity of the soil also varied in relation to the availability of nutrients. Ridge regression was used to quantify the contribution of each soil parameter to the TSS/TA ratio and betalain concentration while taking multicollinearity into account, as these interrelationships can mask the individual contribution of predictors if other standard multiple regression is used.

Ridge Regression was applied to identify the soil parameters most strongly associated with variation in fruit quality, specifically sweetness (TSS/TA ratio) and pigmentation (betalain concentration), while accounting for multicollinearity. This eliminated the instability brought on by correlated predictors and allowed for the quantification of the contributions of each soil parameter to fruit quality.

The standardized ridge regression coefficients (Table 4) revealed that different soil parameters were associated with sweetness and pigmentation. For betalain concentration, organic matter (-2.23) and available nitrogen (-2.04) showed the strongest negative associations, indicating that higher levels of these parameters were linked with reduced pigment accumulation. In contrast, soil pH showed a moderate positive association (+1.42), suggesting that more alkaline soil conditions favor betalain synthesis. Available phosphorus showed a weak coefficient (-0.90), indicating a limited role in determining pigment concentration.

Table 4. Ridge regression results showing the effects of soil parameters on Betalain concentration and TSS/TA ratio

Soil Parameter	Betalain Concentration	TSS/TA Ratio
pH	1.42	-1.06
Organic Matter	-2.23	-4.3
Electrical Conductivity	0.11	-1.98
Available Nitrogen	-2.04	-1.9
Available Phosphorus	-0.9	11.44
Available Potassium	-0.37	4.13

For the TSS/TA ratio (sweetness), available phosphorus had the largest positive standardized coefficient (+11.44), which was higher than all other predictors in the model (Table 4). This clearly indicates that phosphorus was the most influential soil parameter associated with sweetness, with potassium as the secondary contributor (+4.13). Organic matter again showed a strong negative association (-4.30), highlighting its suppressive influence on both sweetness and pigmentation. Electrical conductivity exhibited a negative coefficient (-1.98), consistent with reduced sugar accumulation under higher salinity conditions.

To further examine whether these relationships were linear across the observed range, nonlinear response patterns were explored using threshold analysis (Figure 4 and 5). For betalain concentration (Figure 4), organic matter (Figure 4b) exhibited a nonlinear negative relationship, with predicted betalain levels decreasing progressively at higher organic matter contents. Soil pH (Figure 4c) showed a positive response across the observed range, indicating increased betalain concentration under more alkaline soil conditions. In contrast, available phosphorus (Figure 4d) and potassium (Figure 4e) displayed higher betalain concentration occurring at intermediate nutrient levels.

For the TSS/TA ratio (Figure 5), available phosphorus (Figure 5d) showed a strong positive response, reinforcing its role as the dominant soil parameter associated with fruit sweetness. Potassium (Figure 5e) exhibited a nonlinear response, with reduced TSS/TA values at intermediate concentrations. Electrical conductivity (Figure 5a) showed a clear negative trend, while organic matter (Figure 5b) displayed a nonlinear response with a mid-range optimum. Soil pH (Figure 5c) showed a weak to moderate negative association with the TSS/TA ratio, consistent with the ridge regression results.

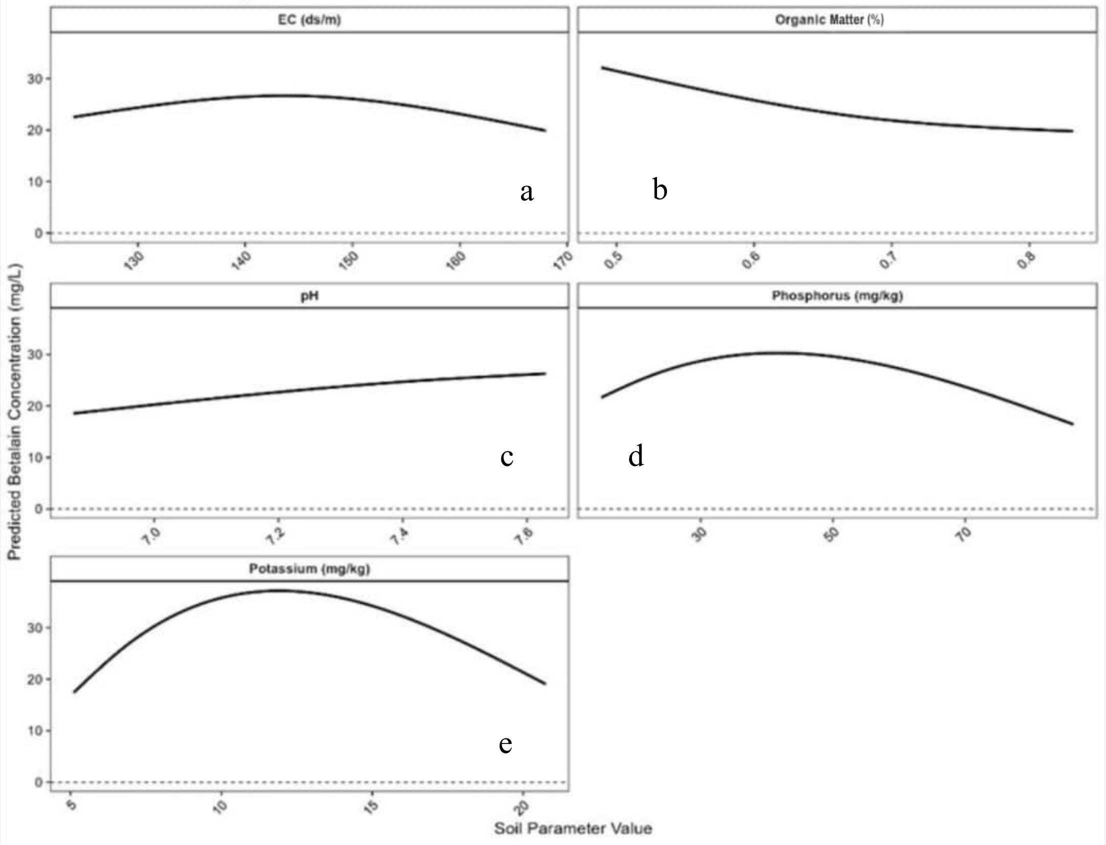


Figure 4. Threshold analysis for Betalain Concentration

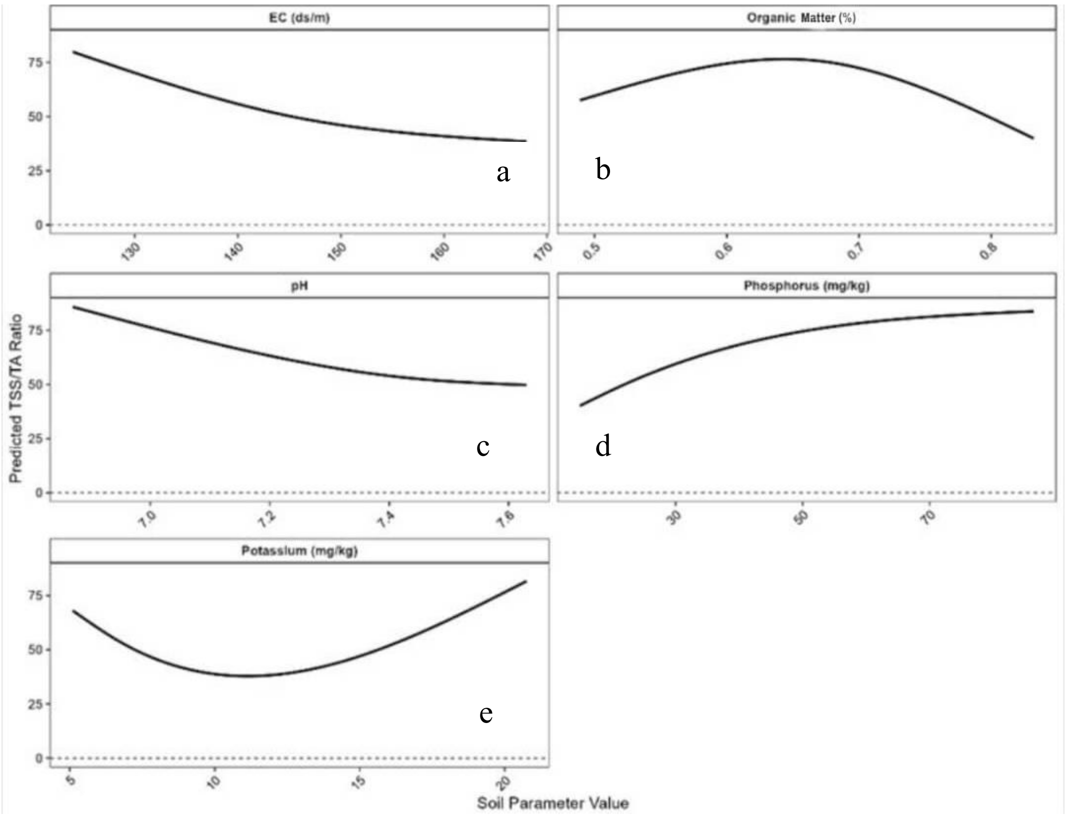


Figure 5. Threshold analysis for TSS/TA ratio

To further complement the threshold analysis, optimal soil parameter values in relation to betalain concentration and TSS/TA ratio were identified and summarized in Table 5. These values were derived from the model-predicted nonlinear response curves (Figure 4 and 5) and represent the soil parameter values at which the predicted response for each quality trait was highest within the observed data range along with their corresponding optimal ranges.

Table 5. Optimal soil parameter values and ranges derived from threshold analysis for maximizing betalain concentration and TSS/TA ratio in white fleshed dragon fruit

Parameters	**Betalain**		**TSS/TA Ratio**	
	Optimal value	**Optimal Range**	**Optimal Value**	**Optimal Range**
pH	7.63	7.5-7.7	6.87	6.7-6.9
Organic Matter (%)	0.84	0.8-0.9	1.1	1.0-1.1
EC (dS/m)	1.43	1.4-1.5	1.24	1.2-1.3
Phosphorus (mg/kg)	41.7	40-45	86.35	80-90
Potassium (mg/kg)	11.91	10-12	20.75	20-22

As shown in Table 5, betalain concentration was associated with higher soil pH (7.63; range: 7.5–7.7), whereas the TSS/TA ratio peaked at a slightly lower pH (6.87; range: 6.7–6.9). Organic matter showed low optimal values for both traits, with 0.84% (range: 0.8–0.9%) for betalain concentration and 1.1% (range: 1.0–1.1%) for the TSS/TA ratio, indicating that increased organic matter levels were consistently associated with reduced fruit quality across sites. Available phosphorus and potassium exhibited substantially higher optimal values for sweetness (86.35 mg/kg P and 20.75 mg/kg K; ranges: 80–90 mg/kg and 20–22 mg/kg, respectively) compared with pigmentation (41.70 mg/kg P and 11.91 mg/kg K; ranges: 40–45 mg/kg and 10–12 mg/kg, respectively), further reinforcing their stronger influence on the TSS/TA ratio than on betalain.

From the trait-specific optimal value and range (table 5) and by identifying overlapping soil parameters where both sweetness and pigment colour were favourable, the joint optimal value and range were derived, representing balanced soil conditions for both fruit quality (betalain and TSS/TA ratio) rather than maximising each trait. The values are presented in Table 6.

Table 6. Joint optimal soil parameter values derived from threshold analysis for maximizing betalain concentration and TSS/TA ratio in white fleshed dragon fruit.

Parameters	Joint Optimal Value	Joint Optimal Range
pH	6.87	6.7-7.0
Organic Matter (%)	0.96	0.90-1.05
EC (dS/m)	1.24	1.20-1.30
Phosphorus (mg/kg)	51.73	50-60
Potassium (mg/kg)	17.87	16-21

As per Table 6, the joint optimal pH was 6.87 (range: 6.7–7.0), where sweetness was favoured while maintaining acceptable pigmentation. The joint optimal organic matter level was 0.96% (range: 0.90–1.05%), confirming that low organic matter levels were consistently associated with improved overall fruit quality. For phosphorus and potassium, the joint optimal values were 51.73 mg/kg (range: 50–60 mg/kg) and 17.87 mg/kg (range: 16–21 mg/kg), respectively, representing intermediate levels that support both sweetness and pigmentation. Electrical conductivity showed a joint optimal value of 1.24 dS/m (range:

1.20–1.30 dS/m), indicating that lower to moderate salinity levels were favorable for overall fruit quality.

Overall, the joint optimal analysis shows that soil conditions that maximise betalain concentration do not fully coincide with those that maximise sweetness. By integrating trait-specific optimal values across study sites, this approach identifies soil parameter ranges that balance colour and sweetness in white fleshed dragon fruit under natural field variability.

3.5 Practical Application and Management Implications

The ridge regression, threshold and joint optimal analysis indicate the need to move away from a one-size-fits-all fertilization approach toward precision management, tailored to the specific market niche of white fleshed dragon fruit.

For growers targeting balanced quality, the joint optimal values indicate maintaining pH at 6.87 (range: 6.7–7.0), organic matter at 0.96% (range: 0.90–1.05%), EC at 1.24 dS/m (range: 1.20–1.30 dS/m), phosphorus at 51.73 mg/kg (range: 50–60 mg/kg), and potassium at 17.87 mg/kg (range: 16–21 mg/kg). These values maximize both betalain content and the TSS/TA ratio. None of the white flesh dragon fruits grown on the study sites have the hypothetical optimal balanced quality of both sweetness and coloration as the fruits grown in BAJO and CRP showed higher coloration in fruits in trade off with sweetness and the fruits grown in FMCL showed the highest sweetness but had low coloration.

For sweetness-focused markets (TSS/TA ratio), phosphorus was identified to be the main key driver with optimal level being 86 mg/kg (range: 80–90 mg/kg) and potassium, with its optimum value being 21 mg/kg (range: 20–22 mg/kg) was the second key driver. Organic matter should be maintained at 1.1% (range: 1.0–1.1%) and EC around 1.24 dS/m (range: 1.20–1.30 dS/m). The white flesh dragon fruits grown at the FMCL farm best represents this scenario having the highest TSS/TA ratio among the three study sites due to the soil chemical conditions of the farm being similar to the hypothetical values which compromises the coloration but accentuates the sweetness.

For visual-appeal markets (higher pigmentation), focus should be given on secondary metabolites and maintaining low vegetative growth. This is done through keeping low soil nitrogen and controlling organic matter. A target of 0.84% (range: 0.8–0.9%) is optimal. Unlike sweetness, betalain production benefits from slightly alkaline conditions with pH of 7.6 (range: 7.5–7.7) and has a much lower phosphorus requirement of 42 mg/kg (range: 40–45 mg/kg). Ridge regression and threshold analysis consistently showed that lower nitrogen enhanced betalain accumulation indicating that excessive dose of nitrogen should be avoided if betalain is to be enhanced. The white flesh dragon fruits grown at CRP and BAJO best explains and represents this scenario having higher betalain values and matching similar soil conditions which favors coloration but comprises sweetness.

This shows that the main trade-off between sweetness and color is not only about nutrients competing with each other, but moreover about certain response thresholds. To achieve balanced fruit quality, it is important to keep key soil parameters within their optimal ranges instead of simply maximizing them.

While this study provides a thorough analysis of the influence of soil parameters on TSS/TA ratio and betalain concentration in dragon fruit, its scope and certain limitations should be considered. The analysis was based on three study sites within a single region of Bhutan. As a result, the findings represent soil-driven relationships observed under local field conditions and may not fully reflect responses under different climates, soil types, or dragon fruit cultivars. Although average climate data during anthesis to harvest period was documented, these variables were not included in regression due to minimal variability among sites. Future studies should incorporate a large number of sites to more explicitly partition soil and climatic effects. In addition, interpretation of the results was constrained by the limited availability of peer-reviewed literature specifically addressing soil chemical parameters–quality relationships in dragon fruit (Hylocereus spp.). Also, the influence of soil physical properties on white fleshed dragon fruit quality was not assessed in this study, as the experimental design focused on chemical soil variability thus, conclusions are restricted to soil chemical drivers of fruit quality.

These results fulfil the study's objective by demonstrating how soil parameters influence dragon fruit quality, revealing critical nutrient thresholds, and providing joint optimal values to balance competing quality traits. Phosphorus and potassium consistently improved sweetness, whereas nitrogen and organic matter reduced both sweetness and pigmentation. Soil pH had a positive effect on betalain concentration up to a saturation point. The nonlinear responses revealed an inherent trade-off between quality traits. Increasing phosphorus and potassium to maximize sweetness tended to reduce colour intensity, while soil conditions that favoured pigment development often compromised sweetness. The joint optimal values therefore provide a scientifically grounded compromise for growers aiming to achieve balanced fruit quality.

Overall, these findings offer clear, context-specific guidance for nutrient management in Bhutan's white fleshed dragon fruit production. While maximizing both sweetness and colour simultaneously remains challenging, precision soil management based on joint optimal values provides a practical pathway to improve overall fruit quality under natural field conditions.

4 Conclusion

To conclude, this study showed that soil chemical parameters are key determinants of dragon fruit quality, influencing both sweetness (TSS/TA ratio) and pigmentation (betalain concentration). Through correlation, principal component analysis, ridge regression, and threshold analysis, soil parameters with strong effects on fruit quality were identified. The results reveal a fundamental trade-off: soil conditions that enhance sweetness tend to reduce pigmentation, whereas conditions that favor pigment accumulation often compromise sweetness. These findings also highlight the need for prior analysis of soil chemical parameters which is crucial for high quality white fleshed dragon fruit cultivation, as optimal soil conditions could be accurately managed to achieve desired fruit quality traits.

To address this trade-off, joint optimal values and ranges for soil chemical parameters, including soil pH (6.87; range: 6.7–7.0), organic matter (0.96%; range: 0.90–1.05%), electrical conductivity (1.24 dS/m; range: 1.20–1.30 dS/m), phosphorus (51.73 mg/kg; range: 50–60 mg/kg), and potassium (17.87 mg/kg; range: 16–21 mg/kg), were derived. These values represent soil conditions under which both sweetness and pigmentation are favorable, rather than maximizing either trait individually. This approach provides a scientifically grounded compromise for overall fruit quality.

These findings offer guided implications for growers where the trait-specific optimal values can be used to target niche markets that emphasise either sweetness, as seen in the white flesh dragon fruits produced at FMCL study site or visual appeal, as seen in the fruits produced by BAJO and CRP study sites, while joint optimal soil ranges and values can guide production toward balanced fruit quality. Together, these results provide an evidence-based framework for precision soil nutrient management in white fleshed dragon fruit cultivation.

The soil chemical compositions at the study sites reflected these quality differences. The FMCL site exhibited approximate available soil nitrogen at 249–225 mg/kg, phosphorus at 67.65–86.25 mg/kg, potassium at 5.10–20.75 and soil pH ranging from 6.87 to 7.15. The CRP site had approximate available soil nitrogen of 280 mg/kg, phosphorus at 14.95–17.75 mg/kg, potassium at 6.15–6.80 mg/kg and soil pH ranging from 7.28 to 7.63. The BAJO site displayed approximate available soil nitrogen at 137–208 mg/kg, phosphorus at 21.90–36.95 mg/kg, potassium at 6.60–7.70 mg/kg and soil pH ranging from 7.44 to 7.61.

Future studies should validate these soil quality relationships across different regions, soil types, and dragon fruit cultivars, and assess the economic implications of adopting specialised versus balanced soil management strategies.

5 Acknowledgement

The authors would like to express their sincere gratitude to the farm managers of Farm Machinery Corporation Limited, Wangdue and Chimipang Royal Project, Punakha for providing the required samples for this study. Warm appreciation also goes out to the National Center for Hydrology and Meteorology (NCHM) for providing the agromet data to be used in this study.

The authors would like to express their heartfelt gratitude to the College of Natural Resources for providing the laboratory required for this study. Acknowledgement also goes out to Agriculture Research and Development Centre, Bajo for the resources and support provided during this study. Special appreciation is given to the staff of ARDC Bajo; Pema Wangchuk, Thinley Wangmo, Sherab Lhamo, Deo Raj Mafchan, and Bikash Gurung for their assistance in field and laboratory data collection.

6 Authors' contribution statement

Nangsel Tshomo and Kuenga Penjor Gyeltshen were involved in the study's conceptualization and protocol design of the manuscript. Nangsel Tshomo was primarily responsible for analysing, interpreting, and visualising the data and drafting the manuscript. Kuenga Penjor Gyeltshen performed the biochemical analysis and data collection.

7 Reference

Alam, M., Biswas, M., Ahmed, J., Hosain, M. A., Alam, A., Khan, M. H. H., & Molla, M. M. (2023). Physico-chemical properties, antioxidant activity and bioactive compounds in edible and non-edible portions of dragon fruit cultivars native to Bangladesh. *Food Research*, *7*(4), 194–203. https://doi.org/10.26656/FR.2017.7(4).243

Bacelar, E., Pinto, T., Anjos, R., Morais, M. C., Oliveira, I., Vilela, A., & Cosme, F. (2024). Impacts of climate change and mitigation strategies for some abiotic and biotic constraints influencing fruit growth and quality. *Plants, 13*(14), 1942. https://doi.org/10.3390/plants13141942

Belbase, P., Jayachandran, K., & Balaji Bhaskar, M. S. (2025). Assessment of Soil and Plant Nutrient Status, Spectral Reflectance, and Growth Performance of Various Dragon Fruit (Pitaya) Species Cultivated Under High Tunnel Systems. Soil Systems, 9(3), 75. https://doi.org/10.3390/soilsystems9030075

Brima, E. I., & Abbas, A. M. (2014). Determination of citric acid in soft drinks, juice drinks and energy drinks using titration. *International Journal of Chemical Studies, 1*(6). http://www.chemijournal.com

Budhathoki, R., Bhutia, K. D., Das, T., Chettri, S., Sharma, L., & Upadhyay, S. (2023). Physico-Chemical Characteristics of Dragon Fruit Hylocereus polyrhizus (Weber) Britton and Rose from Different Locations of Nepal. *Environment and Ecology*, *41*(4C), 2853–2858. https://doi.org/10.60151/envec/jftt4959

Chen, S. Y., Xu, C. Y., Mazhar, M. S., & Naiker, M. (2024). Nutritional value and therapeutic benefits of dragon fruit: A comprehensive review with implications for establishing Australian industry standards. *Molecules, 29*(23), Article 5676. https://doi.org/10.3390/molecules29235676

Chhogyel, N., & Kumar, L. (2018). Climate change and potential impacts on agriculture in Bhutan: A discussion of pertinent issues. *Agriculture & Food Security, 7*(1). https://doi.org/10.1186/s40066-018-0229-6

Fathordoobady, F., Mirhosseini, H., Selamat, J., & Manap, M. Y. A. (2016). Effect of solvent type and ratio on betacyanins and antioxidant activity of extracts from *Hylocereus polyrhizus* flesh and peel by supercritical fluid extraction and solvent extraction. *Food Chemistry, 202*, 70–80. https://doi.org/10.1016/j.foodchem.2016.01.121

Franco, R. K. G., Esguerra, E. B., Tababa, J. L., & Castro, A. C. (2022). Harvest maturity affects the quality and storage behavior of white fleshed dragon fruit [Hylocereus undatus (Haworth) Britton and Rose]. Food Research, 6(2), 423–433. https://doi.org/10.26656/fr.2017.6(2).268

GNT International B.V. (2010). *Composition comprising betalains* (Patent No. WO2010090508A1). World Intellectual Property Organization. https://patents.google.com/patent/WO2010090508A1/en

Islam, M. S., Islam, M. N., Gupto, S. D., & Rahman, M. K. (2025). Assessment of soil fertility in relation to dragon fruit (Hylocereus costaricensis) cultivation in south-eastern region of Bangladesh. Journal of Biodiversity Conservation and Bioresource Management, 10(2), 87–98. https://doi.org/10.3329/jbcbm.v10i2.82334

Jolliffe, I. T., & Cadima, J. (2016). Principal component analysis: A review and recent developments. *Philosophical Transactions of the Royal Society A: Mathematical,* *Physical and Engineering Sciences, 374*(2065), 20150202. https://doi.org/10.1098/rsta.2015.0202

Lata, D., Narayana, C. K., Karunakaran, G., Sudhakar Rao, D. V., & Sane, A. (2022). Maturity determination of red and white pulp dragon fruit. *Journal of Horticultural Sciences, 17*(1). https://doi.org/10.24154/jhs.v17i1.1309

Lee, D. H., Kim, Y. K., Son, Y., Park, G. H., Kwon, H. Y., Park, Y., Park, E. J., Lee, S. Y., & Kim, H. J. (2023). Multivariate analysis among marker compounds, environmental factors, and fruit quality of *Schisandra chinensis* at different locations in South Korea. *Plants, 12*(22), 3877. https://doi.org/10.3390/plants12223877

Li, Y., Li, Q., Yan, Y., Liu, W., Xu, C., Wang, Y., Nan, L., & Liu, X. (2024). Evaluation of soil nutrients and berry quality characteristics of Cabernet Gernischet (Vitis vinifera L.) vineyards in the eastern foothills of the Helan Mountains, China. Frontiers in Plant Science, 15. https://doi.org/10.3389/fpls.2024.1418197

Lindsay, W. L., & Norvell, W. A. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Science Society of America Journal, 42(3), 421–428.

Majidi, H., Minaei, S., Almasi, M., & Mostofi, Y. (2011). Total Soluble Solids, Titratable Acidity and Ripening Index of Tomato In Various Storage Conditions. Australian Journal of Basic and Applied Sciences, 5(12), 1723–1726.

Mondal, T., & Alam, M. (2023). Dragon fruit: Wonder fruit of the 21st century. *Agriculture & Food: E-Newsletter, 5*(4), 530–533. https://www.researchgate.net/publication/369692557

National Statistical Bureau. (2025). *Integrated agriculture and livestock census of Bhutan 2025*. Royal Government of Bhutan.

Nie, S. A., Zhou, Y. Z., Yue, Q. Q., et al. (2025). Characteristics of medium and trace nutrient elements and their impact on fruit quality of *Citrus sinensis* (L.) Osbeck orchard with different soils. *Soils and Crops, 14*(4), 471–479. https://doi.org/10.11689/sc.2025051402

Orlab Instruments Pvt. Ltd. (2025). *Digital soil testing kit (STFR meter).* https://www.orlabindia.com/digital-soil-testing-kit/

Papadakis, I. E., Protopapadakis, E., Dimassi, K. N., & Therios, I. N. (2005). Nutritional Status, Yield, and Fruit Quality of "Encore" Mandarin Trees Grown in Two Sites of an Orchard with Different Soil Properties. Journal of Plant Nutrition, 27(9), 1505–1515. https://doi.org/10.1081/PLN-200025994

Pratiwi, R., Mulyaningsih, R. D., & Hasanah, A. N. (2025). Development of paper based colorimetric method using pigment from red dragon fruit for determination of Cu and Fe. *Scientific Reports, 15*(1), Article 98693. https://doi.org/10.1038/s41598-025-98693-7

Puccinelli, M., Cuccagna, S., Maggini, R., Carmassi, G., Pardossi, A., & Trivellini, A. (2025). Effects of Nitrogen Nutrition on the Nutraceutical and Antinutrient Content of Red Beet (Beta vulgaris L.) Baby Leaves Grown in a Hydroponic System. Agriculture, 15(18), 1914. https://doi.org/10.3390/agriculture15181914

Sadowska-Bartosz, I., & Bartosz, G. (2021). Biological properties and applications of betalains. *Molecules, 26*(9), 2520. https://doi.org/10.3390/molecules26092520

Sahu, A., Kishore, K., Nayak, R. K., Dash, S. N., Sahoo, S. C., & Barik, S. (2023). Influence of potassium on mineral content, yield and quality attributes of dragon fruit (Selenicereus monacanthus) in acidic soil of Eastern tropical region of India. Journal of Plant Nutrition, 46(11), 2621–2636. https://doi.org/10.1080/01904167.2022.2160744

Salam, U., Ullah, S., Tang, Z. H., Elateeq, A. A., Khan, Y., Khan, J., Khan, A., & Ali, S. (2023). Plant metabolomics: An overview of the role of primary and secondary metabolites against different environmental stress factors. *Life, 13*(3), 706. https://doi.org/10.3390/life13030706

Singh, A., Swami, S., Panwar, N. R., Kumar, M., Shukla, A. K., Rouphael, Y., Sabatino, L., & Kumar, P. (2022). Development changes in the physicochemical composition and mineral profile of red fleshed dragon fruit grown under semi-arid conditions. *Agronomy, 12*(2), 355. https://doi.org/10.3390/agronomy12020355

Srivastava, A. K., & Malhotra, S. K. (2014). Nutrient management in fruit crops: Issues and strategies. *Indian Journal of Fertilisers, 10*(12), 72–88.

Sun, H., Huang, X., Chen, T., Zhou, P., Huang, X., Jin, W., Liu, D., Zhang, H., Zhou, J., Wang, Z., Hayat, F., & Gao, Z. (2022). Fruit quality prediction based on soil mineral element content in peach orchard. Food Science and Nutrition, 10(6), 1756–1767. https://doi.org/10.1002/fsn3.2794

Sundarrajan, R. V., Rajangam, J., Saraswathy, S., Gnanasekaran, M., Rajesh, S., Anitha, T., & Sankar, C. (2025). A comprehensive review on impact of climatic change on adaptability and mitigation in fruit crop. *Plant Science Today, 12*(1). https://doi.org/10.14719/pst.6043

Woo, K. K., Ngou, F. H., Ngo, L. S., Soong, W. K., & Tang, P. Y. (2011). Stability of betalain pigment from red dragon fruit (Hylocereus polyrhizus). *American Journal of Food Technology*, *6*(2), 140–148. https://doi.org/10.3923/ajft.2011.140.148

Xie, F., Chen, C., Chen, J., Yuan, Y., Hua, Q., Zhang, Z., Zhao, J., Hu, G., Chen, J., & Qin, Y. (2022). Metabolic profiling of sugars and organic acids, and expression analyses of metabolism-associated genes in two yellow-peel pitaya species. *Plants, 11*(5), 694. https://doi.org/10.3390/plants11050694

Yang, H., Du, T., Mao, X., Ding, R., & Shukla, M. K. (2019). A comprehensive method of evaluating the impact of drought and salt stress on tomato growth and fruit quality based on EPIC growth model. Agricultural Water Management, 213, 116–127. https://doi.org/10.1016/j.agwat.2018.10.010

https://doi.org/10.55925/btagr.26.9107

Exploring the Production Potential and Adaptability of Black Rice Across Bhutanese Agroecological Zone

Deki Lhamo[1], Cheku Dorji[1], Dolay[2], Sonam Deki[3] and Chezang Dendup[4]

Abstract

Black rice is a nutrient-rich, pigmented rice variety with high health-promoting properties, yet its cultivation and adaptability in Bhutan remain largely unexplored. This study evaluated the production potential and adaptability of black rice across four agroecological zones in Bhutan over four years (2021–2024), using research stations and on-farm trials. Experiments included Single plots evaluation for first three years in the research Centre and Randomized Complete Block Designs in the final year. at research centers and single-plot in the final year, with growth and yield parameters recorded, including tillers per hill, plant height, panicle length, days to maturity, and grain yield. Results indicated significant variation ($P \leq 0.05$) in growth and yield across locations, with the highest grain yields observed in dry sub-tropical (Bajo, 2033.31 ± 199.41 kg/acre) and humid sub-tropical (Lingmethang, 1819 ± 282.01 kg/acre) zones, while wet sub-tropical (Samtenling) and highland dry sub-tropical (Tsirang) zones showed lower yields. Black rice exhibited longer maturity periods, fewer tillers, and taller plants compared to improved and local varieties. On-farm trials confirmed that favorable valley conditions in low- to mid-altitude regions support optimal growth and yield. The study demonstrates that black rice can adapt beyond its native southern belt, offering a viable, nutrient-dense alternative for Bhutanese farmers. Strategic promotion and market development are recommended to maximize its economic and nutritional potential.

Keywords: *Black Rice; Agroecological Zones; Yield Performance; Adaptability*

Corresponding email: dlhamo@moal.gov.bt

[1] Agriculture Research and Development Centre, Bajo, Department of Agriculture, Ministry of Agriculture and Livestock

[2] Agriculture Research and Development Sub-Centre, Tsirang, Department of Agriculture, Ministry of Agriculture and Livestock

[3] Agriculture Research and Development Sub-Centre, Lingmithang, Department of Agriculture, Ministry of Agriculture and Livestock

[4] Agriculture Research and Development Centre, Samtenling, Department of Agriculture, Ministry of Agriculture and Livestock

1 Introduction

Rice is the staple food in Bhutan and has historically played a vital role in the country's food security and livelihoods, while remaining closely interwoven with its culinary traditions, cultural heritage, and religious practices. The current estimated per capita consumption is 144 Kg per year. Rice is grown about 23,289.59 acres with the production of 40,804 MT (National Statistics Bureau [NSB], 2024). Over the years, various research centers have introduced and developed over 31 improved, high yielding and disease resistant rice varieties which are made available to the farmers (Department of Agriculture, 2024). However, the overall adoption rate of these cultivars remains around 42% (Ghimiray, 2013).

Alongside these improved varieties, Bhutan also cultivates traditional rice landraces that form an integral part of its rice farming. The traditional Bhutanese rice varieties can be broadly classified as "Bja Maap" (red pericarp varieties) and "Bja Kaap" (white pericarp varieties) (Wangmo, 2019). Of the pigmented rice varieties, black rice has received increasing attention due to its sensory characteristics, its high nutritive value and, mainly, because of its beneficial health properties (Ito & Lacerda, 2019) but still unknown to Bhutanese consumers and growers. Black rice belongs to *Oryza sativa* L., the same species as white rice and red rice (Pratiwi & Purwestri, 2017). Black rice, often referred to as "Forbidden Rice" or "Emperor's Rice," has an uncertain origin but is known to have originated in Asian countries such as China, India, Japan, and Vietnam (Panda et al., 2022). Historically, it was reserved exclusively for emperors and royal families in ancient China, with severe consequences for unauthorized consumption (Sah & Kushwaha, 2016). Also called "Purple Rice" due to its deep purple-black hue, this rice holds both cultural and nutritional significance. Black rice is a rich storehouse of fibers, vitamins, minerals, proteins, and bioactive compounds, making it a valuable addition to the daily diet. Its incorporation not only promotes overall health but also helps reduce the risk of several diseases such as cancer, inflammation, atherosclerosis, obesity, diabetes, constipation, and hepatic disorders (Das, 2023; Sharma, 2024).

Although black rice has been cultivated in small pockets along Bhutan's southern borders with India, its presence within the country remains minimal. Despite its recognized nutritional and cultural value globally, no systematic studies have yet been carried out to evaluate its performance or adaptability under Bhutanese conditions. Considering Bhutan's rice heritage and the rising interest in pigmented rice, this study aims to evaluate the production feasibility of black rice across different agroecological zones.

2 Materials and Method

2.1 Study Sites and Agroecological Zones

The agroecological zones of Bhutan are subcategorized into six major groups corresponding with altitude and climatic conditions (Table 1). These study sites fall under dry, humid, and wet subtropical zones. Temperate and alpine regions were excluded, as rice is not grown in these areas and moreover black rice is native to the wet subtropical zones.

Table 1. Major agroecological zones of Bhutan

Agroecological Zones	Altitude (masl)	Temperature (˚C)		Mean Rainfall (mm)	Proportion of Geographical Area (%)
		Max	Min		
Alpine	3500–7500	12.0	-1.0	<650	28.6
Cool temperate	2600–3600	22.0	1.0	650–850	23.9
Warm Temperate	1800–2600	26.0	1.0	650–850	18.6
Dry Sub-tropical	1200–1800	29.0	3.0	850–1200	13.1
Humid Sub-tropical	600–1200	33.0	5.0	1200–1500	10.2
Wet Sub-Tropical	100–600	35.0	12.0	2500–5500	5.0

Source: RNR Research Strategy and Plan Document (May 1992)

At Bajo, the trial was conducted in the research farm at an altitude of 1200 masl. Bajo is located at latitude 27°29'25"N and longitude 89°53'58"E. The site represents a dry Sub-tropical agroecological zone is characterized by warm summers and cool making frost possible in the coldest months.

The dry-subtropical AEZ (13% of the country's area) is dominated by rice as the main summer crop followed by wheat, mustard and vegetables. Improved crop varieties and use of fertilizers and herbicides are gaining popularity; however, farmyard manure is still applied. High crop yields are obtained compared to other agricultural ecological zones (International Center for Tropical Agriculture [CIAT] & World Bank, 2017). The soils at the Bajo are mainly coarse or fine loamy. Organic carbon is 0.1–1.1% with C:N ratios 3.3–50, and available phosphorus is low to moderate (1–5 ppm), making the soils suitable for crop growth with proper nutrient management (NSSC, 2004). The mean minimum temperature during rice season ranges from 18.7˚C in May to 10.4˚C in November while the mean maximum temperature varies from 28.7˚C in May to 24.8˚C in November. The mean annual rainfall was 557 mm from 2021-2024 (Table 2).

Table 2. Mean temperature and rainfall of trial sites

Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Samtenling (370 masl): Data from 2023-2024												
Mean Rainfall (mm)	0	6.4	167.5	180.5	362.7	973	1171.1	1383.8	433.4	269.1	6.2	7.7
Mean Max. Temp. ˚C	23.8	24.2	27.3	30.4	31	30.8	32.2	31.6	34	30.9	29.9	26
Mean Min. Temp. ˚C	12.8	13.1	18.4	21.5	23.2	23.8	24.4	24.1	24	21	17.4	14.5
Bajo (1200 masl): Data from 2021-2024												
Mean Rainfall (mm)	2.4	4.6	39.7	18.9	44.4	59.9	106.7	119.6	87.1	69.4	0.8	3.8
Mean Max. Temp. ˚C	19.9	20.4	24.9	27.8	28.7	29.5	30.4	29.7	30.0	27.8	24.8	21.7
Mean Min. Temp. ˚C	6.6	8.3	11.9	15.2	18.7	22.0	22.4	22.1	20.7	16.9	10.4	7.4
Lingmithang (650masl): Data from 2023-2024												
Mean Rainfall (mm)	1.8	4.7	54.6	14.8	55.0	96.0	99.5	108.6	50.9	65.8	0.0	1.8
Mean Max. Temp. ˚C	13.5	17.1	19.3	26.6	30.3	31.5	34.1	33.3	35.9	34.0	34.8	20.4
Mean Min. Temp. ˚C	9.9	11.9	16.3	20.1	21.8	25.3	25.1	25.7	26.0	22.3	16.4	12.8
Tsirang (1480 masl): Data from 2021-2024												
Mean Rainfall (mm)	4.3	11.0	42.1	41.6	54.5	346.2	272.7	340.7	151.5	105.0	0.8	2.7
Mean Max. Temp. ˚C	15.2	15.8	19.5	21.6	22.5	23.3	24.1	24.3	24.9	22.6	20.5	18.1
Mean Min. Temp. ˚C	5.6	6.8	10.5	13.1	15.1	18.8	19.6	19.4	18.6	15.6	9.6	7.4

Data Source: National Center for Hydrology and Meteorology

At Samtenling, the trial was conducted in the research farm at an altitude of 370 masl. It is located at latitude 26°54'26"N and longitude 90°25'52"E. The site represents a wet Sub-tropical agroecological zone are located in the Himalayan foothills in the southern belt and are characterized by high humidity and heavy rainfall making it one of the most precipitation-rich areas in the country. Agriculture is the predominant land use in this zone, with rice being the staple crop. However, due to higher rainfall and humidity there are more insect and disease problems in crops. Other crops include maize, mustard, legumes, and various vegetables. In addition to these, citrus fruits, particularly mandarin oranges, are significant cash crops in the region. Largescale winter cropping although technically feasible is normally not practiced due to the scarcity of water.

The top soils at the Samtenling Research and Development Centre are coarse-loamy with common distinct reddish-brown color. It is very acidic with a pH of 5.40 and generally exhibits very low to low inherent fertility. Total nitrogen is low (0.15%), while organic carbon is moderate (1.60%). In contrast, available phosphorus is very high at 68.0 ppm. (NSSC, 2001). The mean minimum temperature during the rice season ranges from 23.8˚C in

June to 17.4˚C in November while the mean maximum temperature varies from 30.8 ˚C in June to 34˚C in November. The mean annual rainfall was 4961.2 mm from 2023-2024 (Table 2).

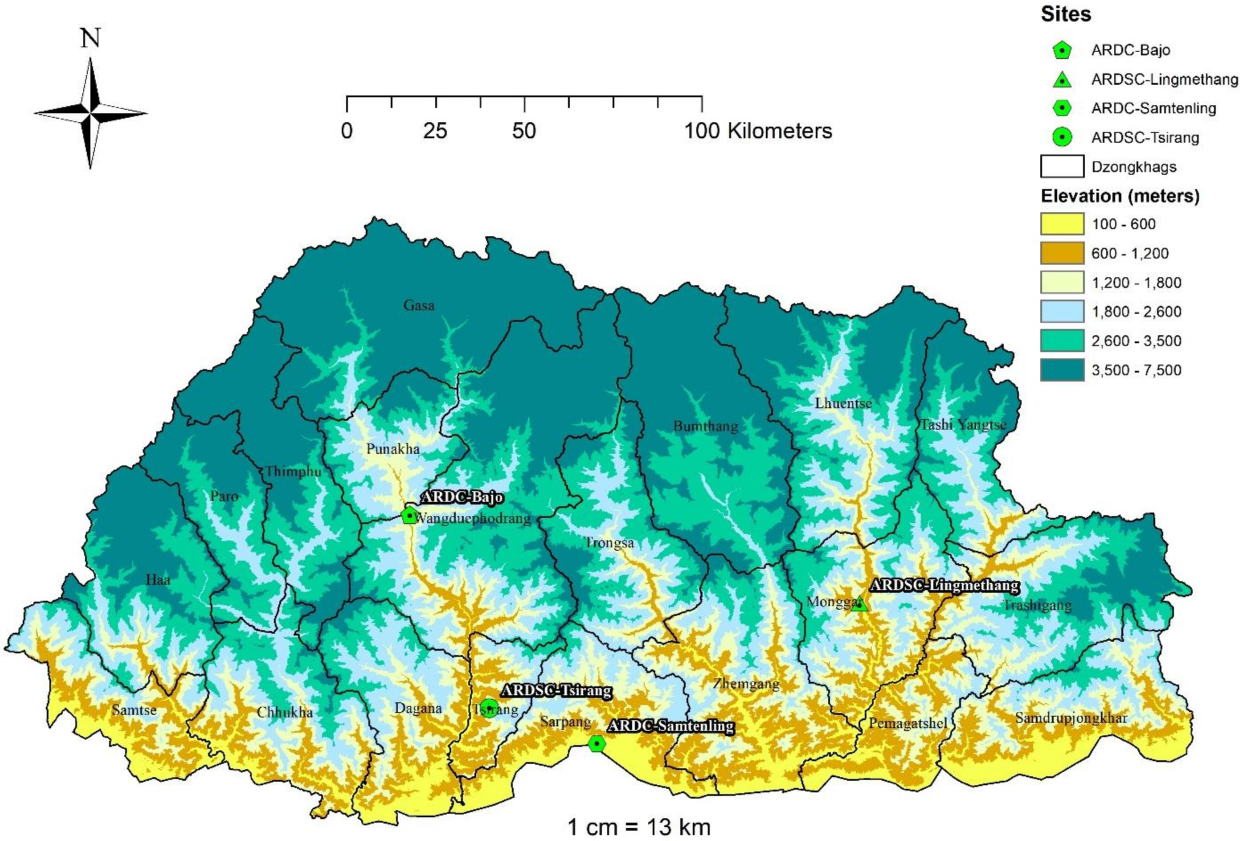


Figure 1. Agroecological zone map of Bhutan with the location of the study sites.

At Lingmethang, the study site is at 27°15'38"N and between 91˚ 10’ 38” and 91°10'45"E at an altitude of 650 masl. It represents a humid -subtropical agroecological zone. Maize and potato intercropping is Very popular. Farmers also cultivate different vegetables, grain legumes, millet, buckwheat, barley and mustard in the dryland (Katwal, 2020). The soils at Lingmethang are mainly sandy loams and sandy clay loams, slightly acidic to neutral (pH 6.32–7.01). The organic carbon and total nitrogen are generally low to moderate and the overall fertility potential and inherent fertility is categorized as slightly poor (NSSC,2003). The mean minimum temperature during rice season ranges from 25.1˚C in July to 12,8˚C in December while the mean maximum temperature varies from 34.1˚C in July to 20.4˚C in December. The mean annual rainfall was 553.4 mm from 2023-2024 (Table 2).

At Tsirang, the study site was at Menchuna ARDSC suited at 26°59'52"N and 90°07'32"E at an altitude of 1480 masl. It represents a dry-subtropical agroecological zone but features a temperate highland tropical climate with dry winters and the wet season is warm, humid, and mostly cloudy. sirang grows a diverse range of crops, including staple cereals like paddy and maize, as well as cash crops such as chili, orange, and cardamom. The soils are mostly sandy loam and slightly acidic (pH 5.01–5.53) with low to moderate organic carbon (0.23–0.58%) and mostly low nitrogen. Available phosphorus and potassium are generally low, giving the soils a low to moderate fertility potential. The mean minimum temperature during rice season ranges from 15.1˚C in May to 9.6˚C in November while the mean maximum temperature varies from 22.5˚C in May to 20.5˚C in November. The mean annual rainfall was 1372.8 mm from 2023-2024 (Table 2).

2.2 Experimental Design

The study was conducted as a Nationally Coordinated Trial (NCT) over four years (2021–2024) at four research centers namely Agriculture Research and Development Centre (ARDC) and Samtenling, Agriculture Research and Development Sub-Centre (ARDSC) Lingmithang, and Tsirang (Figure 1). In its final year, the trial was extended to farmers' fields across multiple locations, including Zomi, Toedwang, and Chubu Gewogs in Punakha District, and Daga, Athang, and Rubesa Gewogs in Wangdue Phodrang District. The trial initially began at ARDC Bajo and ARDSC Tsirang in 2021, and was later expanded to ARDC Samtenling and ARDSC Lingmithang in 2023 and 2024.

For the first three years, all experiments were conducted in large single plots evaluate the feasibility and performance of black rice under different study area. In 2024, the evaluation was carried out using a Randomized Complete Block Design (RCBD) with three treatments and five replications. The treatments included one improved variety and one local variety used as checks. Each replication formed a block to account for field variability, and treatments were randomly assigned to plots within each block using a random number table. Each plot measured 2 m × 3 m (6 m^2), accommodating 96 hills (12 columns × 8 rows) with 25 cm × 25 cm spacing between hills. Plots were separated by 40 cm to minimize inter-plot interference. In 2024, single-plot trials were conducted in farmers' fields to assess the feasibility and performance of black rice under real field conditions.

2.3 Crop management

Healthy seeds of black rice, sourced from Nganglam, Pemagatshel, were sown at a rate of 25 kg per acre. Seedlings were transplanted into the well-puddled experimental plots, and recommended fertilizer rates (30-20-8 kg NPK per acre) were applied. Weeds were controlled through two hand weeding. The crop of each plot was harvested separately at full maturity when 90% of the grains turn golden yellow in color on different dates.

2.4 Data collection

For data collection five plants sample per a plot were selected randomly for the yield parameters such as Tillers /hill, Plant height (cm), Panicle length (cm), Grain /panicle (No) and Empty grain/panicle. Moisture content was measured using digital grain moisture meter at the time of crop harvest and fresh weight of grains of each plot were converted into grain yield ton per acre after adjusting the moisture content at 14% using the formula (1). Straw obtained from each crop cut were weighed separately in kg and finally converted into straw yield ton per acre. Harvest index is the ratio of economic yield to biological yield and was calculated with the formula (2). To measure yield in the farmers field, three crop cuts were taken from an area 6 m^2. Threshing was done manually.

$$Adjusted\ yield\ (kg/acre) = \left(\frac{100-MC_{field}}{100-MC_{standard}}\right) * \frac{Yield\ per\ plot(kg)}{plot\ size\ (\mathrm{m}^2)} * 4046.86\ldots\ (1)$$

Where MC_{field} is moisture content (%) of the sample at harvest

$$Harvest\ Index\ (\%) = \frac{Grain\ yield\ (kg/acre)}{Grain\ yield(kg/acre)+straw\ yield\ (kg/acre)} * 100\ldots\ldots\ldots\ (2)$$

2.5 Statistical Analysis

The data for the 2024 trial were analyzed using the statistical software Statistix 8.1. Analysis of variance (ANOVA) was used to compare means, with the level of significance set at 5% ($p \leq 0.05$).

For the analysis of data spanning four years, analyses were conducted in R software using the lme4 (Bates et al., 2015) and emmeans (Lenth, 2024) packages. The lme4 package is used for fitting linear and generalized linear mixed-effects models, which allow the inclusion of both fixed and random effects and can handle unbalanced datasets. The emmeans package is used to estimate marginal means (least-squares means). A mixed-effects model was applied to assess the effect of location on rice traits over multiple years, accounting for unbalanced data. Location was treated as a fixed effect, while year was included as a random effect. ANOVA was performed within the mixed-model framework using Satterthwaite's approximation to obtain appropriate degrees of freedom and to test the significance of location effects. Estimated means with standard errors (Mean ± SE) were calculated, and pairwise comparisons between locations were performed using the LSD test.

The effect of rainfall and temperature on rice yield was analyzed using a linear mixed-effects model, with rainfall and temperature included as continuous fixed effects and location and year as random effects. The significance of fixed effects was tested using ANOVA with Satterthwaite's approximation.

3 Results and Discussion

3.1 Multi-Year Performance Across Agroecological Zones

3.1.1 Yield

The result showed significant differences for the grain yield ($P \leq 0.05$) across the sites (Table 3). The observed grain yields ranged from 519.06 to 2033.31 kg/ac, with the highest yields recorded at Bajo (dry sub-tropical, 10.4–28.7 ˚C, 557 mm rainfall) and Lingmethang (humid sub-tropical, 12.8–34.1 ˚C, 553.4 mm rainfall), where moderate temperatures and rainfall favored good crop growth and grain development. In contrast, very high rainfall at Samtenling (wet sub-tropical, 17.4–34 ˚C, 4961.2 mm) and cooler conditions at Tsirang (dry sub-tropical highland, 9.6–22.5 ˚C, 1372.8 mm) limited yield. Despite being grown for the first time in the experimental sites, black rice produced appreciable yields.

Notably, the observed yields not only match but, in places like Bajo, exceed the recent national average rice productivity in Bhutan of about 1800 kg/ac (National Statistics Bureau, 2024). The results show the good potential of black rice, especially in low- to mid-altitude regions, although generally, black rice was grown in lowland about 0-600masl. Comparable productivity has been reported internationally, with yields ranging from 1941 kg/acre in India (Chandrika et al., 2024), 2,084 kg/ac in Indonesia (Herliana et al., 2019) and 850–1011 kg/ac in Nepal (Khadka, 2016) indicating that the performance of black rice in Bhutan is consistent with its potential in other Asian contexts. It is considered comparatively less productive than the other rice varieties under normal condition (Borah et al, 2018). Though lower productivity, higher prices are the reason behind in accessibility of nutrient enriched black rice by common people (Tiwari, 2022).

Table 3. Growth and yield performance of black rice across four locations

Location	Altitude (masl)	Years	Tillers (Nos)	Plant height (cm)	Panicle length (cm)	Maturity days	Yield (Kg/ac)
Bajo	1200	2021-2024	10.69 ± 1.26^{a}	135.91 ± 4.57^{b}	19.88 ± 0.66^{a}	150.25 ± 9.38^{a}	2033.31 ± 199.41^{c}
Lingmithang	650	2023-2024	15.43 ± 1.67^{b}	136.64 ± 6.46^{b}	23.23 ± 0.93^{b}	148.12 ± 12.95^{ab}	1819 ± 282.01^{bc}
Samtenling	370	2023-2024	10.36 ± 1.67^{ab}	132.12 ± 6.46^{b}	22.95 ± 0.93^{b}	139.62 ± 12.95^{a}	519.06 ± 282.01^{a}
Tsirang	1480	2021-2024	9.28 ± 1.26^{a}	103.03 ± 4.57^{a}	22.7 ± 0.66^{b}	183.75 ± 9.38^{b}	1038.63 ± 199.41^{ab}
p-value			0.0744	0.0032	0.0343	0.0624	0.0073

* The values presented are estimated marginal means (± SE) obtained via the emmeans package

3.1.2 Number of Tillers

The mean number of tillers across the four study locations varied from 9.28 ± 1.26 to 15.43 ± 1.67, with the highest number recorded at Lingmithang (650 masl) and the lowest at Tsirang (1480 masl). Although the effect of location on tiller number was not statistically significant, a general trend of higher tillering was observed in the lowland sites compared with the highland sites. This finding is consistent with the results reported for black rice in Indonesia, where plants grown in highland environments produced fewer tillers than those cultivated in lowland areas (Purwanto, Hidayati, & Nandariyah, 2018).

3.1.3 Maturity durations

Maturity duration of rice varied across the four locations, ranging from 139.62 ± 12.95 days at Samtenling to 183.75 ± 9.38 days at Tsirang, with Bajo and Lingmithang showing intermediate values (Table 3). Although the differences were marginally non-significant ($p = 0.0624$), the observed variation reflects meaningful environmental influences, particularly altitude and temperature. Shorter maturity at Lingmithang and Samtenling likely resulted from warmer conditions that accelerated plant development, whereas cooler conditions at Tsirang delayed crop maturity. Similar trends were reported by Khanum et al. (2023), where the same rice varieties exhibited different days to maturity across three agroecological zones in Bangladesh, with cooler, higher-altitude regions delaying maturity and warmer, lowland areas accelerating it. Black rice could be categorized as a long-duration cultivar as related by

Nandariyah (2023), black rice, being a landrace, generally has cultivation challenges such as a prolonged vegetative phase and high habitus.

3.1.4 Plant height

Plant height differed significantly among locations (($P \leq 0.05$) (Table 3). Tsirang recorded the shortest mean height (103.03 ± 4.57 cm), while the other three locations had taller plants, ranging from 132.12 to 136.64 cm. Comparatively, black rice plant height has been reported to range from 141–146 cm in India (Sangma et al., 2022), 157.34–164.68 cm in the Philippines (Menardo et al., 2024), and 109.7–114 cm in Egypt (Metwally, 2024). These findings are consistent with observations reported by Tahir et al. (2002) who noted that plant height is primarily determined by the genetic makeup of the cultivar, though environmental factors also play a significant role.

3.1.5 Panicle length

Panicle length differed significantly among locations (($P \leq 0.05$), with the highest at Lingmithang (23.23 ± 0.93 cm) and the lowest at Bajo (19.88 ± 0.66 cm). Samtenling (22.95 ± 0.93 cm) and Tsirang (22.7 ± 0.66 cm) were intermediate (Table 3). These results are consistent with previous studies in Indonesia, where Sudarmayanti et al. (2022), reported that panicle length in rice is strongly influenced by environmental conditions. Notably, one of their experimental sites, Tampak Siring (379 masl), showed a panicle length (22.33 cm) similar to Samtenling (370 masl) in the present study, suggesting that altitude and related environmental factors may have comparable effects on panicle development. Sarker et al. (2011) also reported differences in number of panicles due to climatic parameters. As cited in Sofian et al. (2019), panicle length is one of the important parameters determining the productivity of a rice variety, because longer panicles produce a greater number of grains, which increases the grain weight per plant and can enhance overall rice productivity.

3.1.6 Effect of rainfall and temperature on yield

The effects of rainfall and temperature on rice yield were assessed using a linear mixed-effects model with location and year as random effects (Table 4). Rainfall showed a negative association with yield (estimate = −0.36), suggesting that yield tended to decrease with increasing rainfall. while temperature showed a positive association (estimate = 96.57), indicating a tendency for higher yields at higher temperatures. However, neither effect was statistically significant. The relationships between rice yield and rainfall and temperature are further illustrated in Figures 2 and 3, respectively.

Table 4. Linear mixed-effect regression

Predictor	Estimate	Std. Error	df	t value	p-value
Rainfall	-0.36	0.12	1.40	-2.98	0.14
Temperature	96.57	70.31	1.03	1.37	0.39

Random-effects analysis revealed variability among locations (variance = 67,842.25), whereas inter-annual variability was negligible (variance = 0), suggesting that agro-ecological differences had a much greater influence on rice yield than year-to-year climatic variation.

Although black rice is grown in the southern belt and across the border in India, yield at Samtenling was low. Samtenling received much higher rainfall (5,376 mm in 2023, 4,540 mm in 2025) than Bajo (449 mm; 575 mm). Consistent with the observed negative relationship between rainfall and yield and the positive relationship with temperature, this poor performance can be associated with excessive rainfall under warm wet sub-tropical conditions, which likely constrained yield. In contrast, mid-altitude locations, receiving more moderate rainfall and favorable thermal conditions, achieved higher yields. Overall, the results indicate that rainfall extremes within the southern belt can suppress rice yield, explaining the unexpectedly poor performance at Samtenling.

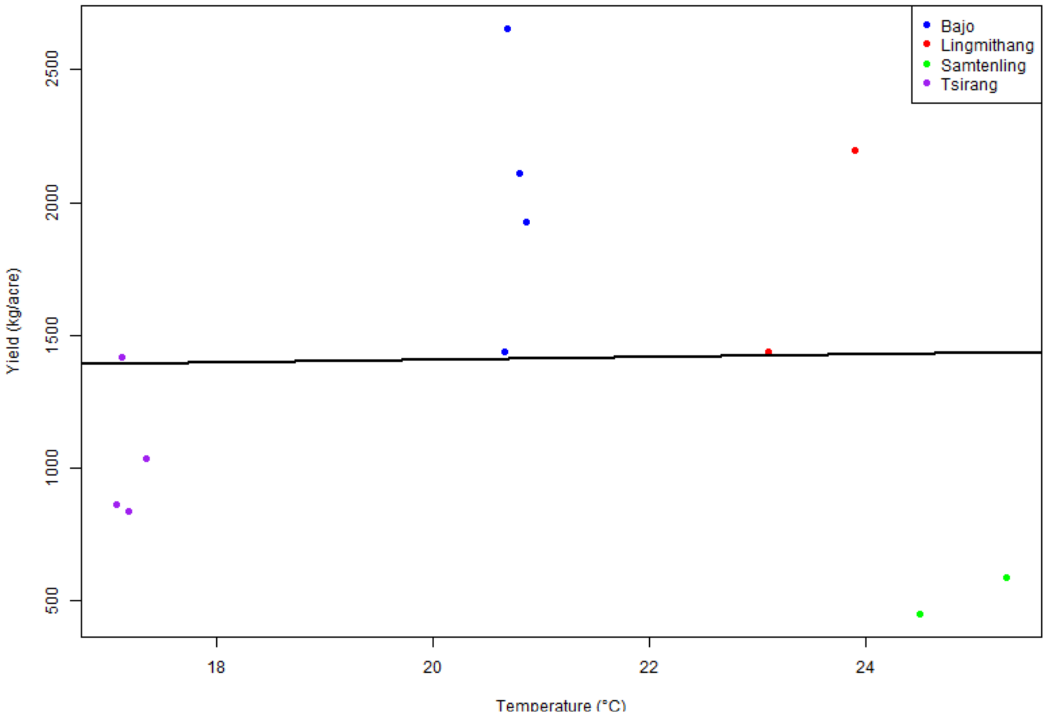


Figure 2. Scatter plot showing effect of temperature on yield

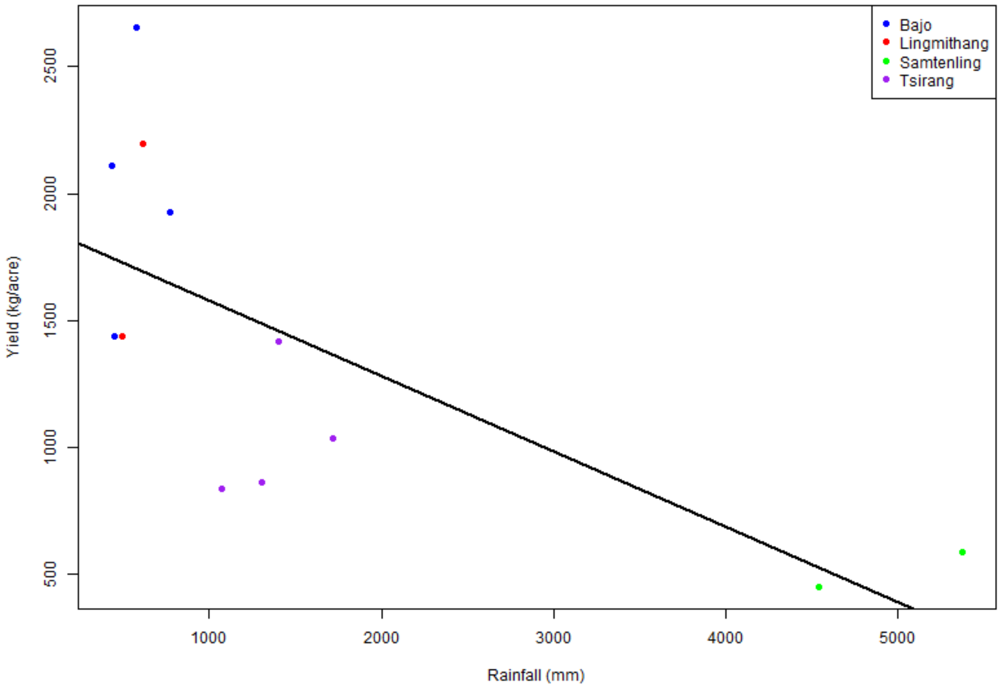


Figure 3. Scatter plot showing effect of rainfall on yield

3.1.7 Varietal Comparison (2024 RCBD)

The ANOVA result (Table 5) shows that significant differences were observed among the three varietal groups across most traits and locations. Black Rice generally produced fewer tillers per hill, for instance, 9.76 in ARDC Bajo and 6.13 in ARDSC Tsirang, compared to local varieties which reached up to 28.05 (Tsirang Zam) and improved varieties with 17.00 (BK3). Plant height of Black Rice was intermediate to high, such as 143.64 cm in Bajo and 142.23 cm in Samtenling, often taller than improved varieties like 128.12 cm (IR20913) and 87.33 cm (BK3), but shorter or comparable to local varieties such as 147.68 cm (Khamtey) and 183.11 cm (Tsirang Zam). Panicle length of Black Rice tended to be slightly shorter than local varieties (19.90 cm in Bajo vs. 23.46 cm for Khamtey) but similar to improved ones like 22.58 cm (IR20913).

In terms of yield, Black Rice consistently produced lower yields, 2655.90 kg/acre in Bajo and 1034.00 kg/acre in Tsirang, compared to local varieties which achieved up to 4181.10 kg/acre (Khamtey) and improved varieties with 2996.50 kg/acre (IR20913). The differences in yield were statistically significant across all locations ($P \leq 0.05$), highlighting that local and improved varieties consistently outyield Black Rice. Days to maturity for Black Rice were generally longer than improved varieties, such as 194 days in Bajo vs. 163 days for IR20913, but varied when compared with local cultivars (174 days for Khamtey).

These results are acceptable and consistent with the known characteristics of Black Rice. Its lower yield, prolonged vegetative phase, and tall stature are inherent unfavorable traits. Being a low-yielding crop (approximately 10% relative to other rice types; Bolay, 2021), Black Rice is naturally rarer and considered less productive under normal cultivation conditions.

Table 5. Comparative performance of black rice, improved, and local varieties across four locations in 2024

Location	Variety	Tillers/hill (Nos.)	Plant Height (cm)	Panicle Length (cm)	Days to Maturity	Yield (kg/acre)
ARDC Bajo	Black Rice	9.76^{a}	143.64^{a}	19.90^{b}	194	2655.90^{c}
	IR20913 (Improved)	14.32^{a}	128.12^{b}	22.58^{a}	163	2996.50^{b}
	Khamtey (Local)	15.12^{b}	147.68^{a}	23.46^{a}	174	4181.10^{a}
	P Value	0.0001	0.0000	0.0001	-	0.0000
ARDSC	Black Rice	6.13^{b}	92.73^{b}	20.66^{c}	187	1034.00^{c}

Tsirang	Wangkhar Ray Kaap-II (Improved)	7.06^{b}	67.60^{c}	23.06^{b}	172	1465.30^{b}
	Chorti (Local)	8.73^{a}	129.27^{a}	24.73^{a}	190	1180.30^{a}
	P Value	0.0048	0.0000	0.0000	-	0.0000
ARDSC Lingmithang	Black Rice	16.80^{b}	136.27^{b}	24.46^{b}	156	2199.5^{b}
	BK3 (Improved)	17.00^{b}	87.33^{c}	23.66^{b}	145	2776.6^{a}
	Tsirang Zam (Local)	28.05^{a}	183.11^{a}	30.62^{a}	150	1940.0^{c}
	P Value	0.0000	0.0000	0.0000	-	0.0000
ARDC Samtenling	Black Rice	10.66^{a}	142.23^{a}	22.31^{ab}	148	452.12^{b}
	Bhur Raykaap 2 (Improved)	9.26^{ab}	114.30^{c}	24.00^{a}	148	585.83^{c}
	Kalo Timburay (Local)	7.06^{b}	121.20^{b}	21.53^{b}	148	322.74^{a}
	P Value	0.0961	0.0003	0.0896	-	0.0068
	*Means followed by same letters are not significant different					

3.2 Performance of black rice in the farmers field in 2024

After analyzing the multi-year production trial data, it was observed that black rice performed relatively well at ARDC-Bajo. Given this performance in the dry subtropical region the evaluation was further extended in 2024 to farmers' fields to assess its performance under actual field conditions, particularly in the Bajo area at an altitude of around 1200 masl, encompassing Wangdue and Punakha districts.

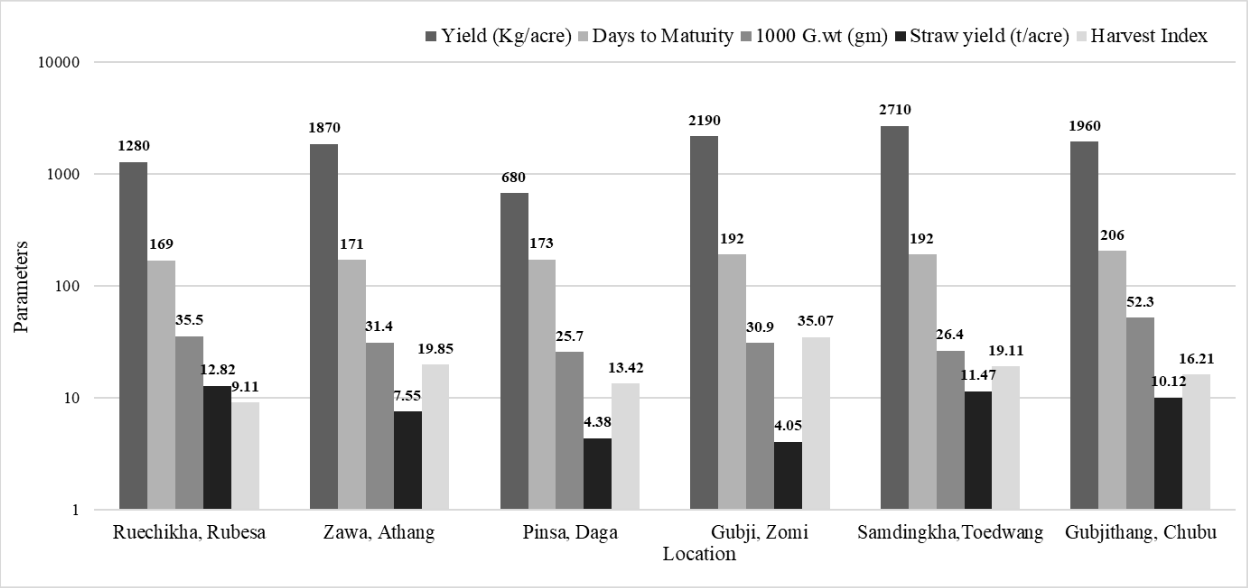


Figure 4. Yield, Days to Maturity, 1000-Grain Weight, Straw Yield, and Harvest Index in farmer's field

The result (Figure. 4) shows that yield of Black Rice across farmers' fields varied widely. Zawa, Athang exhibited the highest number of tillers per hill (11.67), and longest panicle length (23 cm), indicating better vegetative growth and panicle development relative to the other evaluated locations (Figure 5). The highest yields were recorded in Punakha valley sites such as Samdingkha (2710 kg/acre) and Gubji (2190 kg/acre), suggesting that the relatively warm and favorable valley conditions supported better grain filling and productivity. In contrast, yields in sites like Pinsa (778.55 masl) remained low (680 kg/acre), reflecting poor tillering but highest plant height (174.98).

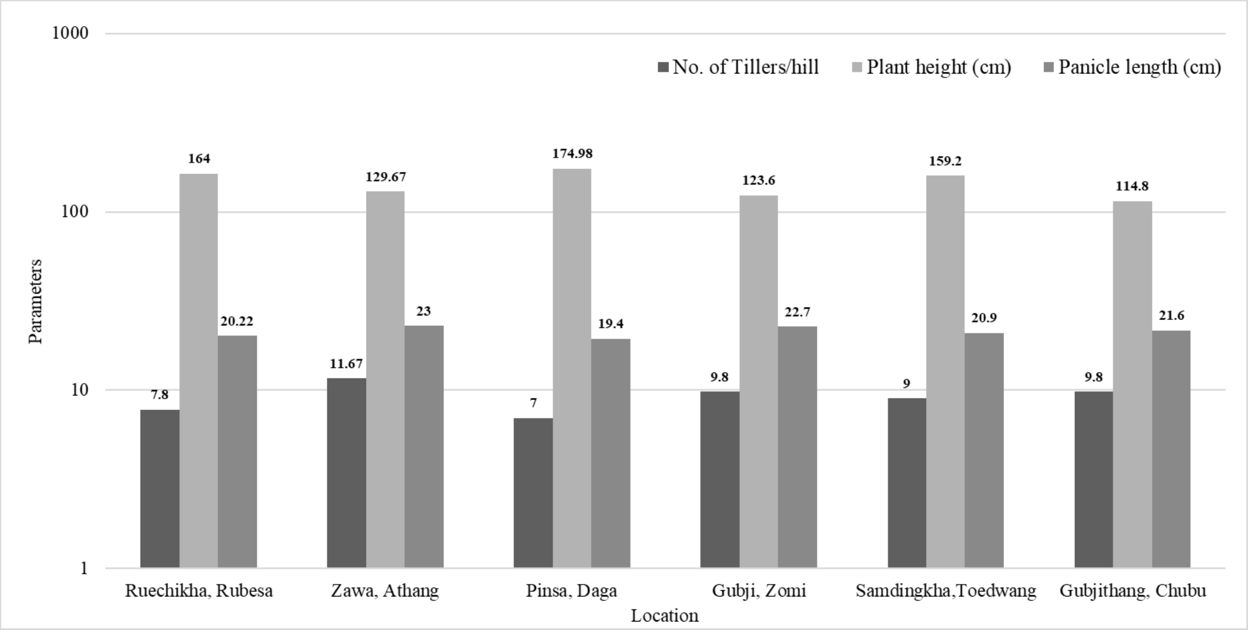


Figure 5. No. of tillers/hill, Plant height, Panicle length in farmer's field

These results highlight the strong influence of location and environment on Black Rice yield. While Punakha sites showed better adaptation, Wangdue Phodrang sites were less productive. Overall, the yield levels observed in farmers' fields (Samdingkha, Toedwang 2710 kg/acre) confirm that the yield were at par to the research station at ARDC- Bajo (2655.9 kg/acre).

3.3 Proximate analysis of black rice vs white rice

Table 6: Comparing the nutrient analysis of black rice with white rice

Sample ID	Total solids	Total ash content	Crude protein	Crude Fat	Total Carb	Energy	N	P	K	Ca	Fe	Vitamin C
Unit	%	%	%	%	%	Kcal/100g	%	ppm	mg	mg	ppm	mg/100g

Black rice	88.14	2.63	8.94	1.75	74.81	350.81	1.43	0.02	8.60	9.00	0.11	0.02
Bk3	96.73	1.22	7.17	0.96	87.38	386.82	1.15	0.01	7.10	9.00	0.19	0.01

The result shows differences in proximate and nutrient composition between black rice and white rice (Bk3). Black rice showed lower total solids but higher ash content than white rice, indicating greater mineral presence. Crude protein and crude fat were also higher in black rice than in white rice. Similar findings have been reported by Abd and Shehata (2010), who observed higher ash, protein, and oil contents in black rice compared to white rice. White rice recorded higher iron content, total carbohydrate content, and energy value (386.82 kcal/100 g) than black rice (350.81 kcal/100 g). Similar findings were reported by Ojha, Mogra, and Kachhawa (2025). Black rice showed higher contents of nitrogen, potassium, phosphorus, and vitamin C, while calcium levels were similar. These findings align with previous reports indicating that, compared to white rice, black rice is richer in minerals such as zinc, iron, phosphorus, and manganese, as well as vitamins including B-complex and E, although vitamin C and calcium were not assessed in that study (Rahim et al., 2022). Overall, the results indicate that black rice provides a more nutrient-dense composition compared to white rice, while white rice serves primarily as an energy source.

3.4 Limitations of studies

- Single-plot data: Observations were collected from a single plot per location, which may limit the generalizability of the findings.
- Limited year coverage: Trials were not conducted uniformly across all years for each location.

4 Conclusion

This study showed that black rice performs best in dry sub-tropical regions such as Bajo (1200 masl), where temperatures and rainfall during the growing season ranged from 10.4–28.7 °C and 557 mm, respectively. It also showed good performance in humid sub-tropical areas like Lingmethang (650 masl), with growing season temperatures of 12.8–34.1 °C and rainfall of 553.4 mm, where relatively higher yields were obtained. Although Tsirang (1480 masl) falls under the dry sub-tropical zone, its higher elevation and other climatic factors likely contributed to its relatively lower performance, while Samtenling (370 masl, wet sub-tropical) exhibited poor adaptation.

These evaluations demonstrate that it can adapt well beyond southern belt, highlighting its strong potential for expansion into other suitable regions of Bhutan. Although black rice is known for certain inherent unfavorable traits, particularly low yield, the evaluation indicates that it has good potential if cultivated under recommended or suitable agro-climatic conditions.

Table 7. Recommended growing season of black rice in different agro-ecological zones

Agroecological zone	Altitude (masl)	Nursery time	Harvest
Dry Subtropical	1200-1800	May	November
Humid Subtropical	600-1200	July	December
Wet Subtropical	100-600	June	November

The cultivation of black rice has been successfully demonstrated to farmers through on-farm trials and can be adopted across suitable agroecological zones, as its package of practices is similar to that of other rice cultivars. Consequently, it has been officially released for cultivation under the recommended growing period (Table 7).

The crop was newly introduced in the trial stations, and it was encouraging to note that farmers were highly receptive to its cultivation. In addition, with the growing trend toward healthier eating habits, black rice fits well as a nutrient-dense and visually distinctive alternative to traditional white rice. Its unique characteristics make it particularly attractive to health-conscious consumers and those seeking premium, specialty food options. Moreover, its lower productivity contributes to higher market prices, restricting access to this nutrient-enriched rice for the general population (Kumari, 2020). However, the market and pricing for black rice in the country remain largely unknown. To fully realize its potential, cultivation efforts should be complemented with proper promotion at the farmers' level, alongside market assessment and strategic planning. Introducing black rice as a nutritious health food could enhance the food and nutritional security of the Bhutanese population. In addition, establishing marketing channels is essential to ensure that farmers can benefit economically from its cultivation.

5 Acknowledgment

The authors would like to sincerely thank the researchers from the collaborating agencies for their timely support, which enabled the successful completion of this study. We also extend our gratitude to all the agricultural extension officers for their cooperation and assistance in ensuring the smooth conduct of the on-farm trials.

6 Authors’ contribution statement

Deki Lhamo was responsible for the implementation of the research and data collection at on-farm Bajo and farmers’ fields, as well as the analysis and interpretation of results and preparation of the draft manuscript. Cheku Dorji contributed to the implementation of the research and data collection at Bajo. Dolay was involved in the implementation of the research and data collection at Tsirang. Sonam Deki contributed to the implementation of the research and data collection at Lingmethang. Chezang Dendup was responsible for the implementation of the research and data collection at Samtenling.

7 References

Abd El-Rahman, S. N., & Shehata, W. M. (2010). Chemical evaluation of black rice compared with white and brown rice. *Journal of Food and Dairy Sciences, 1*(4), 143–149.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software, 67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Borah, N., Athokpam, F. D., Semwal, R. L., & Garkoti, S. C. (2018). Chakhao (Black Rice; Oryza sativa L.): A culturally important and stress tolerant traditional rice variety of Manipur. *Indian Journal of Traditional Knowledge*, *17*(4), 789-794.

Bolay. (2021). *Everything you always wanted to know about forbidden black rice*. https://www.bolay.com/blog/everything-you-always-wanted-to-know-about-forbidden-black-rice

Chandrika, S. S., Sree, P. S., Basha, J. S., & Vani, M. P. (2024). Yield and economics of black rice as influenced by integrated nutrient management practices. *Biological Forum - An International Journal, 16*(2), 106.

International Center for Tropical Agriculture (CIAT), & World Bank. (2017). *Climate-smart agriculture in Bhutan: CSA country profiles for Asia series*. International Center for Tropical Agriculture and World Bank.

Das, M., Dash, U., Mahanand, S. S., Nayak, P. K., & Kesavan, R. K. (2023). Black rice: A comprehensive review on its bioactive compounds, potential health benefits and food applications. *Food Chemistry Advances, 3*, 100462. https://doi.org/10.1016/j.focha.2023.100462

Department of Agriculture. (2024). *Inventory of released and de-notified crops in Bhutan (1988–2024)*. Ministry of Agriculture and Livestock, Royal Government of Bhutan. https://www.doa.gov.bt

Ghimiray, M., Pandey, S., & Velasco, M. L. (2013). *Tracking of improved varieties in South Asia: Bhutan report on rice estimating adoption rate of modern rice varieties in Bhutan.*

Herliana, O., Widiyawati, I., & Hadi, S. N. (2019). The effect of stable manure and seedling number on growth and yield of black rice (*Oryza sativa* L. indica). *Caraka Tani: Journal of Sustainable Agriculture, 34*(1), 13–21. https://doi.org/10.20961/carakatani.v34i1.27098

Ito, V. C., & Lacerda, L. G. (2019). Black rice (*Oryza sativa* L.): A review of its historical aspects, chemical composition, nutritional and functional properties, applications, and processing technologies. *Food Chemistry, 301*, 125304. https://doi.org/10.1016/j.foodchem.2019.125304

Khanum, M. T., Monira, M. S., Hoque, M. A., Rai, P., & Shifa, M. F. (2023). Comparison of growth and yield performance of rice varieties under three agro-ecological zones. *Cognizance Journal of Multidisciplinary Studies, 3*(9), 220–250.

Katwal, T. B., & Bazile, D. (2020). First adaptation of quinoa in the Bhutanese mountain agriculture systems. *PLOS ONE, 15*(1). https://doi.org/10.1371/journal.pone.0219804

Khadka, R. B. (2016). Evaluation of Black rice under different fertilizer doses in western Terai, Nepal. *Agronomy Journal of Nepal*, *4*, 136-141.

Kumari, S. (2020). Black rice: An emerging ‘super food’. *Pantnagar Journal of Research, 18*(1), 1–5.

Lenth, R. V. (2024). *emmeans: Estimated marginal means, aka least-squares means* (R package version 1.10.6) [Computer software]. Comprehensive R Archive Network. https://CRAN.R-project.org/package=emmeans

Menardo, D. V., Percival, O. C., Nathaniel, T. L., Daveson, M. C., & Jackson, A. (2024). Adaptability trial of black rice (*Oryza sativa* L.) under Conner, Apayao condition. *Journal of Rice Research and Developments, 6*(1). https://doi.org/10.36959/973/448

Metwally, T. F., El-Habat, H. B., El-Malky, M. M., & Barakat, A. S. (2014). Comparative studies for grain yield, grain quality, cooking quality and nutritional value traits of black rice variety. *Journal of Plant Production*, *5*(3), 401-414.

Nandariyah, Sukaya, Purnomo, D., Sutarno, Yuniastuti, E., & Az-Zahra, C. D. A. (2023). Study of black rice parents’ performance and the crossing ability. *Caraka Tani: Journal of Sustainable Agriculture, 38*(1), 65–74. https://doi.org/10.20961/carakatani.v38i1.60245

National Statistics Bureau. (2024). *Integrated agriculture and livestock census of Bhutan 2023*. Royal Government of Bhutan. https://www.nsb.gov.bt

National Soil Services Centre (NSSC). (2001). *Technical report on the detailed soil survey of Bhur Farm, Gelephu; Sarpang*. Department of Agriculture, Ministry of Agriculture and Livestock, Royal Government.

National Soil Services Center (NSSC). (2003). *Technical report on the detailed soil survey of RNR Research Sub-Centre, Lingmethang, Mongar*. Department of Agriculture, Ministry of Agriculture and Livestock, Royal Government.

National Soil Services Center (NSSC). (2004). *Technical report on detailed soil survey of Bajo RNR-RC, Bhutan*. Department of Agriculture, Ministry of Agriculture and Livestock, Royal Government.

Ojha, K., Mogra, R., & Kachhawa, K. (2025). Nutritional comparison of boiled black and white rice: Proximate composition, mineral content, and sensory evaluation of the product. *Ecology, Environment & Conservation, 31*. https://doi.org/10.53550/EEC.2025.v31i01s.062

Panda, D. K., Jyotirmayee, B., & Mahalik, G. (2022). Black rice: A review from its history to chemical makeup to health advantages, nutritional properties and dietary uses. *Plant Science Today, 9*(2), 199–214. https://doi.org/10.14719/pst.1817

Pratiwi, R., & Purwestri, Y. A. (2017). Black rice as a functional food in Indonesia. *Functional Foods in Health and Disease, 7*(3), 182–194.

Purwanto, E., Hidayati, W., & Nandariyah. (2018). The yield and quality of black rice varieties in different altitude. *IOP Conference Series: Earth and Environmental Science, 142*(1). https://doi.org/10.1088/1755-1315/142/1/012037

Rahim, M. A., Umar, M., Habib, A., Imran, M., Khalid, W., Lima, C. M. G., Shoukat, A., Itrat, N., Nazir, A., Ejaz, A., Zafar, A., Awuchi, C. G., Sharma, R., Santana, R. F., & Bin Emran, T. (2022). Photochemistry, functional properties, food applications, and health prospective of black rice. *Journal of Chemistry, 2022*, 2755084. https://doi.org/10.1155/2022/2755084

Sah, S. K., & Kushwaha, U. K. S. (2016). Book review: Black rice: Research, history, and development. *Advances in Plants & Agriculture Research, 5*(1), 443. https://doi.org/10.15406/apar.2016.05.00165

Sangma, R. R., Manpoong, C., Sharma, A., Devadas, V. S., Singh, D., & Pandey, H. (2022). Performance of black rice (*Oryza sativa*) varieties grown in Namsai district of Arunachal Pradesh, India. *Research on Crops, 23*(1), 11–14. https://doi.org/10.31830/2348-7542.2022.002

Sarker, D., & Pramanik, B. K. (2011). *Climatic impact assessment: A case study of Teesta Barrage Irrigation Project in Bangladesh*. https://www.researchgate.net/publication/282063859

Sharma, A., Sandal, A., & Sayyed, M. (2024). Pigmented rice: A source of health and longevity. *Annual Research & Review in Biology, 39*(7), 74–82. https://doi.org/10.9734/arrb/2024/v39i72102

Sofian, L., Aryana, I. G. P. M., & Kisman, K. (2019). Appearance of some black rice genotype (*Oryza sativa* L.) in two types of agroecosystems in the dried land of Central Lombok District. *International Journal of Multicultural and Multireligious Understanding, 6*(5), 742. https://doi.org/10.18415/ijmmu.v6i5.1152

Sudarmayanti, B. A., Muliarta, I. G. P., & Sudika, I. W. (2022). Agronomic appearance, genetic parameters of red and black rice (*Oryza sativa* L.) in different environments dry land. *Path of Science, 8*(7), 1–10. https://doi.org/10.22178/pos.83

Tahir, M., & Marwat, K. B. (2002). *Genetic variability of plant and yield characters in rice (Oryza sativa L.)*. https://www.researchgate.net/publication/231608668

Tiwari, H., & Patel, S. V. (2022). *Black rice cultivation in India*. https://www.researchgate.net/publication/362684704

Wangmo, C. (2019). *Characterization catalogue and pictorial varietal descriptions on traditional paddy varieties (Oryza sativa) conserved in the National Crop Genebank*. National Biodiversity Centre, Ministry of Agriculture and Livestock. https://www.biodiversity.bt

https://doi.org/10.55925/btagr.26.9108

Economic Analysis of Winter Chili Subsidy Program in Bhutan: A case study of Dagana and Sarpang Districts

Choney Zangmo[1], Kentaro Kawasaki[2] and Takeshi Sato[2]

Abstract

Chili plays a significant role in Bhutanese cuisine and is the most commonly consumed vegetable. While Bhutan produces an excess of chilies from April to November, production declines during the colder months from December to March (off-season). Previously, until 2016, the country relied on importing fresh chilies from India. However, due to concerns about chemical residue, the government imposed a ban on chili imports and introduced the winter chili subsidy program in 2017. This program aimed to promote self-sufficiency in chili production during the off-season and was implemented in the southern districts of Bhutan, situated between 200 and 1200 meters above sea level. By providing free inputs such as hybrid seeds, mulching plastic, greenhouses, water storage and conveyance equipment, the program successfully stimulated the domestic production of hybrid chili, which was previously non-existent. However, no studies have been conducted thus far to assess the effectiveness of the program. Therefore, the focus is on evaluating the program's impact at the household level, utilizing both descriptive and econometric methods with data from the Dagana and Sarpang districts. The findings indicate a positive trend in the quantity of subsidized inputs and an increase in the number of chili growers. Nonetheless, it is important to note that farmers do not receive sufficient quantities of these inputs. Statistical analysis using t-test reveals that male-headed households receive significantly higher quantities of certain inputs compared to their female counterparts. Furthermore, the regression analyses suggest that the program contributed to increased chili productivity, especially when farms receive enough quantity of seeds and mulching plastic, yields increased by 36-49% and 30-90%, respectively. Additionally, farmers reported that the subsidy program played an important part in expanding their cultivated areas.

Keywords: Winter Chili; Subsidy; Inputs; Productivity; Area; Gender

---

Corresponding email: *czangmo@moal.gov.bt*

[1] Department of Agriculture, Ministry of Agriculture and Livestock, Thimphu

[2] University of Tokyo, Japan

1 Introduction

Agricultural input subsidies are provided to increase crop productivity and household income and create jobs. It is also expected to keep the price of agricultural produce at a reasonable range for the benefit of customers. Agricultural subsidies are vital in developing countries where the majority of the population is dependent on agriculture as the source of livelihood (Hassan Danish et al., 2017).

Schwartz & Clements (1999) defines subsidy as any government assistance that (i) allows consumers to purchase goods and services at prices lower than those offered by a perfectly competitive private sector, or (ii) raises producers' incomes beyond those that would be earned without this intervention. Subsidies are termed differently like assistance, support, transfer, payments, and aids. Subsidies are given in cash or kind or both.

The authors state three important reasons to assess subsidies. The first, subsidy forms one of the major policy instruments of government expenditure. Secondly, at micro level, it affects the farm decisions, income distribution and reduce the flexibility of economy. Thirdly, on a global scale, it distorts the resource allocation by affecting competitiveness. There are evidences of existence of different form of subsidies in countries like India, China, Kenya, Zambia and Malawi to name few.

Subsidies in Bhutan's agriculture sector started with planned development in 1961 as a means to improve farm production and productivity. The farmers were given free seeds, farm tools, plant protection chemicals and fertilizers. These subsidies were gradually phased out with the development pace of the country. From 1998 onwards (beginning of 8th Five Year Plan), the subsidy was given only for producing improved planting materials and transportation of agriculture inputs. Commission of 10% on sales value for seeds and fertilizers is still given to Agriculture Sales and Service Representatives (Ministry of Agriculture and Forests [MoAF], 2021). Subsidy on transportation of seeds and fertilizers is still provided to maintain the same price of these inputs throughout the country. As of now, the majority of subsidies are free supply of inputs. Farmers receive various production input materials which are procured by the government, transported and distributed to them at their sub-districts/villages.

In Bhutan, subsidies are not explicitly termed as subsidy in the book of accounts, as in most countries. It is embedded in various developmental programs in the annual budgets across the governmental agencies at central, district and sub-district levels. So, it is difficult to get a complete picture of how much budget is spent on subsidy programs

Among food crops, chili occupies a uniquely important position in Bhutanese agriculture and food systems. It is consumed in almost every meal and is used primarily as a vegetable rather than a spice. Reflecting its cultural and nutritional significance, chili has been prioritized as one of the seven vegetables under the national food and nutrition security program (Department of Agriculture [DoA], 2022). Ueda & Samdup (n.d.) equated chili with household food security, given its indispensable role in Bhutanese cuisine. It is grown throughout the country, including Dagana and Sarpang districts. It is most commonly traded produce domestically and even exported during summer months. It is consumed as fresh, dried, blanched and dried, powdered, and pickled. It has remained as one of the highest produced vegetables till 2021 (Department of Agriculture [DoA], 2011-2016; Renewable Natural Resources Statistical Division [RNR-SD], 2017-2020). The most commonly grown landraces are suited for warm temperature and cultivated during the months of March to November.

Though chili in other forms is abundantly available year-round, the fresh produce becomes scarce during months of December to March as domestic production is constrained by cold temperature. Thus, December-March are termed as off-season. Until 2016, the demand for fresh chilies was met through imports from India. However, this changed after high pesticide level beyond maximum allowable limit was detected in it and two other vegetables during the routine inspection conducted by the erstwhile Bhutan Agriculture and Food Regulatory Authority, Ministry of Agriculture and Forests. It resulted in ban of these vegetables due to concern for public health safety (Department of Agriculture [DoA], 2018). Thus, the winter chili subsidy program was initiated as an urgent need to meet the domestic demand and secure self-sufficiency during the off season. Winter chilies in Bhutan are those that are produced between December to June next year. The varieties being produced are hybrids imported from India as the landraces cannot yield well during cold months. Though substantial government support has been provided for production of winter chili, no study has been carried out yet to assess the effect of the input subsidies on the chili productivity and area expansion at farm household level. While some literatures have investigated the impact of agricultural input subsidies, empirical evidence has largely focused on Sub-Saharan Africa, particularly Malawi (e.g. Arndt et al., 2016). Studies examining input subsidy programs for import substitution remain limited. Furthermore, in contrast to staple grains, the effectiveness of such programs for high-value vegetable crops has received little attention. This study addresses these gaps by evaluating the winter chili subsidy program in Bhutan.

2 Materials and Methods

2.1 Study Site

The study was conducted in two major winter chili-producing dzongkhags of Sarpang and Dagana districts (Figure 1). A total of 18 sub-districts were covered with nine sub-districts each, to get a representative of the whole districts as they differ in their location, accessibility and the development status. The sub-districts under Dagana were Dorona, Drujeygang, Karmaling, Karna, Larjab, Lhamoizingkha, Nichula, Tsangkha and Tsendagang. Under Sarpang, sub-districts of Chuzanggang, Dekiling, Gakidling, Gelephu, Samtenling, Senggye, Shompangkha, Tareythang and Umling were covered.

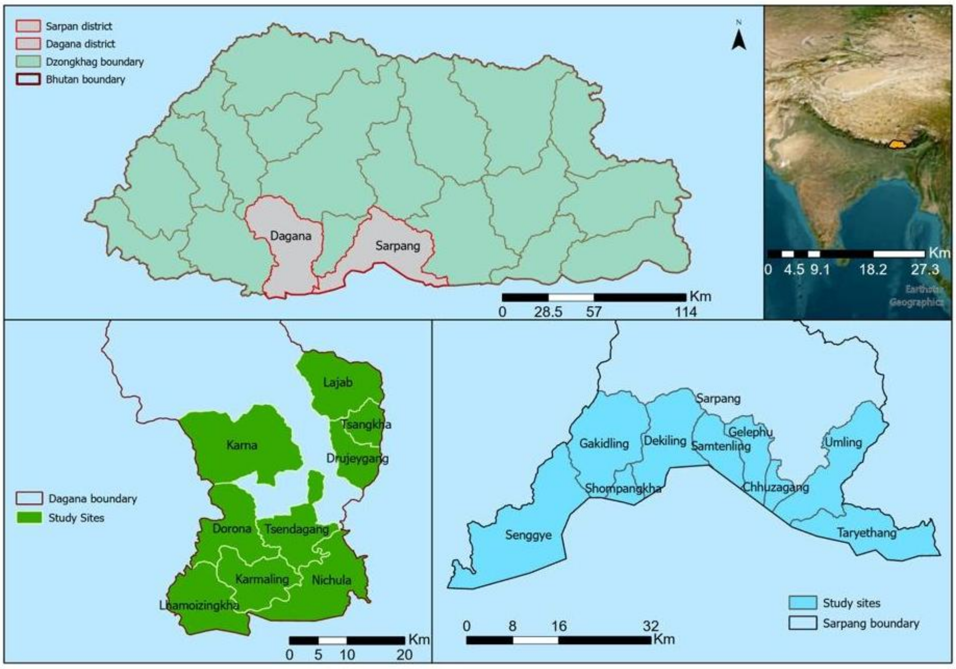


Figure 1. Study area showing Dagana and Sarpang and the sub-districts

Dagana lies between the altitude of 100-4000 meters above sea level (masl) and the main crop of the farmers are maize, vegetables and citrus. It consists of 14 sub-districts and only 11 sub-districts grew winter chili in 2021 as per the records maintained with the district agriculture office. There were 419 chili growers cultivating a total area of 216.2 acres.

Sarpang district lies within an altitude range of 100-1800 masl. It consists of 12 sub-districts, out of which 11 sub-districts grew winter chilies in 2021 except for Chudzom gewog, as per the records maintained with the district agriculture office. There were 678 farmers in total cultivating a total area of 264.55 acres. The main crop of Sarpang farmers is rice, vegetables and areca nut.

2.2 **Sampling method**

Within two districts, the sub-district and villages were selected purposively based on availability of the winter chili growers. As in the study by Huang et al., (2011), a probability proportional sampling was done to select total sample from the sub-districts as per the total chili growing households. Random sampling was done to select the chili growers within these sub-districts. The administrative data maintained with two districts of the chili growers' list was used as sampling frame. There were 419 households in Dagana and 678 households in Sarpang totaling to 1097 households. Yamane (1967:886) formula was used to calculate sample size at 95% confidence interval and 10% precision level as follows:

$$n = \frac{N}{1+N(e)^2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where:

n= Sample size, N= Population, e= Precision level

Therefore,

$$n = \frac{1097}{1+1097(0.1)^2} = 91.6 \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

So, 92 households were selected randomly in total, among which 34 farmers and 58 farmers were from Dagana and Sarpang districts, respectively.

2.3 **Data collection**

A total of 92 households were surveyed from the two districts using Google forms by the author in the months of September and October, 2022 for a period of 51 days. A semi-structured English questionnaire was used to interview the farmers by visiting their houses. The interview was conducted in the local dialects of *Dzongkha, Sharchop* and *Nepali* and answers were recorded in Google forms which were submitted by the end of each interview to the server. The responses were cross-checked later in the evening and clarification was sought in case of wrong or unusual entry via phone call to the respondents. Information on farm characteristics, land area, production and sale of chilies were collected. Detailed information on the input subsidies received from 2017 until 2020 was also collected.

In addition, nine Agriculture Extension Supervisors were also interviewed using semi-structured questionnaire to get their views regarding the program. Information on trend of inputs subsidy, the benefit and disadvantages of the subsidy and their views on crop replaced by chili were collected. The officials play a critical role in operationalization of the program as they are the closest representative of the government. Besides their other responsibilities, they also play a major role in the implementation of subsidy programs. In most cases, they make decisions regarding who would receive the subsidy.

Two officials involved in the winter chili program at Agriculture Research and Development Center (ARDC) of Yusipang in Thimphu and Smartening in Sarpang were also interviewed through open ended questions as expert interviews are known to generate huge and high-quality data (Wang et al., 2019). The objective was to find out the policies and directives for subsidy program implementation process. Huang et al., (2013) found that though there are set of criteria for the allocation of subsidies, the government however doesn't document the allocation process of the subsidies at grassroots level, which can have an impact on the achievement of the intended objective. Interviewing farmers and government officials working at different levels gave an insight into the views of the subsidy recipients and those of experts.

2.4 **Data Analysis**

Ideally, we should compare farms that received subsidies (the treated group) with those that did not (the control group) to estimate policy impacts. However, this approach is not feasible in our study region because all chili farms receive some form of subsidy, leaving no valid control group. Instead, we exploit variation in the type of input subsidy received. For instance, some farms received seed subsidies, while others received mulch subsidies. Focusing on this variation allows us to identify the differential impacts of alternative subsidy instruments.

Additionally, the households ventured into winter chili cultivation only because of the subsidy program and all those farms received some form of subsidy annually, throughout the cultivation period. So, there were no farms to compare the before and after subsidy scenario.

When the outcome variable is cultivated area, we use the change in area before and after subsidy receipt for each farm. This within-farm comparison helps control for time-invariant unobserved heterogeneity and yields more precise estimates of policy impacts. We do not apply the same before and after strategy to yield outcomes, as yields fluctuate substantially over time due to weather and other shocks, making such differences unstable and less informative.

The data cleaning, visualization and analysis was done using stata MP, version 17 and excel. During the analysis, it was found that one of the winter chilies growing farmers residing in the Sarpang district has leased in 30 acres of land. He was a former civil servant turned chili entrepreneur, taking opportunity of the subsidy program and the good price fetched by the produce. However, he was not a representative of typical farm household as he was a transient farmer. Due to his huge cultivated area, which is not usual, the influence on means of data was substantial. So, the outlier was removed and analysis was done for the rest of the 91 households. In addition to data analysis of the farm households, the views shared by the farmers and agriculture experts were used to validate the findings.

The data analysis was done using both descriptive and regression method as follows:

2.4.1 Descriptive analysis

The first part presents a descriptive analysis. We provide an overview of farm characteristics, land holdings, trends in input subsidies, gender, farmers' perceptions of input subsidies, and chili yields, using simple tables, figures, and mean comparisons based on *t*-tests.

2.4.2 Regression analysis

In the regression analysis, the Ordinary Least Square (OLS) was employed to estimate the determinants of area expansion and yield. Specifically, we will estimate the following model:

$$Y_i = c_0 + bX_i + u_i$$

where Y_i is an outcome variable of farm i, c_0 is the constant term, **b** is a vector of coefficients, **X** is a vector of explanatory variables, and u is the error term. We will use the heteroskedasticity-robust standard errors.

We consider two types of outcomes, which are, average yield in 2020 and 2021, and the area expansion of chili from 2020 to 2021. Yield is measured in logarithm to achieve better fit.

X includes input subsidies and farm characteristics. Input subsidies are the amount received in 2019, not 2020 or 2021. The year 2019 is selected for several reasons: First, some inputs, such as mulch and water tank, can be used for several years. Thus, 2019 input subsidy can affect yield or area expansion in 2020 or 2021. Second, there is an endogeneity concern if we use inputs received in 2020 or 2021. Farms who plan to expand area of cultivation will likely request more inputs, which result in reverse causality. Actually, we could not find meaningful results if we use input subsidies in 2020 or 2021.

Some input subsidies are classified into three groups based on the amount of received. For seed, it is classified as "below recommended rate", "within recommended rate", and "above recommended rate" if the amount of subsidy is less than 8 packets per acre, from 8 to 12 packets, and above 12 packets, respectively. For mulch, it is classified as "below recommended rate", "within recommended rate", and "above recommended rate" if the amount of subsidy is less than 4 rolls per acre, from 4 to 8 rolls per acre and above 8 rolls per acre, respectively.

3 Results and Discussion

3.1 Farm households' characteristics

From the total of 91 households, 59 households were headed by male (64.84%) and the rest 32 were female headed households. The main occupation of the majority of the household heads was farming (95.65%). The average age of farmers was 44.24 years, with youngest farmers age at 22 years and the oldest farmer being 73 years. The average family size was 2.48, which is less compared to the national average size of 4 people (Renewable Natural Resources Statistics Division, 2019). The smallest family had just one person, and the largest

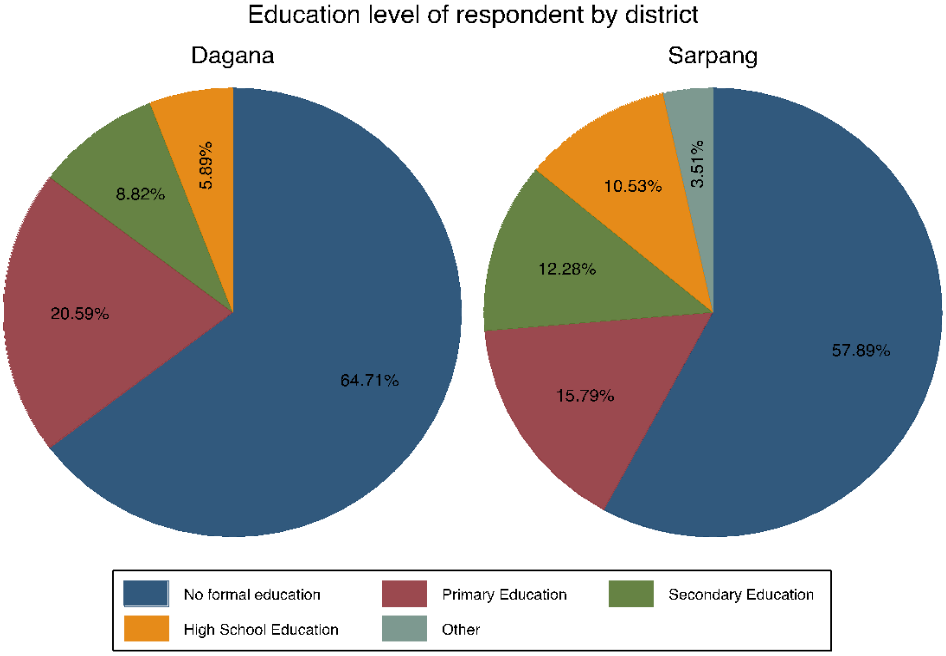


Figure 2. Different education level of the respondents

family had 7 members.

As reflected in the Figure 2, in total 61.3% of the farmers didn't receive any form of education. A total 8.21% of the respondents attended high school, the highest education level achieved in the sample survey. However, none of the female respondents attended high school. Primary education was the most common education received (18.19%) by the respondents. Compared to Dagana, Sarpang had 10.53% respondents who had a high school education which is 4.64% more. Overall, the district also had more respondents receiving some kind of formal education at 43.1% compared to 35.29% in Dagana district. The unique feature of Sarpang respondents was enrollment in non-formal education which constituted 3.51% of the respondents. The proportion of respondents achieving the highest education level as expected, was more in the younger age group at 25.09% to none with high school education above 55 years and above. From 32 female households, 65.63% were uneducated, and the highest level achieved was primary school education. In male households, the percentage of uneducated was lower at 58.63%.

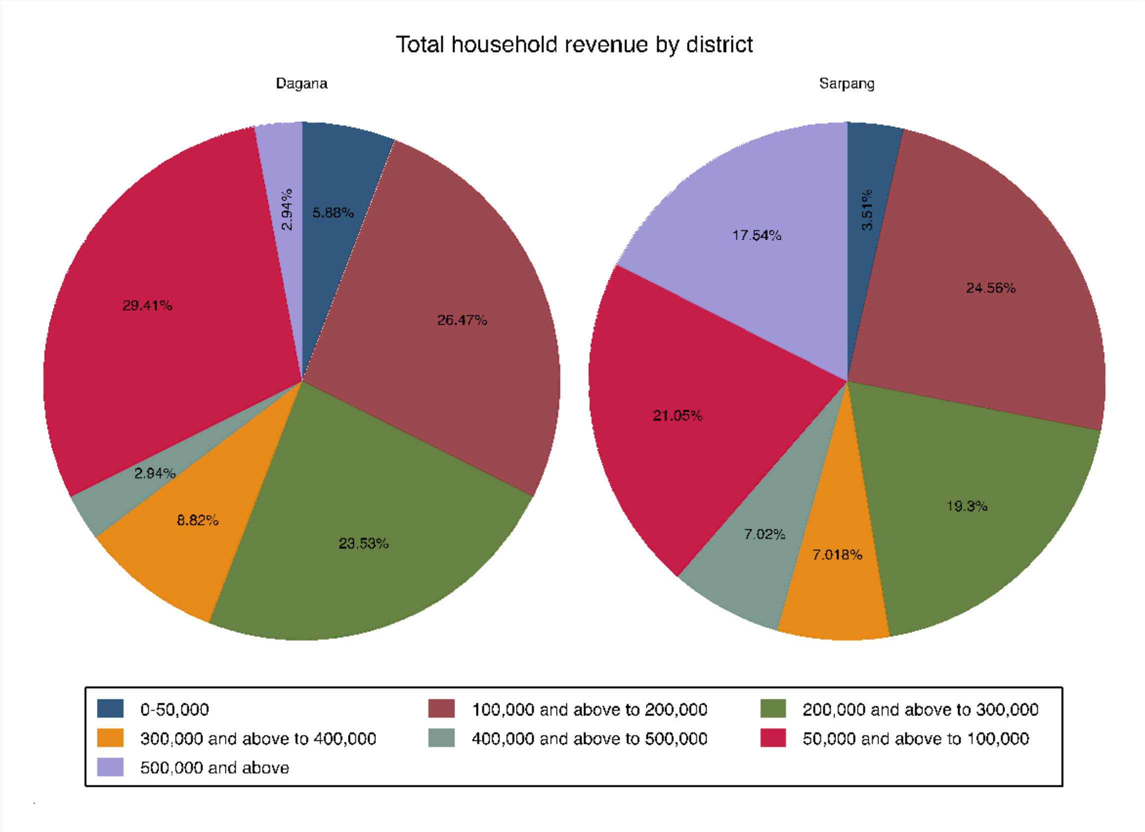


Figure 3. Total household revenue by district

The majority of the household's revenue (25.52%) fell between the range of 100,000 to 200,000 Bhutanese Ngultrum (BTN), followed closely by 25.23% in the range of 50,000-100,000 BTN as depicted in Figure 3. Dagana district had higher percentage of household (5.88%) in lowest revenue range of 0-50,000 BTN in comparison to Sarpang (3.51%). Sarpang district had higher percentage of household (17.54%), having a highest revenue range of 500,000 BTN and above compared to mere 2.94% in the Dagana district. So, households in Sarpang had more proportions of households with higher revenue.

3.2 Land ownership and cultivation area

The majority of farmers owned land between 3-5 acres (31 households) followed by 19 households, each owning land area of 1.5-3 acres and above 5 acres. The rest of the households had less than 1.5 acres.

The average cultivated area was 3.72 acres. On average, households in the Sarpang district cultivated 3.92 acres and Dagana cultivated 3.37 acres. In Sarpang, the highest cultivated area was 13 acres, and in case of Dagana, it was 10 acres. There was not much difference in average cultivated area between male and female headed households.

The average chili cultivated area was 0.37 acres in 2020 and 0.49 acres in 2021. The minimum cultivated area was 0.01 acres and the maximum was 4.2 acres in 2020. In 2021, the cultivated area ranged from 0.03 to 6 acres.

3.3 Input subsidy trend and household coverage

The subsidized input has been increasing for all inputs from 2017 to 2020, as shown in Figure 4. In 2017, households received 52.8 packet of seeds in total compared to 344.8 packets in 2020. Likewise, a total of 123.75 rolls of mulching plastic was received by the households in 2020 compared to 60.8 rolls in 2019. The trend is similar for other inputs. This can be due to increased awareness among farmers and improved planning and execution of the subsidy program. Interviews with sub-district officials also confirmed that there had been an increasing trend. The most common inputs among the households are hybrid seeds and mulching plastic. The least is water harvesting silpaulin sheet. Seeds and mulches are seen as essential and fundamental inputs. Constructing a water harvesting structure entails costs and labor from the farmers and this could be the reason for its unpopularity. It may also be because most farms have access to continuous water sources for irrigation, thus not requiring its storage.

The trend in increase of input subsidy kept pace with the increase in number of chili growers. The plausible reason could be increase in government budget for the subsidy program and also increased awareness among the farmers leading to more demand for subsidy. A similar trend was found in China whereby, the subsidy amount increased to 51.4 billion yuan compared to 100 million yuan in 2002 in an effort of the government to secure food and reduce income gap (Huang et al., 2011). However, in contradiction Hassan Danish et al. (2017) found the decrease in fertilizer subsidy trend in Pakistan after some period.

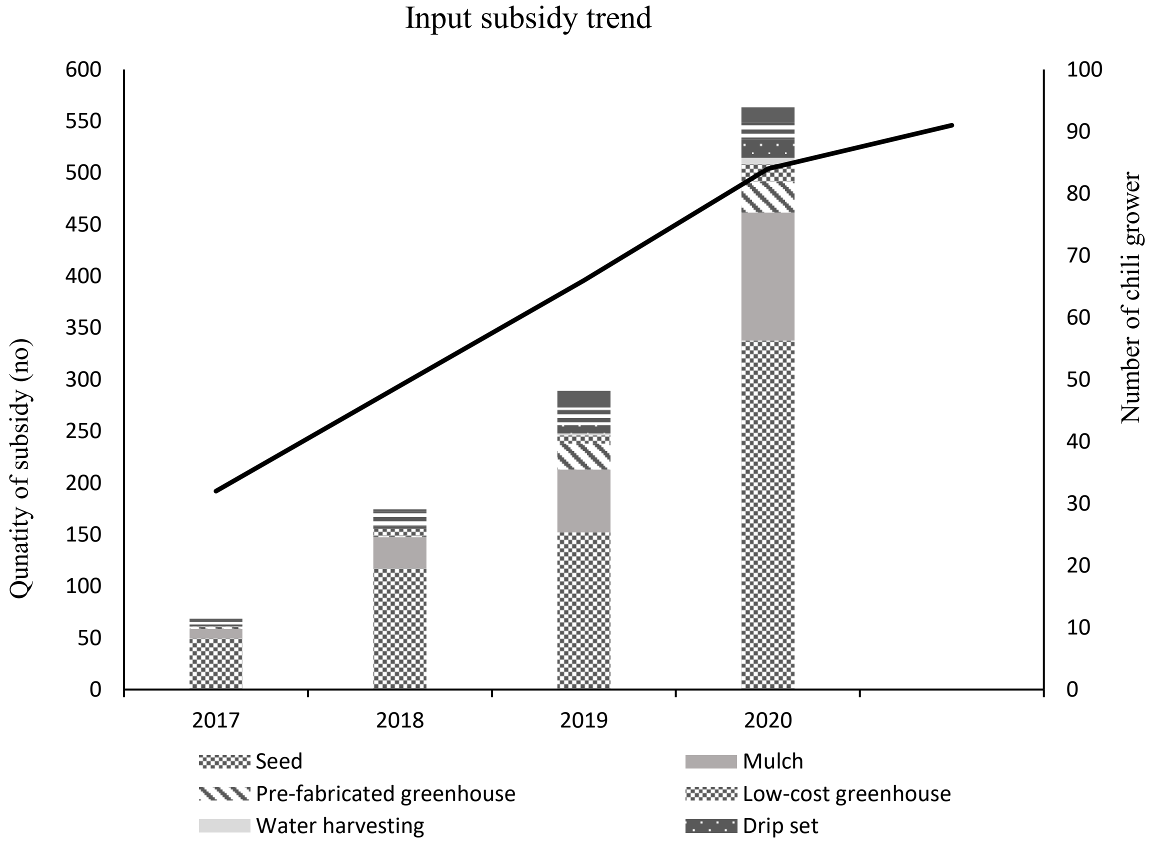


Figure 4. Input subsidy trend and number of chili growers

Note: The line represents input subsidy trend. The left y-axis shows quantity of input subsidy received, while the right y-axis number of farmers who received the subsidy.

3.4 **Difference in inputs by gender of household head**

Table 1 compares the amounts of input subsidies received by gender. With the exception of seeds, male-headed households receive larger quantities of subsidies than female-headed households. For instance, male-headed households received 3.6 rolls per acre of mulching plastic in 2019 compared to 1.8 rolls per acre by female-headed households. Likewise, male-headed households received 5.5 numbers of protected structures per acre compared to 1.8

numbers received by female headed households in the year 2020. *t*-tests indicate that these differences are marginally significant, with *p*-values at 0.1 and $p < 0.05$ respectively.

A similar pattern is observed for micro-irrigation, fencing net, water storage tanks, and prefabricated greenhouses. Although the differences are not always statistically significant, likely due to the small sample size, the consistency of these patterns suggests the presence of gender inequality in the selection of subsidy recipients.

Table 1. Comparison of inputs per acre between different gendered households

Variables	Female			Male			P_value	Star
	Mean	SD	n	Mean	SD	n		
Seed/acre (2020)	10.117	8.248	30	12.236	9.015	54	0.291	
Seed/acre (20)19	6.406	6.261	30	5.386	6.324	54	0.479	
Mulch/acre (2020)	5.317	5.109	30	7.576	7.212	54	0.133	
Mulch/acre (2019)	1.847	2.224	30	3.553	5.353	54	0.100	*
Drip/acre (2020)	0.217	0.806	30	1.542	4.729	54	0.133	
Drip/acre (2019)	0.067	0.365	30	0.356	1.170	54	0.191	
Green net/acre (2020)	0.728	2.030	30	1.589	4.898	53	0.362	
Green net/acre (2019)	0.622	2.172	30	1.129	4.609	53	0.572	
Water harvesting/acre (2020)	1.111	2.290	30	4.317	14.935	54	0.247	
Water harvesting/acre (2019)	0.778	2.109	30	3.542	14.830	54	0.314	
Greenhouse/acre (2020)	1.828	1.984	30	4.142	5.550	54	0.030	**
Greenhouse/acre (2019)	1.228	1.867	30	2.732	5.479	54	0.150	

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

3.5 Farmers' perception of input subsidy

Farmers were asked to choose the most crucial subsidy for chili crop cultivation among various input options, including seed, animal protection structures, protected structures, mulching plastic, training, and water conveyance equipment. Among these options, 29 farmers chose seed as the most vital input subsidy. One of the reasons given for this choice was the difficulty in accessing quality seeds. Mulching plastic was considered important by 18 farmers, while 16 farmers highlighted the significance of protected structures. Surprisingly, capacity-building training was considered the least important among the respondents, with only five farmers identifying it as a valuable support for chili cultivation, as indicated in Table 2. This could be attributed to the widespread diffusion of knowledge and skills among farmers within their own communities.

Table 2. Farmers choice of input subsidy

Important subsidy	**Freq.**	**Percent**
Animal protection structures	10	10.99
Protected structures	16	17.58
Mulching plastics	18	19.78
Not sure	1	1.1
Seed	29	31.87
Trainings	5	5.49
Water conveyance equipment	12	13.19
Total	91	100

3.6 **Yield**

As per the descriptive statistics, the average yield of 2020 and 2021 was 1098.01 kg/acre. The average yield was less in 2021 at 957.35 kg/acre compared to 1243.72 kg/acre in 2020. Nonetheless, the average yield in both the years in the two districts was above the national average of 900 kg/acre as shown in Figure 5. The average yield of Dagana was 1568.04 kg/acre and that of Sarpang was 891.86 kg/acre. So, the average yield of Sarpang was less by 75.82%.

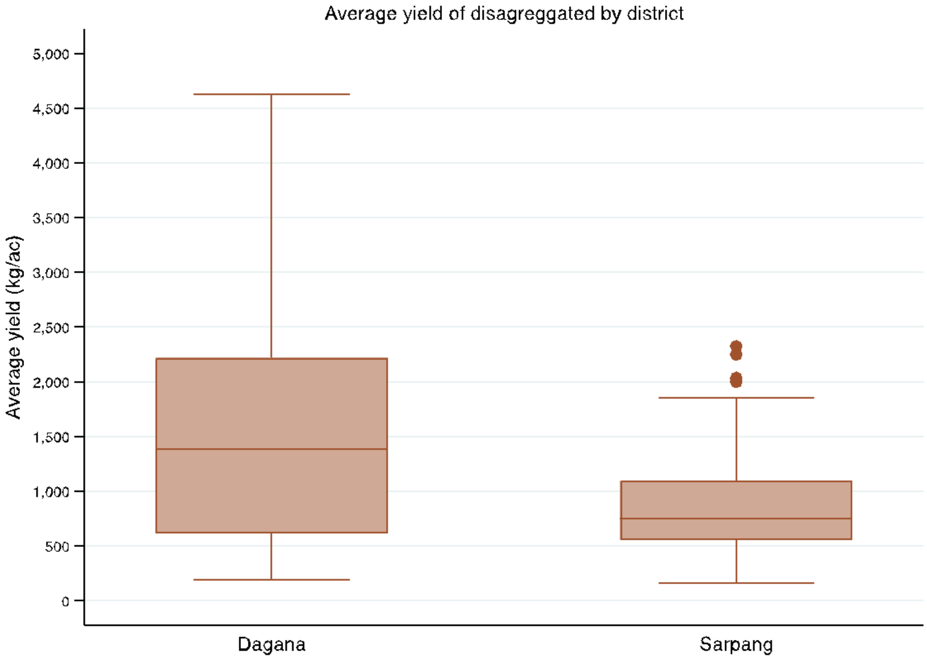


Figure 5. Difference of chili yield between two districts

The data presented in Figure 5 illustrates the distribution of household yields in Dagana and Sarpang. In Dagana, approximately 50% of households had yields ranging from 600 to 2200 kg/acre, while in Sarpang, the range was narrower, from 500 to 1100 kg/acre. The median yield in Dagana was around 1400 kg/acre, whereas in Sarpang, it was approximately 700 kg/acre. In Dagana, the upper quartile yield, representing the top 25% of households, ranged from 2200 kg/acre to a maximum of 4623.33 kg/acre. On the other hand, in Sarpang, the upper quartile yield ranged from 1100 kg/acre to a maximum of 1800 kg/acre.

3.7 Economic analysis of input subsidy under different levels of seed and mulch use

The economic analysis focuses only on seed and plastic mulch, as these subsidized inputs have a short economic life and require replacement within one to two production cycles. In contrast, other subsidized inputs (protected structure, drip and water harvesting tank) have a useful life of three years or more. So, restricting the analysis to annually replaceable subsidized inputs allow for a more accurate estimation of recurring production costs and returns attributable to the input subsidy.

The input use levels are defined solely by the quantity of subsidized inputs received and subsequently used by the households and are categorized as below recommended rate (benchmark), within recommended rate, and above recommended rate.

Incremental yield gains associated with higher levels of subsidized input use are derived from the regression analysis results, which estimate the marginal yield response to seed and mulch application while accounting for other independent variables too. These estimated yield differentials are used to compute gross revenue, additional returns, and benefit cost ratios under different subsidy use scenarios.

The results show that when production costs increased by Nu. 1260 for increasing the seed use from under the recommended level to within the recommended level, the additional revenue generated was Nu. 95,305.33 and generated a benefit cost ratio of 75.6 indicating that for every one ngultrum spent, it generated a revenue of Nu. 76. However, the benefit cost ratio decreases to 59.2 when the seed use increased above the recommended level, indicating lower return to cost compared to when seeds are used at recommended level though there is increase in yield. This is similar to the findings by Panhwar et al. (2011), of increase in the yield of sunflower with increasing seed rate. Likewise for the use of mulch within the recommended rate from that of below the recommended rate, the benefit cost ratio was 12.9. And unlike the seed use, the benefit cost ratio increased to 19.5 when used above the recommended level. The increase in benefit is due to the gain in productivity with increasing input use. So, this analysis indicates that benefit cost ratios improve consistently as farmers move from below to within and above recommended input levels of both seed and mulch, indicating strong economic efficiency of the winter chili subsidy program. Farmers utilizing subsidized inputs below recommended rates forgo significant potential income despite the availability of inputs at zero cost.

Table 3. Cost benefit analysis of subsidized seed and mulch under different levels of use

Cost						Benefit					
Input type	Quantity (gm/roll per acre)	Level	Input price (Nu/gm)	Cost (Nu/acre)	Additional cost (Nu/acre)	Yield (kg/acre)	Yield (% change)	Output price (Nu/kg)	Revenue (Nu/acre)	Additional revenue (Nu/acre)	Benefit Cost Ratio
Seed	42	Below RR	45	1890	0 (Benchmark)	1244	(average yield)	214	266216	0 (Benchmark)	(Benchmark)
	70	Within RR	45	3150	1260	1689.35	35.80%	214	361521.3	95305.33	75.6
	91	Above RR	45	4095	2205	1853.56	49%	214	396661.8	130445.84	59.2
Mulch	3	Below RR	2070	6210	0 (Benchmark)	1244	(average yield)	214	266216	0 (Benchmark)	(Benchmark)
	6	Within RR	2070	12420	6210	1617.2	30.40%	214	346080.8	79865	12.9
	9	Above RR	2070	18630	12420	2376.04	91.10%	214	508472.6	242257	19.5

3.8 Regression analysis

The regression results are presented in Table 4. The number of observations was 91 after dropping missing observations. It should be noted that, in the sample used in this study, all farmers received some form of subsidy. Therefore, the results of the analysis do not represent the effects of subsidies compared to receiving no subsidy, but rather the relative effects across different types of subsidies. Consequently, an insignificant coefficient should not be interpreted as indicating that a subsidy has no effect; instead, it should be interpreted as indicating no differential effect compared with other types of subsidies.

For area expansion, none of the variables are statistically significant, likely due to the small sample size though the farmers reported a total area expansion of 24.07 acres due to the subsidies. Nevertheless, the t-statistics for male-headed households and distance to the market are relatively large, suggesting that these variables are marginally insignificant. The signs of the coefficients indicate that male farmers are more likely to expand the cultivated area of chili, whereas farmers located in more remote villages are less likely to do so. Among the subsidy variables, the t-statistics for seed provision are relatively large, indicating that farmers who receive more seeds are more likely to expand their cultivated area, compared to farms who receive other types of subsidies. Thus, it is comparable to findings of significant positive effect of subsidies on allocation of land to targeted crops (Chibwana et al., 2012; Demirdogen et al., 2022; Yi et al., 2015). In contrast, Huang et al. (2011) found that there was no change in the area under grain cultivation though farmers received grain subsidies. Smale & Thériault (2022) also found that subsidy didn't have any effect of area under cowpea when it was planted as main crop and it reduced the area under crop when it was gown as intercrop.

In the yield regression, many input subsidy variables are significant. First, both seed variables are positive and statistically significant. This is similar to the findings of significant positive impact of subsidy on crop yield (Ali et al., 2019; Chibwana et al., 2010; Denning et al., 2009; Karamba & Winters, 2015; Shively, G. E., & Ricker-Gilbert, J, 2013). The positive impact of seed for increase in productivity can be attributed to the higher quality of hybrid seeds, which are pure and has high germination ability and produces true to its type plants. Since yield is measured in logarithmic form, the coefficients can be interpreted as percentage changes. The yield increased by 36% and 49% when farms use the recommended rate and above the recommended rate, respectively. Similarly, mulch increased the yield by 30% to 90%, depending on the amount received. Protected structures, such as greenhouse also increased yield by 3.6%. The coefficient on the number of water storage tanks is negative, which is counterintuitive. This negative sign likely reflects underlying water scarcity. When water availability is insufficient, yields decline and farmers are more likely to request water storage tanks.

In yield model, farm characteristics are not statistically significant, however, relatively large t-statistics are observed for "Chili is the main crop" and "Sarpang." The former likely captures farming skills, as farms specializing in chili production tend to have greater experience and management capacity, resulting in higher yields. The latter reflects regional differences, as shown in the previous section, chili yields in Sarpang are substantially lower than those in Dagana. The average yield for two years of Sarpang district was 891.86 kg/acre compared to 1568.04 kg/acre in Dagana. The factors cited by the agriculture experts for high productivity in Dagana were raising of chili nurseries in plug trays/plastic cups and inside the greenhouses, ensuring high survival rate and higher productivity as roots don't get disturbed while transplanting. In Sarpang, most farmers kept nurseries on the beds or seeds sown in some boxes and sheltered under the house roofing. Overall, the regression results suggest that providing sufficient input subsidies, particularly seeds and mulch can increase yield and, to some extent, cultivated area, thereby contributing to higher domestic production.

Table 4. Regression results

	Area expansion	**log(yield)**
Subsidy received in 2019		
Seed: Within recommended rate (dummy)	0.121	0.358
	[1.43]	[2.15]**
Seed: Above recommended rate (dummy)	0.187	0.49
	[1.33]	[2.92]***
Mulch: Within recommended rate (dummy)	0.09194	0.304
	[0.77]	[1.68]*
Mulch: Above recommended rate (dummy)	-0.02221	0.911
	[-0.15]	[2.35]**
Protected structure (number/acre)	-0.01156	0.036
	[-1.31]	[2.33]**
Water storage tank (number/acre)	-0.002	-0.014
	[-1.27]	[-3.61]***
Farm characteristics		
Altitude (m)	0.000	0.000
	[-0.61]	[-0.10]
Male HH head (dummy)	0.196	-0.061
	[1.66]	[-0.31]
Age of respondent	0.003	-0.006
	[0.42]	[-0.82]
Educated (> 7 years) (dummy)	0.015	-0.108
	[0.12]	[-0.72]
Family members engaged in farming	0.001	-0.08
	[0.01]	[-1.03]
Years into chili cultivation	-0.001	-0.002
	[-0.09]	[-0.10]
Chili is main crop (dummy)	0.05	0.299
	[0.21]	[1.49]
Distance from the market: 15 to 20 km (dummy)	-0.189	-0.115
	[-1.66]	[-0.40]
Distance from the market: > 20 km (dummy)	-0.053	0.07
	[-0.50]	[0.40]
Sarpang (dummy)	0.13	-0.301
	[1.04]	[-1.48]
Constant term	**0.248**	**7.03**
	[0.75]	[17.06]***
Observations	82	82
R-squared	0.157	0.351

Note: Robust t-statistics in brackets *** p<0.01, ** p<0.05, * p<0.1.

4 Conclusion

The Subsidy program in chili increased the productivity and simultaneously reduced the cost of inputs. The households cultivating chili as the main crop were able to generate higher revenue. Though there was no difference in effect of different subsidized inputs on chili cultivated area expansion, there are evidences from farmers' report that subsidy did contribute to area expansion. The domestic production has been partially able to fulfill the demand for fresh green chilies during the months of December to March, consistent with the finding by Jayne & Rashid (2013) in ability of the subsidy program to substitute imports and raise food self-sufficiency. So, it can be stated that the program objective of self-sufficiency has been partially achieved. However, there is a need of other supports like effective measures to control pests and diseases and proper nursery management to increase the productivity as the average yield is much lower than the potential yield of 5000 kg/acre achieved in on-station farms. There are still challenges in producing sufficient quantities that need to be addressed through proper research and development (R&D).

There was difference in quantities of subsidized inputs received per acre between the different gendered households. The male households received more quantities of mulch and prefabricated greenhouse compared to the female headed households. Despite the fact that Bhutan has more female farmers than males working in agriculture, the chili subsidy program benefitted male headed households more. It is evident from this study that they were not only able to access more subsidized inputs but also earned more revenue from chili than their female counterparts. So, a better targeted program would contribute to higher total production through enhanced production and productivity of the female farmers. This finding is important for consideration when similar subsidy program is planned in future.

Most studies concluded that public investment effectively raises agricultural productivity compared to subsidies in Asia (Akber & Paltasingh, 2019; Jayne et al., n.d.; Jayne & Rashid, 2013). Thus, it is recommended that spending should shift from that of subsidy to investment in crop R&D and infrastructure development. There is a trade-off between subsidies and investment due to limited resources. Accordingly, Bhutan's government also needs to formulate a proper exit strategy for this program.

Lastly, the study on agricultural input subsidies for cash crops in Asia remains limited, so this study makes a substantial contribution not only to Bhutan's local policy but also to the broader research literature.

There are two main limitations in this study. First, the data collected on yield fell during the pandemic time (2020 and 2021). As also mentioned by the farmers, the yield estimates might be underestimated since portions of the harvest were lost due to complete breakdown of market chain. Second, small sample size could result in imprecise estimates in the regression results. With a small sample, the regression has low power to detect true effects. As a result, variables that truly matter may appear statistically insignificant ("false negative").

5 Acknowledgement

We would like to thank the Director and Chiefs of Department of Agriculture, District Agriculture Officers, and Extension Officials of Dagana and Sarpang districts for their support rendered during my data collection. We are also grateful to all the farmers who participated in my survey. Additionally, our gratitude to the agriculture experts in the Vegetable Program and researchers of Agriculture Research and Development Center of Samtenling and National Center for Organic Agriculture, Yusipang for sharing their expert views.

The author would also like to thank the JDS for funding this research. Without the fund support, this work wouldn't have been possible.

6 Author's contribution statement

Choney Zangmo was responsible for conception and design of the study, implementation of research and data collection, analysis and interpretation of result, while Kentaro Kawasaki and Takeshi Sato provided guidance and support in use of econometric models, verification of result and review of the manuscript.

7 References

Arndt, C., Pauw, K., & Thurlow, J. (2016). The economy-wide impacts and risks of Malawi's farm input subsidy program. *American Journal of Agricultural Economics, 98*(3), 962-980. https://doi.org/10.1093/ajae/aav048

Akber, N., & Paltasingh, K. R. (2019). Investment, subsidy and productivity in Indian agriculture: An empirical analysis. *Agricultural Economics Research Review, 32*(conf), 13. https://doi.org/10.5958/0974-0279.2019.00014.4

Ali, A., Rahut, D. B., & Imtiaz, M. (2019). Affordability linked with subsidy: Impact of fertilizer subsidy on household welfare in Pakistan. *Sustainability, 11*(19), 5161. https://doi.org/10.3390/su11195161

Chibwana, C., Fisher, M., Jumbe, C., Masters, W. A., & Shively, G. (2010). Measuring the impacts of Malawi's farm input subsidy program. *SSRN Electronic Journal*. https://doi.org/10.2139/ssrn.1860867

Chibwana, C., Fisher, M., & Shively, G. (2012). Cropland allocation effects of agricultural input subsidies in Malawi. *World Development, 40*(1), 124–133. https://doi.org/10.1016/j.worlddev.2011.04.022

Demirdöğen, A., Olhan, E., & Hasdemir, M. (2022). Heterogeneous impact of agricultural support policies: Evidence from Turkey. *Environment, Development and Sustainability, 24*(10), 12203–12225. https://doi.org/10.1007/s10668-021-01941-9

Denning, G., Kabambe, P., Sanchez, P., Malik, A., Flor, R., Harawa, R., Nkhoma, P., Zamba, C., Banda, C., Magombo, C., Keating, M., Wangila, J., & Sachs, J. (2009). Input subsidies to improve smallholder maize productivity in Malawi: Toward an African green revolution. *PLOS Biology, 7*(1), e1000023. https://doi.org/10.1371/journal.pbio.1000023

Department of Agriculture, Ministry of Agriculture and Forests. (2011–2016). *Agriculture statistics (Annual reports)*. Royal Government of Bhutan. Retrieved from https://doa.gov.bt/agriculture-statistics/

Department of Agriculture. (2022). *Transformation of agriculture through crop prioritization: A strategy document for 2022–2027*. Ministry of Agriculture and Forests, Royal Government of Bhutan. https://doa.gov.bt/guidelines-and-manuals/

Hassan Danish, M., Tahir, A. M., & Azeem, M. S. H. (2017). Impact of agriculture subsidies on productivity of major crops in Pakistan and India: A case study of fertilizer subsidy (Report No. 427). Punjab Economic Research Institute.

Huang, J., Wang, X., & Rozelle, S. (2013). The subsidization of farming households in China's agriculture. *Food Policy, 41*, 124–132. https://doi.org/10.1016/j.foodpol.2013.04.011

Huang, J., Wang, X., Zhi, H., Huang, Z., & Rozelle, S. (2011). Subsidies and distortions in China's agriculture: Evidence from producer-level data. *Australian Journal of Agricultural and Resource Economics, 55*(1), 53–71. https://doi.org/10.1111/j.1467-8489.2010.00527.x

Jayne, T. S., Mason, N. M., Burke, W. J., & Ariga, J. (n.d.). *Agricultural input subsidy programs in Africa: An assessment of recent evidence*. https://doi.org/10.22004/ag.econ.259509

Jayne, T. S., & Rashid, S. (2013). Input subsidy programs in sub-Saharan Africa: A synthesis of recent evidence. *Agricultural Economics, 44*(6), 547–562. https://doi.org/10.1111/agec.12073

Karamba, R. W., & Winters, P. C. (2015). Gender and agricultural productivity: Implications of the farm input subsidy program in Malawi. *Agricultural Economics, 46*(3), 357–374. https://doi.org/10.1111/agec.12169

Ministry of Agriculture and Forests. (2021). *Cost sharing mechanism*. Royal Government of Bhutan. https://www.doa.gov.bt/guidelines-on-cost-sharing-mechanism-for-the-rnr-sector-twelfth-five-year-plan-2019/

Panhwar, H. A., Laghari, G. M., Kaleri, A. A., Soothar, M. K., Panhwar, A. A., Soothar, J. K., Rajput, M. M., Abro, A., & Soothar, J. K. (2017). Effects of seed rates on the growth and yield of different sunflower varieties. *Pure and Applied Biology*, *6*(4), 1189–1197. https://doi.org/10.19045/bspab.2017.600127

Renewable Natural Resources Statistical Division. (2017-2020). *Agriculture statistics (Annual reports)*. Ministry of Agriculture and Forests, Royal Government of Bhutan. https://doa.gov.bt/agriculture-statistics/

Ricker-Gilbert, J., Jayne, T. S., & Shively, G. (2012). Do fertilizer subsidies boost staple crop production and reduce poverty across the distribution of smallholders in Africa? Quantile regression results from Malawi. https://doi.org/10.22004/AG.ECON.126742

Ricker-Gilbert, J., Jayne, T. S., & Shively, G. (2013). Addressing the "wicked problem" of input subsidy programs in Africa. *Applied Economic Perspectives and Policy, 35*(2), 322–340. https://doi.org/10.1093/aepp/ppt001

Schwartz, G., & Clements, B. (1999). Government subsidies. *Journal of Economic Surveys, 13*(2), 119–148. https://doi.org/10.1111/1467-6419.00079

Shively, G. E., & Ricker-Gilbert, J. (2013). *Measuring the impacts of agricultural input subsidies in Sub-Saharan Africa: Evidence from Malawi's Farm Input Subsidy Program*. Purdue Policy Research Institute Policy Briefs, 1(1), Article 4. https://docs.lib.purdue.edu/gpripb/vol1/iss1/4

Smale, M., & Thériault, V. (2022). Input subsidy effects on crops grown by smallholder farm women: The example of cowpea in Mali. *Oxford Development Studies, 50*(3), 244–258. https://doi.org/10.1080/13600818.2021.2008892

Ueda, A., & Samdup, T. (n.d.). *Chili transactions in Bhutan: An economic, social and cultural perspective*. https://archivenepal.s3.amazonaws.com/

Yi, F., Sun, D., & Zhou, Y. (2015). Grain subsidy, liquidity constraints and food security—Impact of the grain subsidy program on the grain-sown areas in China. *Food Policy, 50*, 114–124. https://doi.org/10.1016/j.foodpol.2014.10.009

Yamane, T. (1967). Statistics: An introductory analysis (2nd ed.). Harper & Row. https://archive.org/details/statisticsanintr0000taro

https://doi.org/10.55925/btagr.26.9109

Systematic Literature Review: Application of Unmanned Aerial Vehicle in Agriculture

Thinley Gyeltshen[1], Kinzang Thinley[1], Pema Yangdon[1], Sherab Lhamo[2], Nangsel Tshomo[2], Tshering Penjor [1], Domang [1] and Tenzin Rabgay[1]

Abstract

In recent years, the application of Unmanned Aerial Vehicle (UAV) or drone technology in agriculture has gained popularity. However, in Bhutan, its application is far beyond reach. This systematic literature review synthesizes research on UAV applications in agriculture following PRISMA 2020 guidelines. From an initial 300 records (sourced from Google Scholar, Sci-Hub, and printed literature), 138 peer-reviewed studies met the inclusion criteria and were catalogued in Zotero. Findings are organized into five primary domains, which include crop monitoring and management, agrochemical spraying, crop damage assessment, surveying and mapping, and phenotyping, each encompassing specific secondary themes. Compared with satellites, manned aircraft, and ground systems, UAVs provide higher spatial and temporal resolution, operational flexibility, and cost-effectiveness, leveraging RGB, multispectral, hyperspectral, LiDAR, and thermal sensors. Empirical evidence demonstrates UAV utility for soil nutrient estimation, early pest and disease detection using vegetation indices (e.g., NDVI), precision spraying, rapid damage assessments, high-accuracy mapping, and enhanced phenotyping and yield estimation when combined with machine learning. Notable limitations include short flight time, regulatory constraints, weather sensitivity, and challenges in scaling plot-level results regionally. A key research gap identified is the absence of standardized, scalable methodologies and multi-location validation to translate plot-level UAV findings into reliable regional monitoring systems.

Keywords: *Remote sensing; UAVs; UAS, Agriculture; Application*

Corresponding email: thinleyg@moal.gov.bt

[1] Agriculture Research and Development Centre, Wengkhar, Department of Agriculture, Ministry of Agriculture and Livestock

[2] Agriculture Research and Development Centre, Bajo, Department of Agriculture, Ministry of Agriculture and Livestock

1 Introduction

Advanced farming techniques, such as precision agriculture and smart farming, now serve as the foundation for sustainable agricultural practices (Budiharto et al., 2019; Tsouros et al., 2019). They utilize state-of-the-art communication and remote sensing systems, integrated with AI-powered data analysis and decision-making strategies (Istiak et al., 2023; Prakash et al., 2022; Tsouros et al., 2019). Over recent years, significant technological advancements in Unmanned Aerial Vehicle (UAV) have led to their widespread adoption and application in precision farming (Istiak et al., 2023).

Unmanned Aerial Vehicles (UAVs), commonly referred to as drones, are operated remotely and equipped with advanced tools such as multispectral cameras, sensors, and communication systems integrated with intelligent decision-making capabilities (Ampatzidis et al., 2020). They are designed to handle tasks like data collection, analysis, and execution (Eskandari et al., 2020; Rejeb et al., 2022). Compared to satellites and piloted aircraft, UAVs offer a more technologically advanced, cost-effective, efficient, and real-time solution for performing actions (Islam et al., 2021; Tsouros et al., 2019).

The growing adoption of UAVs in precision agriculture is driven by several advantages over traditional remote sensing methods. Tsouros et al. (2019) reported that satellite imagery often falls short due to its low spatial resolution and infrequent capture intervals, making it unreliable for consistent agricultural monitoring. Moreover, delays between image acquisition and availability further limit its utility, while environmental factors like cloud cover frequently obstruct data collection. Similarly, the use of manned aircraft (MA) is limited by high operational costs, making it unfeasible for frequent flights to obtain multiple crop images (Tsouros et al., 2019). In addition, UAVs are highly significant as they can operate more effectively than ground-based (GB) systems since they can survey extensive areas in a short time, without causing any damage (Guan et al., 2019; Rejeb et al., 2022). The commonly used sensors for UAVs are Visible light sensors (RGB), Multispectral sensors, Hyperspectral sensors, Light detection and ranging sensors (LiDAR), and Thermal sensors (Tsouros et al., 2019).

Bhutan recognizes the vast potential of utilizing Unmanned Aerial Vehicles to transform its agricultural sector and meet the nation’s growing food demand. By 2023, the agriculture and livestock sectors are expected to provide sustenance for an estimated 837, 288 people, requiring significant production growth across key commodities compared to 2021 production (Ministry of Agriculture and Livestock [MoAL], 2023). Cereals production must rise to 289, 748 metric tons, reflecting an approximately 78% increase from 162, 931 metric tons. Similarly, the combined output of vegetables, fruits, roots, tubers, mustard, and spices is projected to reach 140,160 metric tons, reflecting a 13% increase from 124, 116 metric tons.

To meet these demands, Bhutan will need to significantly enhance agricultural and livestock production while ensuring farmers' incomes are sufficient to maintain food affordability (MoAL, 2023). However, achieving this ambitious goal is challenged with several obstacles in particular with an increasing amount of arable land left fallow, farm-labor shortage caused by increasing number of migrations to other service sectors, and persistent human-wildlife conflicts (National Statistical Bureau [NSB], 2023).

One significant way to address these challenges and enhance production is to adopt modern farming technologies, such as UAVs and other evolving smart and precision farming innovations. Strategic integration of these advanced tools will not only address the immediate challenges but also support long-term agricultural sustainability and productivity. Thus, this study aims to explore different applications of UAVs in the agriculture sector and guide the policy makers while framing the guidelines and developing policy related to drone usage and application in Bhutan.

2 Materials and Method

2.1 Gathering of Literature

This article was developed through a systematic literature review of studies conducted by various authors across different regions of the world. The review process adopted PRISMA 2020 guidelines (Page et al., 2020), encompassing three primary stages: identification of relevant journal articles, screening of collected literature, and inclusion of eligible studies (Figure 1).

Identification Phase: Relevant literature was sourced from Google Scholar, Sci-Hub, and printed academic books. A total of 300 articles were initially identified using keywords such as remote sensing, UAS, UAV, drone, agriculture, vegetative index, Agrisoft, and Pix4D.

Before screening, 66 articles were excluded: 50 due to duplication, 5 for being unrelated to agriculture, and 11 for lacking proper authorship.

Screening Phase: The remaining 234 articles underwent rigorous screening. Of these, 100 were excluded based on predefined eligibility criteria: 67 for low methodological quality, 13 for restricted access (non-open access), and 16 for insufficient information content. These exclusions primarily involved non-peer-reviewed publications, inaccessible full texts, and studies published more than 15 years before 2025. However, the excluded articles were revisited to retrieve any potentially overlooked information. As a result, 4 of the 100 excluded articles were reinstated for inclusion.

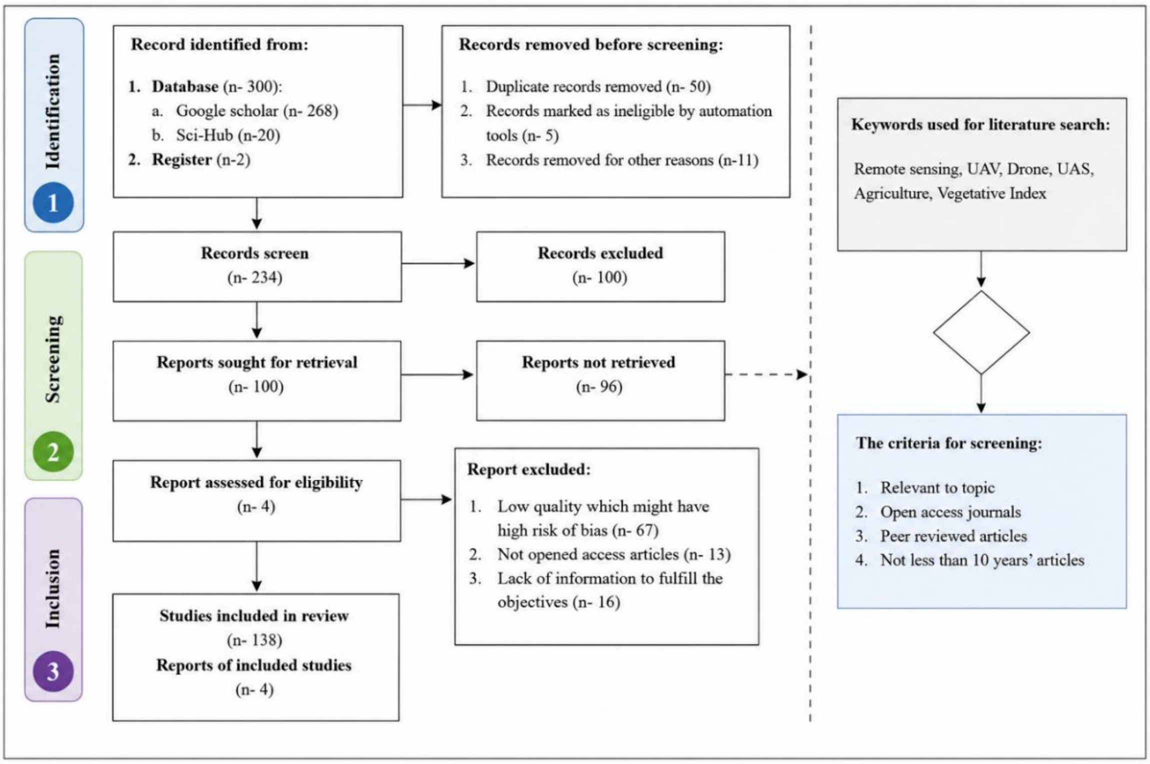


Figure 1. Process of PRISMA 2020 for systematic review

Source: Adapted from Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD. *The PRISMA 2020 statement: an updated guideline for reporting systematic reviews.*

Inclusion Phase: A total of 138 articles were ultimately selected for inclusion in this systematic review (Figure 1). These articles were deemed highly relevant to the study's objective, which focuses exclusively on drone applications in agricultural crop farming, excluding livestock-related research. Particular emphasis was placed on the methodological approaches and identified research gaps within the selected studies to ensure comprehensive data extraction. All included articles were catalogued using the open-access reference management software Zotero, facilitating accurate in-text citation and bibliographic referencing.

2.2 Conceptual Framework

Based on existing literature, the application of drones in agriculture is structured within a conceptual framework comprising two hierarchical levels: primary and secondary thematic areas (Figure 2). The primary thematic areas represent the core categories of drone utilization, each encompassing specific secondary thematic areas that reflect distinct use cases. Five primary thematic domains were identified: (i) crop monitoring and management, (ii) agrochemical spraying, (iii) crop damage assessment, (iv) surveying and mapping, and (v) phenotyping characterization. Each domain is further subdivided into concise secondary themes, as illustrated in Figure 2. The results and discussion section of this study are organized according to these thematic classifications to effectively address the research objectives.

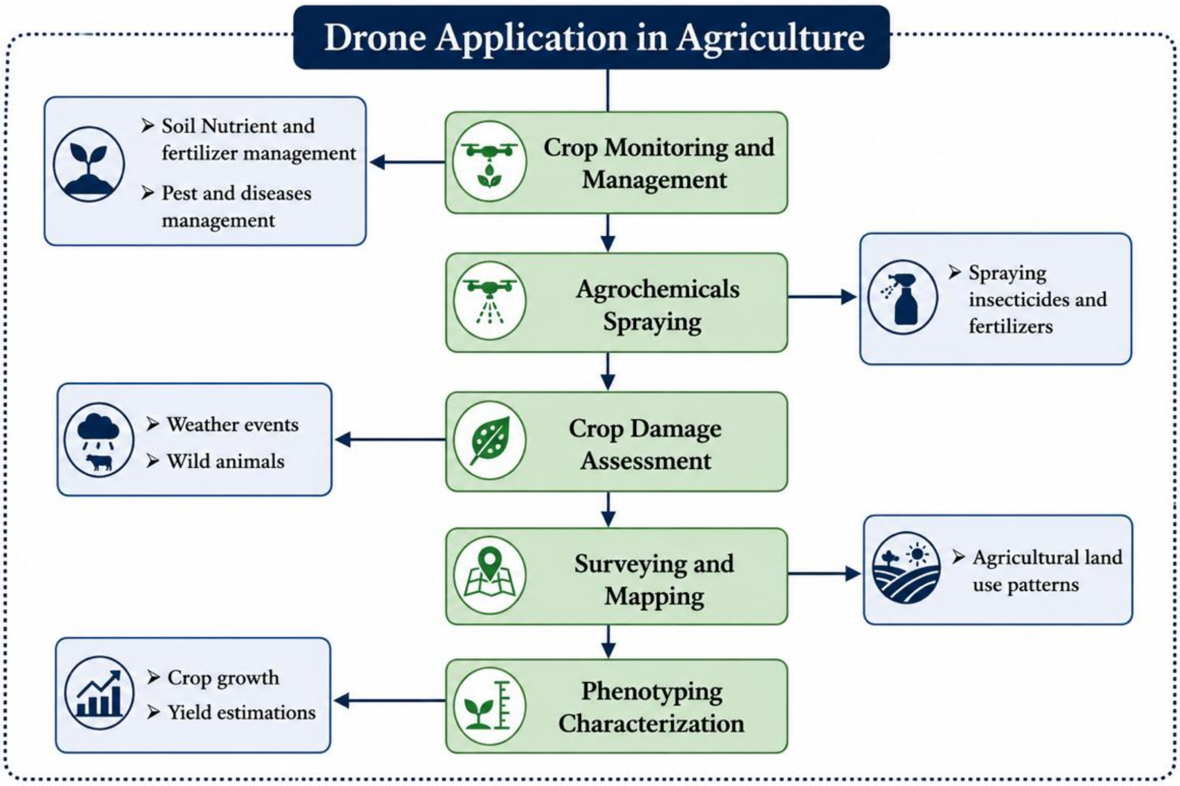


Figure 2. Conceptual framework for drone application in agriculture

3 Results and Discussion

3.1 Crop Monitoring and Management

Monitoring and managing crop health is crucial, as soil health, pests, and diseases can lead to substantial economic losses by decreasing yield and quality (Tsouros et al., 2019). This section will cover UAV use in Soil nutrient and fertilizer management, and Pest and Disease management

3.1.1 Estimating Soil Nutrient and Fertilizer Management

Two important markers of soil fertility and health are soil organic matter (SOM) and soil total nitrogen (STN). STN is necessary for plant growth, while SOM improves soil structure, water-holding ability, and nutrient retention. Farmers can increase crop yields and optimize nutrient management techniques by precisely predicting these characteristics (Yang et al., 2021).

Yang et al. (2021) conducted a study in Northeast China using a DJI M600 Pro UAV, equipped with a Resonon Pika L hyperspectral camera for the collection of samples on SOM and STN. They applied the particle swarm optimization (PSO) technique to refine the input weights and bias parameters of the extreme learning machine (ELM) model, enhancing its capability to predict soil organic matter (SOM) and soil total nitrogen (STN) with greater precision. The optimized PSO–ELM framework, developed using the selected preference bands, demonstrated superior predictive performance, achieving R^2 of 0.73 and RPD of 1.91 for SOM, and R^2 of 0.63 with an RPD of 1.53 for STN- when compared to the support vector machine (SVM), partial least squares regression (PLSR), and conventional ELM models which commonly used root mean square error (RMSE), and mean absolute error (MAR). The findings offer valuable insights for advancing soil nutrient monitoring through imaging spectrometry in precision agriculture.

3.1.2 Pest and Disease Management

Drones play a crucial role in detecting crop stress caused by pests and diseases. By capturing high-resolution aerial images over large areas in a short time, UAVs enable efficient monitoring of pest and disease outbreaks at various crop growth stages. These images provide valuable data for early detection and management of such outbreaks.

One key application of UAV imagery is the extraction of vegetation indices, such as the Normalized Difference Vegetation Index (NDVI) (Candiago et al., 2015). NDVI is calculated using the ratio of near-infrared (NIR) light to visible red light (Candiago et al., 2015). In comparison to healthy plants, stressed or unhealthy plants reflect more visible light and less NIR light (Peñuelas and Filella, 1998). The use of UAVs enhances precision agriculture practices, enabling targeted spraying of pesticides and accurate monitoring of the intervention's effectiveness over time (Tsouros et al., 2019). This targeted approach not only optimizes resource usage but also minimizes environmental impact. Table 1 provides an overview of the types of UAVs used for pest detection across different crops. Based on rotor configuration, unmanned aerial vehicles (UAVs) are classified and designated differently. For example, Zhang et al. (2019) employed a quadrotor drone equipped with RGB and multispectral sensors (RGB+M), comprising three visible and three multispectral bands, to assess fall armyworm infestation in wheat. The imagery was used to evaluate outbreak severity and to generate spatial maps that supported precision pesticide application as reported by growers.

Table 1. Different types of UAVs are used for Pest detection in different crops

Platform details	Type	Spectral resolution	Sensor details	No. of spectral bands	Field observation	Plant name	Arthropod common name	Reference
md4-1000, Microdrones	four rotors	RGB	α ILCE-5100L with an E 20 mm F2.8 lens, Sony	3	Visual inspection of images	Grape	Cotton jassid	Del-CampoSanchez et al. 2019
Aeryon Scout, Aeryon Labs Inc	four rotors	RGB + M	Photo3S, Aeryon Labs Inc. + ADCLite, Tetracam Inc	3 + 3	Outbreak reported by grower	Wheat	Fall armyworm	Zhang et al. 2014
Spreading Wings S800, SZ DJI Technology Co.	Six rotors	M	Mini-MCA6, Tetracam Inc.	6	Damage assessments	Potato	Colorado potato beetle	Hunt and Rondon 2017, Hunt et al. 2017

Note: *RGB- Red, Green, Blue; M- Multispectral*

3.2 Spraying of Agrochemicals

One significant application of drones in agriculture is the spraying of agrochemicals, such as pesticides and fertilizers, across farmland (Hunt et al., 2010; Neupane & Baysal-Gurel, 2021; Prakash et al., 2022). UAVs equipped with specialized sprayers disperse fluids through nozzles in the form of fine droplets under controlled pressure (Baysal-Gurel, 2021). This pressure is generated by a spray motor, ensuring effective and uniform application (Velusamy et al., 2022). As shown in Table 3.2, the 3CD-15 and WSZ-0610 drones differ in performance due to variations in their technical configurations. The 3CD-15 drone is equipped with four spray nozzles and operates at a maximum speed of 6 m/s with a spray rate of 0.54 L/min, carrying up to 15 L of chemicals and achieving a maximum flight time of 20 minutes (Table

2). In contrast, the WSZ-0610 drone has two spray nozzles, a lower maximum speed of 4 m/s, and a spray rate of 0.72 L/min while carrying 10 L of chemicals for a similar flight duration (Table 2). Owing to its higher payload capacity and operational speed, the 3CD-15 drone is more suitable for large-scale farm operations than the WSZ-0610 model.

Table 2. Drones used in spraying pesticides in recent times

Types of drones	Volume of pesticides (L)	Max. Flight time full load (min)	Max. speed (m/s)	Discharge rate (L/min)	No. of nozzles
DJI Agrus MG-1S	10	10	12	0.379	4
3WQF120-12	12	30	5	0.8	2
3CD-15	15	20	6	0.54	4
WSZ-0610	10	20	4	0.72	2
HY-B-15L	15	15	4.5	0.38	5

Source: Adapted from Borikar et al. (2022)

Compared to traditional methods like speed sprayers or wide-area sprayers, UAVs offer superior efficiency and reduced pesticide usage. The quantity of pesticides applied per hectare has a direct correlation with environmental pollution and worker health risks (Chin et al., 2023; Geipel et al., 2014; MoAL, 2023; Tetila et al., 2020). By minimizing pesticide consumption, UAVs help address these challenges. For instance, they can achieve large-scale decontamination, covering up to 50 hectares per day, while requiring only about 10 minutes to spray 0.5 hectares of farmland (Kim et al., 2019). Thus, this precision and efficiency make UAVs a highly effective solution for modern, sustainable agriculture (Table 3). Chen et al. (2020) employed drones for the control of rice planthoppers and reported a control success rate of 90.8%. This high level of effectiveness demonstrates the practical applicability of drone-based pest management in real-world agricultural settings.

Table 3. Effectiveness of managing agricultural pests using drones

Crop	Chemicals	Pest	Unit	Control efficiency	References
Rice	Treatment 1, 3, 5	Rice planthoppers	%	90.8	(Chen *et al.*, 2020)
Wheat	Deltamethrin	Sunn pest	%	96	(Sheikhigarjan *et al.*, 2024)
Sugarcane	Chlorfenapyr, chlorantraniliprole, and lufenuron.	Fall armyworm	%	94.94	(Song *et al.*, 2020)

3.3 Crop Damage Assessment

Accurate crop damage assessment is essential for determining actual losses caused by extreme weather events or wild animal activity. This process is particularly important to assess the causes and to enable appropriate intervention. Moreover, for insurance companies, it ensures timely and fair compensation for losses (Cao et al., 2020; Rejeb et al., 2022; Su et al., 2018). Traditional methods of damage assessment are often time-consuming and labour-intensive (Su et al., 2018). In contrast, UAVs enable rapid and precise crop damage assessments, significantly reducing the time required to evaluate losses (Baluja et al., 2012; Candiago et al., 2015; Cen et al., 2019; Geipel et al., 2014; Istiak et al., 2023; Tsouros et al., 2019).

3.3.1 Weather Events

Drones equipped with thermal cameras and multispectral sensors are highly effective in detecting the impacts of extreme weather conditions, such as temperature extremes (frost, heat) and precipitation anomalies like flooding or drought (Geipel et al., 2014; Negash et al., 2019; Tsouros et al., 2019). For instance, thermal sensors can identify temperature anomalies, enabling the pinpointing of frost-affected areas, while NDVI values are instrumental in detecting crops under drought stress (Hunt et al., 2010; Tsouros et al., 2019). The practical applications of vegetation indices for crop assessment under various weather events are as follows. These are based on a study by Budiharto et al. (2019), Islam et al. (2021) and Tsouros et al. (2019).

- **Drought**: Use NDVI, NDWI, and SAVI to monitor stressed areas and prioritize irrigation (Budiharto et al., 2019).
- **Floods**: Apply NDWI to locate waterlogged areas and NDVI to track crop recovery (Costa et al., 2020).
- **Hailstorms**: Employ NDVI and EVI to identify broken or damaged canopies (Tsouros et al., 2019).
- **Frost or Heat Stress**: Monitor CI and GNDVI for signs of chlorophyll loss or degradation (Kim et al., 2019; Meinen & Robinson, 2021).

3.3.2 Wild Animals

Drones equipped with high-resolution RGB, multispectral, thermal, LiDAR, or hyperspectral sensors and cameras play a critical role in assessing crop damage caused by wild animals

(Robinson, 2021). These advanced imaging tools enable early detection, detailed analysis, and precise monitoring of crop conditions, helping farmers take timely action.

- **RGB Cameras**: They capture high-definition images of the crops in natural colour. It can be used to visually assess physical damage from animal activity (e.g., grazing, trampling) (del Cerro et al., 2021; Mogili & Deepak, 2018).
- **Multispectral Cameras**: They capture data in multiple colour bands of the electromagnetic spectrum, typically including visible light, near-infrared (NIR), and sometimes red-edge and shortwave infrared (SWIR), which will help in identifying areas where animals have caused harm, based on differences in vegetation health (Hunt et al., 2010).
- **Thermal cameras:** They measure temperature differences across the field, capturing heat signatures. It identifies areas with potential water stress or compacted soil from animal activity, which can affect temperature regulation of plants (Cen et al., 2019; Tetila et al., 2020)
- **LiDAR (Light Detection and Ranging):** It uses laser pulses to create highly detailed 3D maps of the crop field. It can help assess ground-level disturbances caused by animal movement, such as tracks or grazing marks (Negash et al., 2019).
- **Hyperspectral Sensors:** They capture a wide range of wavelengths, from visible to infrared, allowing for more detailed analysis of plant health. It helps to detect stress in plants due to grazing or trampling, allowing for early intervention (Neupane & Baysal-Gurel, 2021).

By utilizing these sensors, drones provide comprehensive insights into crop health and damage, ensuring efficient monitoring and timely responses to minimize losses caused by wild animals (Table 4). Dobosz et al. (2023) evaluated the use of drones equipped with various sensors for assessing crop damage. To ensure accuracy and replicability, areas of crop damage smaller than 3 m² were excluded from the field study. Among the three drones tested, the highest assessment performance was achieved by the drone using a DSM-based filter with a threshold value of 0.4, yielding a performance of 118.4%. This drone demonstrates potential for rapid crop damage assessment following natural disasters in the country.

Table 4. Evaluation accuracy of various methods in crop damage assessment

Method	Estimated crop damage area (% of the reference area)	Remarks

NDVI based- filter value threshold- 0.12	224.4 (39.2)	Area below 3m^2 is omitted
DSM- based- filter value threshold- 0.4	678.7 (118.4)	
CART based- DSM filter value threshold- 0.73	309.2 (54)	

Note: numbers in parenthesis are in percentage *Source: Adapted from (Dobosz et al., 2023)*

3.4 Surveying and Mapping

Surveying and mapping are other areas where UAVs are widely used. Under this section, the application of UAVs in agricultural land use is explained in detail.

Drones play important roles, and it has multiple advantages in various types of industries, particularly surveying and mapping, in today's rapidly changing technological landscape (Espinoza et al., 2017; Meinen & Robinson, 2021; Rejeb et al., 2022). It offers high-resolution, cost-effective, and efficient spatial data collection for land use planning and resource management (Rejeb et al., 2022). They enable participatory mapping, fostering community involvement in identifying traditional land resources, land rights, and boundaries (International Fund for Agricultural Development [IFAD], 2009). Traditionally, surveyors had to rely on ground-based methods, which were both time-consuming and expensive (Tech Collective, 2024).

National Land Commission [NLC], 2025) categorized Bhutan's land into four micro land use zones: *1) Kamzhing:* A land with or without bench terraced which can be used for the production of crops, establishment of orchards, plantations and pasture development, *2) Protected Chhuzhing:* An irrigated and bench terraced land used for cultivation of paddy, *3) Regulated Chhuzhing:* An irrigated and bench terraced agricultural land that are outside of *Protected Chhuzhing*, which can be used for paddy production as well as for other crops, and *4) Agricultural Leased Land:* This term refers to leased state land for commercial agriculture and cattle production, with the right to use but not ownership.

Compared to conventional satellite and ground-based methods, drones overcome limitations like cloud cover, high costs, and time inefficiencies (Mogili & Deepak, 2018; Tsouros et al., 2019). The accuracy rate of UAVs in land use applications is given in Table 5. Thus, it supports precision agriculture, enhancing productivity, tenure security, and informed decision-making for sustainable agricultural practices at local and regional levels (Kachamba *et al*., 2016).

Table 5. Accuracy rate of UAVs in the Agriculture Land Use study

Image composite	Overall accuracy (%)
RBG	74
RBG+NIR	80
RBG+NIR+DSM	88

Source: Adapted from (Chen et al., 2020)

3.5 Phenotyping Characterization of Crops

One key application of drones in agriculture is crop phenotyping and characterization. Amongst many applications, the emphasis is given to crop yield estimation and crop growth assessment.

3.5.1 Crop Yield Estimation

Accurate crop yield and quality assessments are critical for decision-makers at national and regional levels to enable timely decisions (Tao et al., 2020). Such data is essential for crop insurance, delivery estimates, harvest planning, quality optimization, storage requirements, and financial management (Horie *et al.*, 1992). A reliable crop yield prediction model empowers farmers to make informed decisions on different farming methods for maximizing profit.

One effective approach for achieving precision in yield estimation is the application of remote sensing (RS) technologies, which include ground-based, satellite-based, and UAV (Unmanned Aerial Vehicle)-based platforms. Among these, UAVs offer significant advantages over the other two in terms of data quality, cost-effectiveness, and operational flexibility (Gago et al., 2015; Ju & Son, 2018; K.c. et al., 2021; Tao et al., 2020). For example, ground-based platforms are labour-intensive, time-consuming, and risk damaging crops during data collection, while satellite platforms often face challenges such as mixed pixels, long observation intervals, and low spatial and temporal resolution (Tao et al., 2020).

In contrast, UAV imagery, particularly when integrated with machine learning (ML) techniques, can enhance assessment precision and reduce or even eliminate the need for terrestrial surveys. The accuracy of crop yield and quality estimation improves as the crop progresses through its growth stages, highlighting the importance of timely sensing (Ballester et al., 2017). The precision rates for wheat yield estimation at various plant growth stages are provided in Table 6, and common vegetative indices used for different plants are provided in Table 7. Across all wheat growth stages, the most effective assessment method is combining spectral indices methods with a drone equipped with hyperspectral sensors. This method has achieved R^2values of 0.4 at the jointing stage, 0.65 at the flagging stage, and 0.75 at the flowering stage, respectively, when compared with manual measurements and traditional spectral indices (Tao et al., 2020).

Table 6. Winter wheat yield estimation in different growth stages by using partial least squares regression (PLSR)

Growth Stage	Information	R^2	RMSE (Kg/Ha)	NRMSE (%)
Jointing	SIs	0.35	1415.6	26.8
	SIs+H	0.37	1364.4	25.8
	SIs+H_{CSM}	0.4	1287.3	24.4
Flagging	SIs	0.57	1155.7	21.9
	SIs+H	0.62	1102.2	20.9
	SIs+H_{CSM}	0.65	1069.6	20.3
Flowering	SIs	0.7	989.4	18.8
	SIs+H	0.74	891.9	16.9
	SIs+H_{CSM}	0.75	875.3	16.5

Note: *SI- Spectral Indices, H- Ground measured plant height, H_{CSM}- UAV-based hyperspectral images, RSME- Root-Mean-Square error, NRMSE- Normalized Root-Mean-Square Error*

Source: Adapted from (Tao et al., 2020)

Table 7. Vegetative Indices (Spectral Indices) used for different crop growing stages

SN	Vegetation Index	Formulation	Scale of Application	Estimated parameter	References
1	Normalized Difference Vegetation Index	NDVI = (NIR -R)/ (NIR + R)	Crown	Biomass, vegetation density	(John Wilson Rouse *et al.*, 1974)
2	Red edge difference vegetation index (REDVI)	REDVI = NIR -RE	Crown	Vegetation coverage	(Cao *et al.*, 2013)
3	Normalized Difference Red-Edge	NDRE = (NIR - RE)/(NIR + RE)	Leaves	Biomass	(Barnes *et al.*, 2000)
4	Red edge chlorophyll index (CIRE)	CIRE = (NIR/RE) - 1	Leaves	Chlorophyll	(Anatoly A Gitelson *et al.*, 2005)

Note: *Vegetation indices (G = green, R = red, RE = red edge, NIR = near infrared)*

3.5.2 Crop Growth Assessment

The assessment of crop growth is a crucial aspect of agriculture, as it directly influences productivity in designated land areas. Traditionally, researchers relied on ground-level observations to identify, select, and cultivate crops with favourable genetic and physical traits. However, collecting extensive, high-quality phenotypic data in open-field conditions remains a significant challenge due to its labour-intensive and time-consuming nature (Holman et al., 2016).

In recent years, UAS have emerged as an important tool for crop growth assessment. For example, Nebiker et al. (2008) demonstrated an early milestone by integrating UAVs with cost-effective multispectral cameras for remote sensing to assess the health of grapevine crops. Similarly, Hunt et al. (2010) utilized multispectral UAV imagery for crop monitoring, identifying a strong correlation between the leaf area index and the green Normalized Difference Vegetation Index (green NDVI).

Ampatzidis & Partel (2019) advanced this approach by developing a method that combines UAVs, multispectral imaging technology, and deep learning-based convolutional neural networks to analyze phenotypic traits in citrus plants. Their method achieved remarkable results, with a precision of 99.9% and a recall of 99.7% for identifying and counting 4,916 citrus trees in an orchard. Additionally, they estimated canopy sizes with 85.5% accuracy and identified, mapped, and counted tree gaps with 100% precision and 94.6% recall (Table 8).

Table 8. Comparison of manual and UAV technologies in measuring the plant growth and gaps

UAV based remote sensing technique	Number of detections	Ground truth	Precision (%)	Recall (%)	F-Score (%)
Plant growth	4904	4916	99.9	99.7	98.8
Plant gap	106	112	100	94.6	97.3

Source: Adapted from Ampatzidis & Partel (2019)

Likewise, Holman et al. (2016) introduced and evaluated an approach to quickly estimate crop height and growth rate using multi-temporal, ultra-high spatial resolution (1 cm/pixel), 3D digital surface models of crop field trials. These models were generated through Structure from Motion (SfM) photogrammetry, utilizing aerial imagery captured during multiple flights

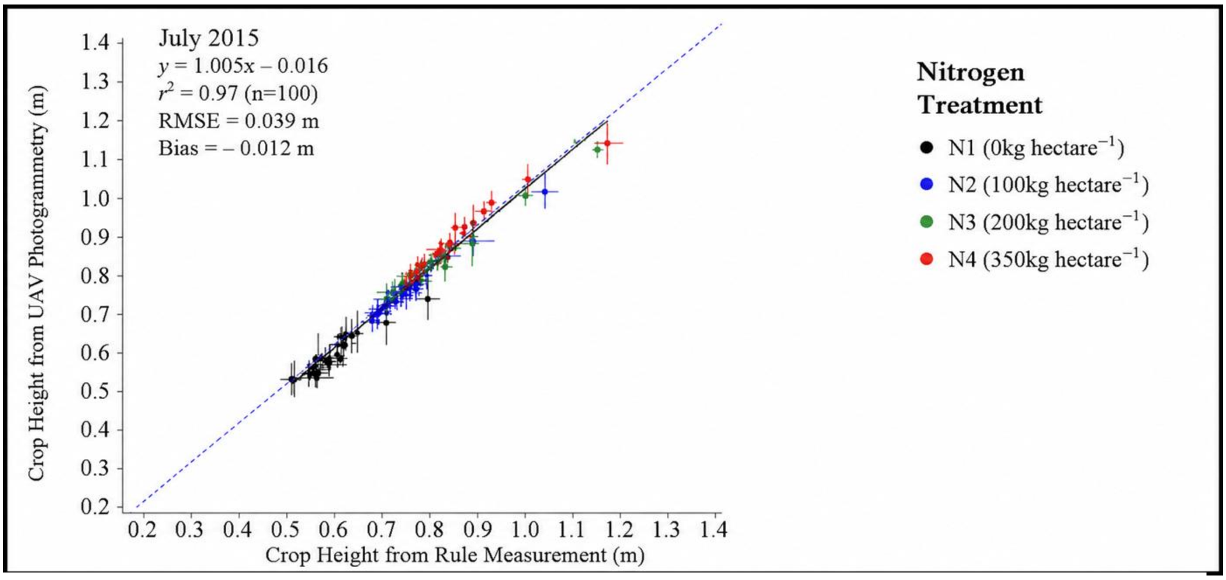


Figure 3. Comparison between manual vs UAVs for crop growth assessment. *Source: Adapted from Holman et al. (2016)*

of an Unmanned Aerial Vehicle (UAV) equipped with an RGB camera. The result showed that the UAV-generated surface model with the highest precision and the Terrestrial laser scanner (TLS) both demonstrated a Root Mean Squared Error (RMSE) of 0.03 meters when compared to the traditional manual 2-meter rule approach, indicating the high applicability of UAVs in measuring the crop height (Figure 3). The model demonstrates an accuracy rate of 97% (r^2 =0.97) at a 0.05 significance level which proves its application in crop height measurement.

The most used software for data collection and processing in UAV applications for agriculture is PIX4D and Agisoft. Figure 4 illustrates the data acquisition and analysis workflow for agricultural drone applications using Agisoft PhotoScan or Cyclone 8.1 (Holman et al., 2016). This workflow applies to field data collected with UAVs as well as LiDAR systems. After importing the data, a critical step is to assess data quality and align the data based on geo-coordinates to generate a Digital Surface Model (DSM). Using the DSM, prescriptions for various agricultural applications are prepared according to the type of intervention and subsequently assigned to the drone for implementation in the selected fields and crops. To evaluate the effectiveness of UAV-based applications, statistical analyses are conducted using different models to compare the precision and performance of drones equipped with various sensors.

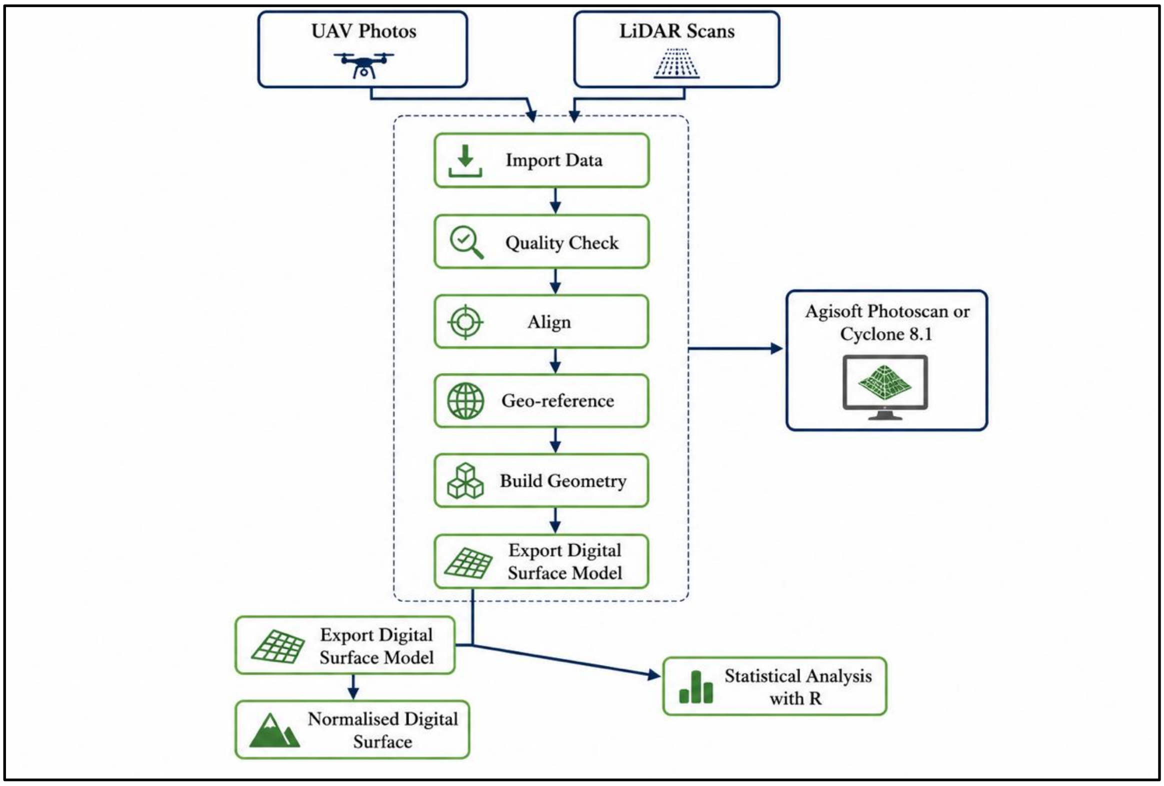


Figure 4. Workflow for data collection and processing. *Source: Adapted from Holman et al., (2016)*

3.6 Limitations of UAVs in Agriculture

- The sustainable flight time of UAVs is relatively short, and in long-distance flight missions, batteries need to be replaced midway to complete flight operations (Eskandari et al., 2020).
- UAVs are predominantly utilizing for estimating crop yields at the field-plot level; however, extending these estimates to regional scales presents greater challenges. While plot-level yield data can serve as a basis for regional yield calculations, such extrapolation may introduce considerable bias due to substantial variations in growing conditions across different plots (Kim et al., 2019).
- There will be local flight restrictions, which will prevent the UAV from performing normal flight operations (Bah et al., 2018; Rejeb et al., 2022).
- It is highly affected by weather factors, especially during rainy and stormy days when data collection cannot be achieved. It will result in missing plant growth data (Holman et al., 2016; Tetila et al., 2020).

4 Conclusion

This review demonstrated that UAVs had matured into versatile tools for crop farming, offering superior spatial-temporal resolution, cost-effectiveness, and operational flexibility compared with satellite, manned aircraft, and ground-based approaches. The peer reveiewed articles used in this study showed that UAVs were effective for soil nutrient and fertilizer management, early pest and disease detection through vegetation indices, targeted agrochemical spraying, rapid crop damage assessment, detailed surveying and mapping, and high-throughput phenotyping and yield estimation, especially when combined with machine learning and hyperspectral or multispectral sensors. However, persistent limitations must be addressed before widespread adoption: limited battery life and flight time, weather sensitivity leading to missed observations, and risks of bias when scaling plot-level findings to regional estimates. To realize UAVs' potential, coordinated efforts are needed to standardize methodologies, validate models across environments, strengthen regulatory frameworks, and invest in training and infrastructure. For countries facing food security and land use challenges, such as Bhutan, integrating UAV technology into national agricultural programs can support targeted interventions, improve resource efficiency, and inform policy. Future research should prioritize scalable approaches and operational trials that bridge experimental results and practical implementation. Addressing these gaps will accelerate adoption and maximize agricultural benefits from UAV technologies.

5 Acknowledgement

We gratefully acknowledge the Agriculture Research and Innovation Division (ARID) and GovTech for providing us with the opportunity to familiarize ourselves with drone applications in agriculture. We also extend our sincere thanks to Bhutan AeroTech for delivering practical training in drone operation and handling across diverse climatic conditions. Finally, we are deeply grateful to the colleagues and management of ARDC Wengkhar for supporting our participation in the adoption of this emerging technology within the agricultural sector.

6 Authors' contribution statement

Thinley Gyeltshen, Kinzang Thinley, and Pema Yangdon- Study conception and design, analysis of research based on thematic areas, and drafting of manuscript. Domang, Tenzin Rabgay, Dr. Tshering Penjor, Sherab Lhamo, and Nangsel Tshomo- Information gathering and formulation of conceptual framework.

7 References

Ampatzidis, Y., & Partel, V. (2019). UAV-based high throughput phenotyping in citrus utilizing multispectral imaging and artificial intelligence. *Remote Sensing, 11*(4), 410. https://doi.org/10.3390/rs11040410

Ampatzidis, Y., Partel, V., & Costa, L. (2020). Agroview: Cloud-based application to process, analyze and visualize UAV-collected data for precision agriculture applications utilizing artificial intelligence. *Computers and Electronics in Agriculture, 174*, 105457. https://doi.org/10.1016/j.compag.2020.105457

Bah, M. D., Hafiane, A., & Canals, R. (2018). Deep learning with unsupervised data labeling for weed detection in line crops in UAV images. *Remote Sensing, 10*(11), 1690. https://doi.org/10.3390/rs10111690

Baluja, J., Diago, M. P., Balda, P., Zorer, R., Meggio, F., Morales, F., & Tardaguila, J. (2012). Assessment of vineyard water status variability by thermal and multispectral imagery using an unmanned aerial vehicle (UAV). *Irrigation Science, 30*(6), 511–522. https://doi.org/10.1007/s00271-012-0382-9

Budiharto, W., Chowanda, A., Gunawan, A. A. S., Irwansyah, E., & Suroso, J. S. (2019). A review and progress of research on autonomous drone in agriculture, delivering items and geographical information systems (GIS). In *2019 2nd World Symposium on Communication Engineering (WSCE)* (pp. 205–209). IEEE. https://doi.org/10.1109/WSCE49000.2019.9041004

Candiago, S., Remondino, F., De Giglio, M., Dubbini, M., & Gattelli, M. (2015). Evaluating multispectral images and vegetation indices for precision farming applications from UAV images. *Remote Sensing, 7*(4), 4026–4047. https://doi.org/10.3390/rs70404026

Cao, Y., Li, G. L., Luo, Y. K., Pan, Q., & Zhang, S. Y. (2020). Monitoring of sugar beet growth indicators using wide-dynamic-range vegetation index (WDRVI) derived from UAV multispectral images. *Computers and Electronics in Agriculture, 171*, 105331. https://doi.org/10.1016/j.compag.2020.105331

Cen, H., Wan, L., Zhu, J., Li, Y., Li, X., Zhu, Y., Weng, H., Wu, W., Yin, W., Xu, C., Bao, Y., Feng, L., Shou, J., & He, Y. (2019). Dynamic monitoring of biomass of rice under different nitrogen treatments using a lightweight UAV with dual image-frame snapshot cameras. *Plant Methods, 15*(1), 32. https://doi.org/10.1186/s13007-019-0418-8

Chen, P.-C., Chiang, Y.-C., & Weng, P.-Y. (2020). Imaging using unmanned aerial vehicles for agriculture land use classification. *Agriculture, 10*(9), 416. https://doi.org/10.3390/agriculture10090416

Chin, R., Catal, C., & Kassahun, A. (2023). Plant disease detection using drones in precision agriculture. *Precision Agriculture, 24*(5), 1663–1682. https://doi.org/10.1007/s11119-023-10014-y

Costa, L., Nunes, L., & Ampatzidis, Y. (2020). A new visible band index (vNDVI) for estimating NDVI values on RGB images utilizing genetic algorithms. *Computers and Electronics in Agriculture, 172*, 105334. https://doi.org/10.1016/j.compag.2020.105334

Dobosz, B., Gozdowski, D., Koronczok, J., Žukovskis, J., & Wójcik-Gront, E. (2023). Evaluation of maize crop damage using UAV-based RGB and multispectral imagery. *Agriculture, 13*(8), 1627. https://doi.org/10.3390/agriculture13081627

Eskandari, R., Mahdianpari, M., Mohammadimanesh, F., Salehi, B., Brisco, B., & Homayouni, S. (2020). Meta-analysis of unmanned aerial vehicle (UAV) imagery for agro-environmental monitoring using machine learning and statistical models. *Remote Sensing, 12*(21), 3511. https://doi.org/10.3390/rs12213511

Espinoza, C. Z., Khot, L. R., Sankaran, S., & Jacoby, P. W. (2017). High-resolution multispectral and thermal remote sensing-based water stress assessment in subsurface irrigated grapevines. *Remote Sensing, 9*(9), 961. https://doi.org/10.3390/rs9090961

Gago, J., Douthe, C., Coopman, R. E., Gallego, P. P., Ribas-Carbo, M., Flexas, J., Escalona, J., & Medrano, H. (2015). UAVs challenge to assess water stress for sustainable agriculture. *Agricultural Water Management, 153*, 9–19. https://doi.org/10.1016/j.agwat.2015.01.020

Geipel, J., Link, J., & Claupein, W. (2014). Combined spectral and spatial modeling of corn yield based on aerial images and crop surface models acquired with an unmanned aircraft system. *Remote Sensing, 6*(11), 10335–10355. https://doi.org/10.3390/rs61110335

Guan, S., Fukami, K., Matsunaka, H., Okami, M., Tanaka, R., Nakano, H., Sakai, T., Nakano, K., Ohdan, H., & Takahashi, K. (2019). Assessing correlation of high-resolution NDVI with fertilizer application level and yield of rice and wheat crops using small UAVs. *Remote Sensing, 11*(2), 112. https://doi.org/10.3390/rs11020112

Holman, F. H., Riche, A. B., Michalski, A., Castle, M., Wooster, M. J., & Hawkesford, M. J. (2016). High throughput field phenotyping of wheat plant height and growth rate in field plot trials using UAV-based remote sensing. *Remote Sensing, 8*(12), 1031. https://doi.org/10.3390/rs8121031

Horie, T., Yajima, M., & Nakagawa, H. (1992). Yield forecasting. *Agricultural Systems, 40*(1), 211–236. https://doi.org/10.1016/0308-521X(92)90022-G

Hunt, E. R., Hively, W. D., Fujikawa, S. J., Linden, D. S., Daughtry, C. S. T., & McCarty, G. W. (2010). Acquisition of NIR-green-blue digital photographs from unmanned aircraft for crop monitoring. *Remote Sensing, 2*(1), 290–305. https://doi.org/10.3390/rs2010290

International Fund for Agricultural Development. (2009). *Traditional land resources, land rights, and boundaries*.

Islam, N., Rashid, M. M., Pasandideh, F., Ray, B., Moore, S., & Kadel, R. (2021). A review of applications and communication technologies for Internet of Things (IoT) and unmanned aerial vehicle (UAV)-based sustainable smart farming. *Sustainability, 13*(4), 1821. https://doi.org/10.3390/su13041821

Istiak, M. A., Syeed, M. M. M., Hossain, M. S., Uddin, M. F., Hasan, M., Khan, R. H., & Azad, N. S. (2023). Adoption of unmanned aerial vehicle (UAV) imagery in agricultural management: A systematic literature review. *Ecological Informatics, 78*, 102305. https://doi.org/10.1016/j.ecoinf.2023.102305

Ju, C., & Son, H. I. (2018). Multiple UAV systems for agricultural applications: Control, implementation, and evaluation. *Electronics, 7*(9), 162. https://doi.org/10.3390/electronics7090162

Kachamba, D. J., Ørka, H. O., Gobakken, T., Eid, T., & Mwase, W. (2016). Biomass estimation using 3D data from unmanned aerial vehicle imagery in a tropical woodland. *Remote Sensing, 8*(11), 968. https://doi.org/10.3390/rs8110968

K.C., S., Ninsawat, S., & Som-ard, J. (2021). Integration of RGB-based vegetation index, crop surface model and object-based image analysis approach for sugarcane yield estimation using unmanned aerial vehicle. *Computers and Electronics in Agriculture, 180*, 105903. https://doi.org/10.1016/j.compag.2020.105903

Kim, J., Kim, S., Ju, C., & Son, H. I. (2019). Unmanned aerial vehicles in agriculture: A review of perspective of platform, control, and applications. *IEEE Access, 7*, 105100–105115. https://doi.org/10.1109/ACCESS.2019.2932119

Meinen, B. U., & Robinson, D. T. (2021). Agricultural erosion modelling: Evaluating USLE and WEPP field-scale erosion estimates using UAV time-series data. *Environmental Modelling & Software, 137*, 104962. https://doi.org/10.1016/j.envsoft.2021.104962

Ministry of Agriculture and Livestock. (2023). *Food and nutrition security policy of Bhutan*. Royal Government of Bhutan. https://www.moal.gov.bt/policy/

Mogili, U. R., & Deepak, B. B. V. L. (2018). Review on application of drone systems in precision agriculture. *Procedia Computer Science, 133*, 502–509. https://doi.org/10.1016/j.procs.2018.07.063

National Statistics Bureau. (2023). *Integrated agriculture and livestock census report*. Royal Government of Bhutan.

Nebiker, S., Annen, A., Scherrer, M., & Oesch, D. (2008). *A light-weight multispectral sensor for micro UAV: Opportunities for very high resolution airborne remote sensing*. https://www.semanticscholar.org/paper/A-Light-weight-Multispectral-Sensor-for-Micro-UAV-Nebiker-Annen/955a70058c6fa1b1a3fbfa17c5aef6818068e024

Negash, L., Kim, H.-Y., & Choi, H.-L. (2019). Emerging UAV applications in agriculture. In *2019 7th International Conference on Robot Intelligence Technology and Applications (RiTA)* (pp. 254–257). IEEE. https://doi.org/10.1109/RITAPP.2019.8932853

Neupane, K., & Baysal-Gurel, F. (2021). Automatic identification and monitoring of plant diseases using unmanned aerial vehicles: A review. *Remote Sensing, 13*(19), 3841. https://doi.org/10.3390/rs13193841

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T., Mulrow, C. D., Shamseer, L., & Moher, D. (2020). Mapping of reporting guidance for systematic reviews and meta-analyses generated a comprehensive item bank for future reporting guidelines. *Journal of Clinical Epidemiology, 118*, 60–68. https://doi.org/10.1016/j.jclinepi.2019.11.010

Peñuelas, J., & Filella, I. (1998). Visible and near-infrared reflectance techniques for diagnosing plant physiological status. *Trends in Plant Science, 3*(4), 151–156. https://doi.org/10.1016/S1360-1385(98)01213-8

Prakash, S., Kumar, M., Jat, D., Jyoti, B., Subeesh, A., Agrawal, K. N., Tiwari, P. S., Mehta, C. R., Singh, P. L., & Singh, K. (2022). *Applications of drones in agriculture: Status and scope*. Indian Council of Agricultural Research. http://krishi.icar.gov.in/jspui/handle/123456789/80715

Rejeb, A., Abdollahi, A., Rejeb, K., & Treiblmaier, H. (2022). Drones in agriculture: A review and bibliometric analysis. *Computers and Electronics in Agriculture, 198*, 107017. https://doi.org/10.1016/j.compag.2022.107017

Su, J., Coombes, M., Liu, C., Guo, L., & Chen, W.-H. (2018). Wheat drought assessment by remote sensing imagery using unmanned aerial vehicle. In *2018 37th Chinese Control Conference (CCC)* (pp. 10340–10344). IEEE. https://doi.org/10.23919/ChiCC.2018.8484005

Tao, H., Feng, H., Xu, L., Miao, M., Yang, G., Yang, X., & Fan, L. (2020). Estimation of the yield and plant height of winter wheat using UAV-based hyperspectral images. *Sensors, 20*(4), 1231. https://doi.org/10.3390/s20041231

Tetila, E. C., Machado, B. B., Astolfi, G., Belete, N. A. de S., Amorim, W. P., Roel, A. R., & Pistori, H. (2020). Detection and classification of soybean pests using deep learning with UAV images. *Computers and Electronics in Agriculture, 179*, 105836. https://doi.org/10.1016/j.compag.2020.105836

Tsouros, D. C., Bibi, S., & Sarigiannidis, P. G. (2019). A review on UAV-based applications for precision agriculture. *Information, 10*(11), 349. https://doi.org/10.3390/info10110349

Velusamy, P., Rajendran, S., Mahendran, R. K., Naseer, S., Shafiq, M., & Choi, J.-G. (2022). Unmanned aerial vehicles (UAV) in precision agriculture: Applications and challenges. *Energies, 15*(1), 217. https://doi.org/10.3390/en15010217

Wood, S. A., Robinson, P. W., Costa, D. P., & Beltran, R. S. (2021). Accuracy and precision of citizen scientist animal counts from drone imagery. *PLOS ONE, 16*(2), e0244040. https://doi.org/10.1371/journal.pone.0244040

Yang, X., Bao, N., Li, W., Liu, S., Fu, Y., & Mao, Y. (2021). Soil nutrient estimation and mapping in farmland based on UAV imaging spectrometry. *Sensors, 21*(11), 3919. https://doi.org/10.3390/s21113919

Zhang, H., Wang, G., Lei, Z., & Hwang, J.-N. (2019). Eye in the sky: Drone-based object tracking and 3D localization. In *Proceedings of the 27th ACM International Conference on Multimedia (MM '19)* (pp. 899–907). Association for Computing Machinery. https://doi.org/10.1145/3343031.3350933

https://doi.org/10.55925/btagr.26.9110

Impact of Blanching and Drying on the Nutritional and Antibacterial Quality of *Moringa oleifera* Leaf Powder

Karma Rigyel[1] and Wang Gyeltshen[2]

Abstract

Moringa oleifera leaves are a vital nutritional resource in Bhutan, yet their high perishability necessitates effective post-harvest processing to prevent rapid spoilage and nutrient loss. This study evaluated the effects of various blanching methods (boiling, steaming, and no blanching) and drying techniques (sun, shade, and oven-drying) on the proximate, phytochemical, and antibacterial properties of the resulting leaf powder. Using a 3 x 3 factorial design, the research identified significant interactions between processing treatments. Results indicated that shade-drying consistently outperformed other drying methods in mineral and protein retention. Specifically, boiling combined with shade-drying maximized crude protein and calcium content, whereas steaming with shade-drying was superior for preserving total phenolics and flavonoids. Conversely, boiling led to the greatest reduction in vitamin C and tannins due to thermal leaching. Notably, the combination of steaming and sun-drying yielded the most potent antibacterial activity against Staphylococcus aureus and Escherichia coli. These findings demonstrate that no single processing protocol optimizes all quality parameters simultaneously; instead, methods must be tailored to the intended end-use of the powder. The study concludes that steaming with shade-drying is ideal for maximizing antioxidant and medicinal functionality, while boiling with shade-drying is preferred for enhancing protein-based nutritional security. These results provide actionable processing protocols for smallholder farmers to improve value-added Moringa products and enhance local food security.

Keywords: *Antibacterial activity, Blanching methods, Drying techniques, Moringa oleifera, Phytochemical retention*

Corresponding author: karmarigyel1314@gmail.com

[1] College of Natural Resources, Royal University of Bhutan

[2] College of Natural Resources, Royal University of Bhutan

1 Introduction

Moringa oleifera, commonly known as the “miracle tree”, has gathered global attention for its exceptional nutritional and therapeutic properties (Khan & Ali, 2023). Native to the Indian subcontinent and widely cultivated across tropical and subtropical regions, its leaves are particularly valued for their dense concentration of essential micronutrients and bioactive compounds (Sundaram & Babu, 2024). These include vitamin C, beta-carotene, calcium, potassium, iron, and a diverse range of phytochemicals such as phenolic acids and flavonoids (Khan et al., 2022). Owing to these components, the leaves exhibit potent antioxidant, anti-inflammatory, and antimicrobial activities, positioning *Moringa* as a promising functional food capable of addressing malnutrition and promoting public health in developing countries (Fahey, 2005).

Despite this nutritional richness, the high perishability of fresh *Moringa* leaves due to their elevated moisture content presents a significant barrier to their storage, distribution, and year-round utilization. Post-harvest processing, particularly through blanching and drying, plays a critical role in preserving leaf quality and extending shelf life. Blanching is essential to inactivate endogenous enzymes that accelerate nutrient degradation; however, it also risks the leaching of water-soluble nutrients if not properly controlled. While steaming is often cited as a gentler mechanism than boiling (Oduro et al., 2008), the subsequent drying technique, whether sun, shade, or oven-drying, further dictates the final nutrient retention and microbial safety of the powder (Batiha et al., 2020).

Current research indicates that the specific combination of pretreatment and drying significantly alters the phytochemical profile, yet standardized practices for these interactive factors remain limited (Abano et al., 2020). This lack of consistency in processing methods leads to high variability in the nutritional profile of *Moringa*-based products, limiting their reliability as effective dietary supplements (Moyo et al., 2011).

To address this gap, this study systematically evaluated the synergistic effects of different blanching and drying methods. The primary objectives were:

1. To assess the impact of boiling and steaming blanching methods on the nutritional composition of Moringa leaves.
2. To compare the effectiveness of sun-drying, shade-drying, and oven-drying in preserving the nutrients.

3. To evaluate the interactions between blanching and drying methods on the proximate, nutritional, phytochemical compositions and antibacterial activities.

The study was guided by the following hypotheses:

- Steaming followed by shade-drying results in significantly higher retention of heat-sensitive nutrients and phytochemicals compared to boiling and sun-drying.
- Significant interactions exist between blanching and drying methods, whereby the efficacy of a drying technique is dependent on the initial blanching pretreatment.

By identifying the optimal processing combination, this research aims to support the development of standardized post-harvest protocols that ensure nutrient density and product consistency, ultimately contributing to improved food security and value-addition in the agricultural sector.

2 Materials and Method

2.1 Study site and experimental design

Fresh *Moringa oleifera* leaves were collected approximately in equal amounts of 1 kilogram from 9 different trees aged 3 to 5 years old from Sampheling Gewog, Chukha Dzongkhag. It is located at an elevation of 300 masl (26°50’34.7” N 89° 25' 41.1” E). The research was carried out at the laboratory of College of Natural Resources (CNR), Lobesa, under Punakha Dzongkhag.

As shown in Table 1 the samples collected were divided into 9 portions weighing each at 500 grams. The portions were then subjected to different blanching methods. Dried samples were then turned to fine powder using a food grinder. The samples were collected in cubed polyphenol bottles separately and labelled.

Table 1. Treatment allocation

Treatment	Blanching Method	Drying Method	Replications
T1	Boiling (100°C, 1 min)	Oven-drying	3
T2	Boiling (100°C, 1 min)	Shade-drying	3
T3	Boiling (100°C, 1 min)	Sun-drying	3
T4	No Blanching	Oven-drying	3
T5	No Blanching	Shade-drying	3

T6	No Blanching	Sun-drying	3
T7	Steaming (100°C, 3 min)	Oven-drying	3
T8	Steaming (100°C, 3 min)	Shade-drying	3
T9	Steaming (100°C, 3 min)	Sun-drying	3
Total			**27**

2.2 Methods

The methods used in this research were described and recommended by the Laboratory Manual for Analysis of Agricultural Commodities and Food Products CNR, Royal University of Bhutan and the 20th edition of the Association of Official Analytical Chemists' Official Method of Analysis, Arlington, Virginia.

2.2.1 Moisture Content

The gravimetric method of measuring moisture content is a methodical process that uses controlled drying to calculate the percentage of moisture in food or agricultural samples. After adding a 2 g sample to the crucible, the total weight was determined. Following an overnight drying process at 105°C to remove any remaining moisture, the crucible and sample were cooled in a desiccator and weighed again to determine their dried weight. The following formula was used to determine the moisture content:

$$Moisture\ Content\ (\%) = \frac{(W_{sample} - W_{dried})}{W_{sample}} * 100 \text{ ....} (1)$$

where:

- W_{Dried} = weight of the sample after oven drying (g)
- W_{Sample} = initial weight of the sample used (g)

2.2.2 Total Ash

Each sample weighed 2 g after being put into empty crucibles. To burn off all the organic matter, the crucible containing the sample was heated to 550°C for six hours in a muffle furnace. The samples were weighed and carefully cooled in a desiccator. The following equation was used to determine the amount of ash:

$$Total\ Ash\ (\%) = \frac{(W_{final} - W_{crucible})}{W_{sample}} * 100 \text{ ....} (2)$$

where:

- W_{final} = weight of the crucible containing the ash after incineration (g)
- $W_{crucible}$ = weight of the clean, empty crucible before adding the sample (g)
- W_{sample} = initial weight of the sample before incineration (g)

2.2.3 Crude Protein

The Kjeldahl method for crude protein determination involves three sequential steps: digestion, distillation, and titration. During digestion, 0.25 g of the sample was mixed with 10 ml of concentrated sulfuric acid and 3-4 g of catalyst, then heated in a digestion block pre-set to 420°C until the mixture turned clear. After cooling, 10 ml of distilled water was added to dissolve salts. The sample was distilled into a 250 ml conical flask using an automated distillation unit. Finally, the distillate was titrated with 0.1 N HCl using a mixed indicator to quantify nitrogen content.

$$Nitrogen\ (\%) = \frac{(V_s - V_b) * N * 14.007}{(W * 1000)} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$Crude\ Protein\ (\%) = Nitrogen\ N\% \ * \ 6.25 \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

where:

- V_s = Volume of standard acid solution used to titrate the sample (mL)
- V_b = Volume of standard acid solution used to titrate the blank (mL)
- 14.007 = Atomic weight of Nitrogen (g/mol)
- N = Precise normality of the standard acid titrant solution
- W = Initial weight of the dry sample (g)
- 6.25 = Empirical protein conversion factor based on standard plant/animal tissue nitrogen ratios

2.2.4 Crude Fat

Crude fat content was determined using the Soxhlet extraction method. Clean beakers were initially rinsed and placed in a hot air oven at approximately 100°C to ensure complete drying. Once dried, they were cooled in a desiccator to room temperature and weighed to obtain the initial weight (W1). A sample weighing between 2 g (W) was placed into a thimble, which was then inserted into the thimble holder. Approximately 80 ml of petroleum ether was added to each beaker. The system was run at a temperature 10-20°C above the solvent's boiling point for 45-60 minutes, followed by solvent recovery at a higher temperature (120°C). Beakers were rinsed, re-dried, cooled in a desiccator, and weighed again (W_2) to determine fat content gravimetrically.

$$Crude\ Fat\ (\%) = \frac{(W_2 - W_1)}{W} * 100 \ldots\ldots\ldots\ldots\ldots\ldots(5)$$

where:

- W1 = initial weight of the clean, dry beaker (g)
- W2 = final weight of the beaker containing the extracted fat (g)
- W = precise weight of the initial sample used for extraction (g)

2.2.5 Carbohydrate

The carbohydrate content of a food was not determined directly but obtained by the method of differences as shown below.

$$Carbohydrate\ (\%) = 100 - (\%\,Ash + \%Moisture + \%Protein + \%fat + \%fibre)..\ (6)$$

where:

- Moisture: The water content is removed by drying the sample
- Ash: The inorganic mineral residue left after burning the sample
- Protein: The crude protein percentage calculated from your total nitrogen
- Fat: The crude fat or ether extract component
- Fiber: The crude or dietary fiber content

2.2.6 Phosphorus

The procedure for determining phosphorus content begins with weighing approximately 3 g of the sample in a crucible. The sample was then dried for at least 12 hours in a 105°C hot air-forced oven. After the dry sample was weighed and heated to 450°C for 6 to 8 hours in a muffle furnace, the ash sample was moved to a 250 ml conical flask. 10 ml of diluted HNO3 were used to dissolve the ash residue, which was then agitated for 5 to 10 minutes. After passing through filter paper into a 100 ml volumetric flask, this solution was diluted with distilled water to the appropriate level. 10 ml of vanadomolybdate solution was added to a 5 ml aliquot of this sample in a 50 ml volumetric flask. The solution was then diluted with distilled water to the appropriate level and thoroughly shaken. A yellow hue that lasts for several days appears after 30 minutes of standing, and a spectrophotometer was used to measure the absorbance at 420 nm.

2.2.7 Potassium

A 5 ml aliquot sample was obtained and put in a 50 ml volumetric flask to measure the potassium concentration. The capacity was increased to 50 ml by adding distilled water. The sample material was not added to a blank solution, which was made in the same way. A calibrated flame photometer that had been used to create a standard curve was used to atomize the sample and the blank. Lastly, the standard curve was used to calculate the sample's potassium level based on the measured absorbance.

2.2.8 Calcium

A 50 ml volumetric flask was filled with a 5 ml aliquot sample to measure the calcium concentration. 50 ml was the final capacity after adding distilled water. In the same way, but without the sample material, a blank solution was made. On a calibrated flame photometer that had been used to create a standard curve, the sample and the blank were both atomized. Lastly, using the standard curve, the observed absorbance was used to calculate the sample's calcium concentration.

2.2.9 Sodium

A 5 ml aliquot sample was obtained and put in a 50 ml volumetric flask to measure the sodium concentration. The capacity was increased to 50 ml by adding distilled water. The sample material was not added to a blank solution, which was made in the same way. A calibrated flame photometer that had been used to create a standard curve was used to atomize the sample and the blank. Lastly, the standard curve was used to calculate the sample's salt content based on the measured absorbance.

2.2.10 Iron

2.5 g of the sample were weighed and reduced to ash by the muffle furnace for six hours to analyse it. After treating the ash with 10 ml of nitric acid, the mixture was filtered. After that, 10 ml of the produced sample solution were collected, and 8 ml of new buffer, 1 ml of hydroxylamine hydrochloride, and 5 ml of 1,10-phenanthroline were added. For fifteen minutes, this solution was left in a dark environment to allow the colour to develop. The solution was then diluted with 100 ml of distilled water, and a spectrophotometer was used to measure its absorbance at 510 nm.

2.2.11 Magnesium

After weighing 2 g of the material into a sanitized crucible, it was reduced to ash for four hours at 550°C in a muffle furnace until a white or grey ash was produced. 5 mL of 20% hydrochloric acid (HCl) was added to the ash after the crucible had cooled in a desiccator. Distilled water was then added to bring the volume up to 50 ml. The minerals were dissolved by thoroughly mixing this mixture. 1 mL of the digested sample was pipetted into a clean flask, diluted with 19 mL of distilled water, and then 5 mL of ammonium buffer solution was added to get the pH down to about 10 before the Eriochrome Black T indicator was added to determine the magnesium content. The solution was then titrated with 0.01 M EDTA until the colour changes from pink to blue, indicating the endpoint.

2.2.12 Vitamin C

Place the 5 g sample into a suitable blender and make up to 50 ml of 3% metaphosphoric acid (HPO_3). The mixture was blended until it was thoroughly homogenized. Filter the blended mixture to remove any solid particles and obtain a clear liquid extract. Took an aliquot 2-10 ml of the filtrate and titrate with the standardized DCPIP dye solution to a pink end point.

2.2.13 Total Phenolic Content

To find TPC, take 1 ml of the sample solution and add 1 ml of diluted Folin-Ciocalteu phenol reagent. The ingredients were mixed by gently shaking the flask. Following five minutes of incubation in a dark environment, three ml of a 20% Na_2CO_3 solution were added to the mixture, and it was gently shaken to ensure it was well combined. After that, the mixture was left to incubate for 30 to 45 minutes at room temperature in a dark environment. Finally, using a reagent blank as a reference, the absorbance of the solution was measured at 765 nm using a UV-Visible spectrophotometer.

2.2.14 Total Flavonoid Content

Using 0.5 ml of the sample solution, 2.15 ml of ethanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M sodium acetate, and 2.8 ml of distilled water were added to measure the total flavonoid concentration. After thoroughly mixing the solution using a vortex mixer, it was allowed to sit at room temperature for half an hour. Lastly, a spectrophotometer was used to measure the absorbance of the sample solutions and a blank at 415 nm.

2.2.15 Total Tannin Content

0.1 ml of the sample solution was obtained, and 0.5 ml of Folin-Ciocalteu phenol reagent and 1 ml of 35% sodium carbonate (Na_2CO_3) solution were added to measure the total tannin content. After that, distilled water was added to dilute the solution to the 10 ml mark, and it was well mixed by shaking. To finish the reaction, the mixture was left to stand at room temperature for half an hour. Following this incubation period, a UV/visible spectrophotometer was used to measure the absorbance of the sample and standard solutions at 700 nm in comparison to a blank (a mixture devoid of sample extract or tannic acid).

2.2.16 Antibacterial Activities

Using distilled water, bacterial suspensions were made for each Staphylococcus and Escherichia coli strain, and the turbidity was adjusted to a 0.5 McFarland standard, or around 1 × 10^8 CFU/mL. A sterile inoculation loop was then used to scatter each bacterial suspension evenly onto Mueller Hinton agar plates. After dissolving crude and fractional extracts in the proper solvents, solutions with concentrations of 0, 25, 50, 75, and 100 mL were created. To ensure uniform spacing, wells with a diameter of 10 mm were made in the inoculated agar plates using a sterile cork borer. 100 µL of each extract solution was carefully added into the respective wells, with 100 µL of gentamycin solution (0.1 mL) serving as a positive control and 100 µL of 70% ethanol as a negative control. To promote diffusion, the plates are left to stand at room temperature for two hours before being incubated for twenty-four hours at 37°C. Following incubation, a calliper or ruler was used to measure the zones of inhibition surrounding each well in ml. Additional test tubes containing extract concentrations of 25, 50, 75, and 100 mL are created to calculate the Minimum Inhibitory Concentration (MIC). Each tube was filled with 1 mL of bacterial suspension, and the MIC was determined by incubating the tubes at 37°C for 24 hours. ml was used to measure the zone of inhibition.

2.3 Statistical Analysis

The study employs a factorial experimental design, where each combination of blanching and drying methods represents a distinct treatment, resulting in nine treatment groups. The independent variables include the blanching method (No blanching, Boiling, Steaming) and drying method (Sun-drying, Shade-drying, Oven-drying).

The dependent variables are the proximate, nutrients, phytochemical compositions, and antibacterial activities of the Moringa leaves. Data was analysed using JASP statistical software. Shapiro-Wilk tests were performed within JASP to check the normality of the data. Levene's tests were performed within JASP to check the homogeneity of variance of the data.

Analysis of Variance (ANOVA): A two-way ANOVA was conducted within JASP to determine the individual and interactive effects of the blanching and drying treatments on the proximate, nutritional, phytochemical compositions, and antibacterial activities.

Post-Hoc Tests: When significant effects were observed in the ANOVA, Tukey's Honest Significant Difference (HSD) test was used to compare mean differences between treatment groups. Significance Level was determined at $p < 0.05$.

3 Results and Discussion

This study assessed the effects of different blanching treatments (boiling, steaming, and no blanching) and drying methods (oven-drying, sun-drying, and shade-drying) on the proximate composition, mineral content, phytochemicals, and antibacterial activity of *Moringa oleifera* leaf powder. The data, analyzed through two-way ANOVA and Tukey's HSD test, revealed significant interactions between treatments, underscoring the combined influence of processing methods on leaf quality. This work provides a foundation for optimizing processing techniques to maintain the integrity and benefits of *Moringa oleifera* leaves for food and therapeutic applications.

3.1 Proximate Composition

3.1.1 Moisture

A statistically significant interaction between blanching and drying methods profoundly influenced the moisture content of *Moringa oleifera* leaf powder. Boiled, shade-dried samples (T2) exhibited the highest moisture content at 15.08 ± 0.80% (Table 2), attributable to water absorption during boiling, which increases tissue permeability (Sallau et al., 2012), coupled with the slower evaporation rates inherent to shade-drying under ambient conditions (Alakali et al., 2015). Conversely, non-blanched, sun-dried samples (T6) recorded the lowest moisture content at 3.93 ± 1.25%, reflecting the rapid dehydration facilitated by direct solar radiation and elevated temperatures. Elevated moisture levels in shade-dried samples predispose the powder to microbial proliferation, compromising shelf life (Hassan et al., 2015). However, this characteristic could be advantageous in applications requiring rehydration, such as in fresh food formulations or culinary preparations where texture is paramount.

3.1.2 Total Ash

The interaction between blanching and drying methods significantly affected total ash content, an indicator of mineral retention. Non-blanched, oven-dried samples (T4) demonstrated the highest ash content at 17.99 ± 0.39 g/100 g, underscoring the efficacy of oven-drying in preserving heat-stable minerals compared to alternative drying methods (Potisate et al., 2014). In contrast, boiled, shade-dried samples (T2) exhibited the lowest ash content at 11.90 ± 0.14 g/100 g, due to the leaching of water-soluble minerals during boiling (Joshi & Mehta, 2010). This loss is exacerbated by the prolonged drying duration of shade-drying, which facilitates further mineral diffusion. High ash content is a desirable trait for developing nutrient-dense food products, particularly for populations at risk of mineral deficiencies, highlighting the suitability of non-blanching and oven-drying for such applications.

3.1.3 Crude Protein

A significant interaction between blanching and drying methods was observed for crude protein content. Boiled, sun-dried samples (T3) exhibited the highest protein content at 35.42 ± 0.60 g/100 g, due to boiling-induced disruption of cellular matrices, which enhances protein extractability (Mutiara et al., 2013). Sun-drying, characterized by moderate temperatures, minimizes protein denaturation compared to oven-drying's higher thermal exposure or shade-drying's prolonged duration (Emelike et al., 2015). Conversely, non-blanched, shade-dried samples (T5) recorded the lowest protein content at 18.21 ± 1.06 g/100 g, suggesting that the absence of thermal pretreatment and extended drying time may promote protein degradation or reduced extractability. These findings position boiling followed by sun-drying as an optimal strategy for producing protein-rich *Moringa* leaf powder, suitable for formulating protein-fortified dietary supplements or functional foods targeting protein-energy malnutrition.

Table 2. Effect of different treatments on proximate composition

Treatments	Moisture (%)	Total Ash (g/100g)	Crude Protein (g/100g)
T1	6.297±1.365	15.257±0.172	25.98±1.711
T2	15.077±0.803	11.903±0.139	26.273±1.095

T3	7.51±1.801	15.89±0.213	35.417±0.597
T4	6.993±2.671	17.99±0.392	23.317±0.797
T5	7.55±2.164	17.84±0.299	18.21±1.062
T6	3.93±1.247	17.1±0.229	20.92±1.704
T7	5.353±1.688	17.267±0.208	27.5±0.887
T8	10.557±1.861	17.6±0.180	21.647±0.578
T9	5.603±2.398	17.66±0.400	28.023±0.497

Each value is a mean ± standard deviation of three replicates.

3.1.4 Crude Fat

The interaction between blanching and drying methods significantly influenced crude fat content. Boiled, oven-dried samples (T1) exhibited the highest fat content at 6.63 ± 0.31 g/100 g, due to boiling's ability to release bound lipids from cellular structures (Ogunsina et al., 2011), coupled with oven-drying's controlled environment, which minimizes oxidative degradation (Roshanak et al., 2016). In contrast, boiled, shade-dried samples (T2) had the lowest fat content at 4.17 ± 0.21 g/100 g, due to the prolonged exposure to ambient conditions facilitating lipid oxidation. Higher fat content enhances the energy density of *Moringa* leaf powder, making it a valuable ingredient for energy-rich fortified foods, particularly in resource-constrained settings where caloric intake is a concern.

3.1.5 Carbohydrate

Carbohydrate content was significantly affected by the interaction of blanching and drying methods. Non-blanched, shade-dried samples (T5) recorded the highest carbohydrate content at 44.76 ± 3.10 g/100 g, reflecting minimal leaching of water-soluble carbohydrates during processing and effective preservation under shade-drying's mild conditions (Oyeyinka & Oyeyinka, 2018). Conversely, boiled, sun-dried samples (T3) exhibited the lowest carbohydrate content at 30.29 ± 1.41 g/100 g, due to leaching of soluble sugars during boiling (Wickramasinghe et al., 2020) and accelerated degradation under sun-drying's higher temperatures. High carbohydrate content positions *Moringa* leaf powder as a viable carbohydrate source for dietary supplements, particularly for energy-dense formulations targeting carbohydrate-deficient diets.

3.2 Nutrient and Mineral Composition

3.2.1 Available Phosphorus

A significant interaction between blanching and drying methods influenced phosphorus content. Boiled, oven-dried samples (T1) exhibited the highest phosphorus content at 300.23 ± 22.60 mg/100 g, due to thermal processing enhancing phosphorus bioavailability by breaking down phytate complexes (Glover-Amengor & Mensah, 2012). Oven-drying's controlled conditions further preserve this mineral compared to shade- or sun-drying. Non-blanched, shade-dried samples (T5) recorded the lowest phosphorus content at 83.71 ± 0.54 mg/100 g (Table 3), suggesting minimal disruption of phosphorus-binding compounds without thermal pretreatment. These findings highlight the potential of boiling and oven-drying for producing phosphorus-rich *Moringa* products.

3.2.2 Potassium

While no significant interaction was observed, the main effects of blanching and drying methods significantly influenced potassium content. Non-blanched, shade-dried samples (T5) exhibited the highest potassium content at 107.7 ± 22.55 mg/100 g, reflecting minimal leaching and effective preservation under shade-drying's low-temperature conditions (Abano et al., 2020). In contrast, steamed, oven-dried samples (T7) recorded the lowest potassium content at 39.37 ± 3.82 mg/100 g, due to steam-induced leaching and oven-drying's thermal effects. High potassium content is crucial for formulating functional foods aimed at supporting cardiovascular health, underscoring the efficacy of shade-drying for potassium retention (Alakali et al., 2015).

3.2.3 Sodium

No significant interaction was observed, but main effects of blanching and drying methods significantly affected sodium content. Non-blanched, oven-dried samples (T4) exhibited the highest sodium content at 81 ± 1 mg/100 g, reflecting effective retention under controlled drying conditions. Boiled, sun-dried samples (T3) recorded the lowest sodium content at 59.33 ± 1.53 mg/100 g, likely due to sodium leaching during boiling (Sánchez-Machado et al., 2010). These findings suggest that non-blanching and oven-drying are optimal for sodium-rich *Moringa* products, which may be valuable in regions where sodium intake needs supplementation.

Table 3. Effect of different treatments on nutrient composition

Treatments	Phosphorus (mg/100g)	Potassium (mg/100g)	Sodium (mg/100g)

T1	300.225±22.596	68.3±16.250	66±1.000
T2	260.125±29.867	102.733±10.769	63±1.000
T3	250.05±14.396	88.267±18.413	59.333±1.528
T4	242.975±27.137	53.8±27.921	81±1.000
T5	83.709±0.539	107.7±22.554	79±1.000
T6	193.7±44.296	103.3±14.646	74.833±1.607
T7	288.2±20.740	39.367±3.821	75±1.000
T8	249.2±9.094b	69.833±33.403	72±1.000
T9	197.575±27.701	86.367±11.448	70±1.000

Each value is a mean ± standard deviation of three replicates.

3.2.4 Calcium

A strong interaction between blanching and drying methods significantly influenced calcium content. Boiled, oven-dried samples (T1) exhibited the highest calcium content at 242.12 ± 1.46 mg/100 g (Table 5), due to boiling’s ability to dissolve oxalate complexes, thereby enhancing calcium bioavailability (Sallau et al., 2012). Oven-drying’s controlled environment further preserves this mineral. Non-blanched, shade-dried samples (T5) recorded the lowest calcium content at 152.25 ± 0.50 mg/100 g, suggesting limited disruption of calcium-binding compounds without thermal pretreatment. High calcium content is critical for developing *Moringa*-based products to support bone health, particularly in populations with limited dairy access (Sundaram & Babu, 2024).

3.2.5 Vitamin C Content

A significant interaction affected vitamin C content. Non-blanched, sun-dried samples (T6) exhibited the highest vitamin C content at 28.74 ± 0.50 mg/100 g, reflecting minimal thermal degradation without blanching and effective preservation under sun-drying’s rapid drying conditions. Boiled, sun-dried samples (T3) recorded the lowest vitamin C content at 10.93 ± 0.60 mg/100 g, likely due to thermal degradation and leaching during boiling (Igbokwe et al., 2020). These findings highlight the importance of non-blanching and sun-drying for preserving vitamin C, a critical antioxidant for immune health.

Table 4. Effect of different treatments on nutrient composition

Treatments	Calcium (mg/100g)	Vitamin C (mg/100g)	Iron (mg/100g)	Magnesium (mg/100g)

T1	242.117±1.464	24.227±1.195	10.633±0.375	174.133±2.548
T2	216.563±3.353	15.818±0.622	7.483±0.391	124.027±5.857
T3	237.577±1.501	10.927±0.599	16.385±0.293	163.2±2.234
T4	235.337±2.719	26.166±0.578	12.233±0.254	184.487±3.788
T5	152.25±0.503	18.14±0.363	8.095±0.251	100.227±1.687
T6	178.93±2.290	28.739±0.495	5.87±0.491	110.523±3.713
T7	239.637±0.189	16.059±0.756	32.106±0.391	179.043±3.937
T8	211.07±2.618	15.105±0.889	27.708±0.683	155.497±3.760
T9	212.743±0.96	21.997±0.794	13.873±0.298	198.957±2.582

Each value is a mean ± standard deviation of three replicates.

3.2.6 Iron

A significant interaction influenced iron content (Table 4). Steamed, oven-dried samples (T7) exhibited the highest iron content at 32.11 ± 0.39 mg/100 g, due to steaming's ability to reduce binding compounds, enhancing iron bioavailability (Keshinro & Ketiku, 2018). Oven-drying further preserves this mineral. Non-blanched, sun-dried samples (T6) recorded the lowest iron content at 5.87 ± 0.49 mg/100 g, suggesting limited disruption of iron-binding compounds without thermal pretreatment. High iron content is essential for addressing iron deficiency anemia, positioning steaming and oven-drying as optimal for iron-rich *Moringa* products.

3.2.7 Magnesium

A significant interaction affected magnesium content. Steamed, sun-dried samples (T9) exhibited the highest magnesium content at 198.96 ± 2.58 mg/100 g, due to steaming enhancement of magnesium availability and sun-drying's rapid preservation, which minimizes losses (Gopalakrishnan et al., 2016). Non-blanched, shade-dried samples (T5) recorded the lowest magnesium content at 100.23 ± 1.69 mg/100 g, reflecting limited disruption of magnesium-binding compounds. These findings suggest that steaming and sun-drying are optimal for magnesium-rich *Moringa* products, supporting muscle and nerve function.

3.3 Phytochemical Composition

3.3.1 Total Phenolic Content

No significant interaction was observed, but main effects of blanching and drying methods significantly influenced TPC. Steamed, shade-dried samples (T8) exhibited the highest TPC (Figure 1) at 102.19 ± 2.33 mg GAE/g DW, due to steaming's enhancement of phenolic extraction by disrupting cellular structures (Razzak et al. 2022) and shade-drying's minimal thermal degradation (Iqbal & Bhanger, 2016). Non-blanched, oven-dried samples (T4) recorded the lowest TPC at 60.12 ± 1.22 mg GAE/g DW, reflecting thermal degradation under oven-drying's higher temperatures. High TPC is associated with antioxidant activity, making steamed, shade-dried *Moringa* powder a valuable ingredient for functional foods targeting oxidative stress-related conditions.

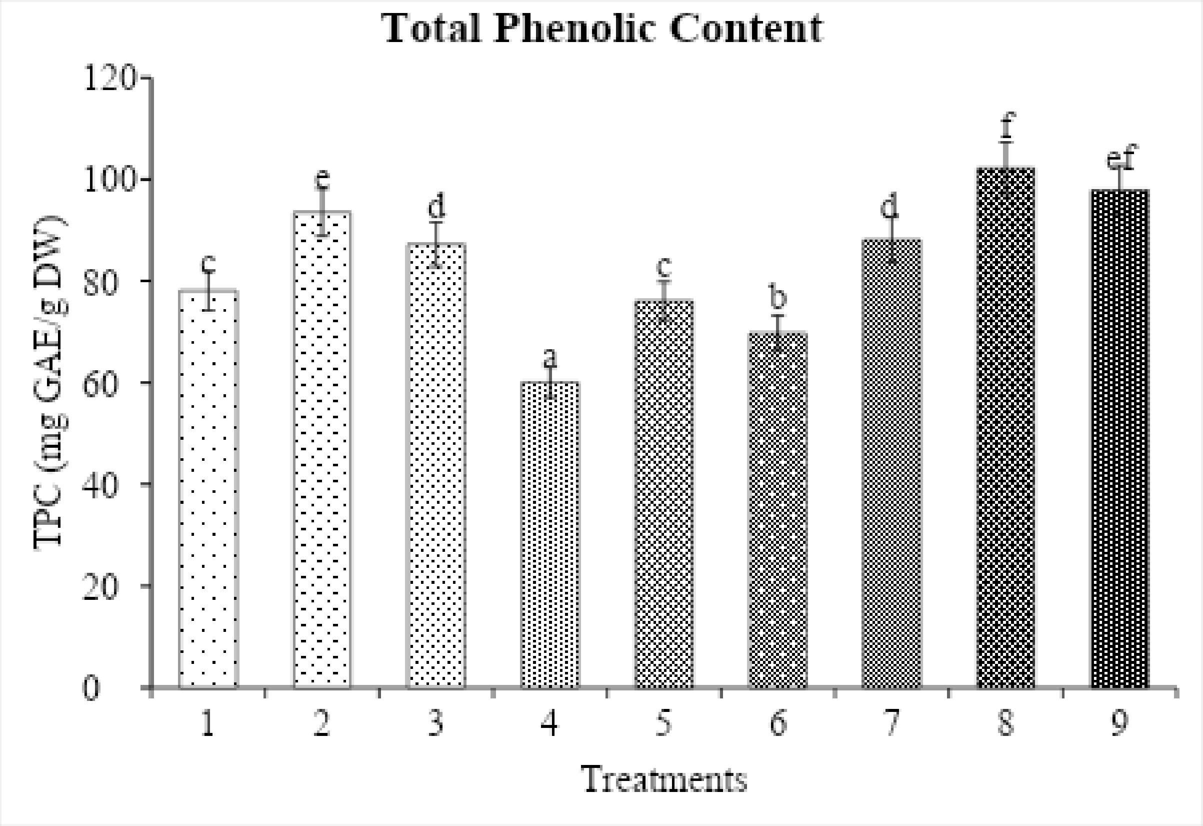


Figure 1: Effect of different treatments on Total Phenolic Content (TPC)

The error bars are the standard error of the mean. Different superscripts along the column indicate significant difference of the mean (P ≤ .05).

3.3.2 Total Flavonoid Content

No significant interaction was observed, but main effects significantly affected TFC. Steamed, shade-dried samples (T8) exhibited the highest TFC at 41.45 ± 2.56 mg QE/g DW, reflecting steaming ability to enhance flavonoid availability and shade-drying's preservation of these heat-sensitive compounds (Makkar & Becker, 2007). Non-blanched, oven-dried samples (T1) recorded the lowest TFC at 26.22 ± 3.56 mg QE/g DW, due to thermal degradation. High TFC contributes to anti-inflammatory and antioxidant properties, underscoring the potential of steaming and shade-drying for flavonoid-rich *Moringa* products.

3.3.3 Total Tannin Content

A significant interaction influenced TTC. Non-blanched, sun-dried samples (T6) exhibited the highest TTC at 22.37 ± 0.78 mg TAE/g DW, reflecting minimal leaching without blanching and effective preservation under sun-drying's rapid conditions. Boiled, oven-dried samples (T1) recorded the lowest TTC at 10.37 ± 0.58 mg TAE/g DW, likely due to tannin leaching during boiling (Kasolo et al., 2018). While high tannin content acts as an anti-nutrient, reducing mineral bioavailability (Samtiya et al., 2020), it also contributes to antioxidant and antimicrobial properties, necessitating a balance in processing methods based on intended applications.

3.3.4 Antibacterial Activities

A significant interaction between blanching and drying methods profoundly influenced the antibacterial activity of *Moringa oleifera* leaf powder. Steamed, shade-dried samples (T8) demonstrated the highest zones of inhibition (Table 5) against *Escherichia coli* (15.00 ± 0.57 mm) and *Staphylococcus aureus* (18.83 ± 0.79 mm), attributable to their elevated phenolic (102.19 mg GAE/g DW) and flavonoid (41.45 mg QE/g DW) contents, which disrupt bacterial cell membranes through oxidative stress and enzyme inhibition (Cowan, 1999). Boiled, oven-dried samples (T1) exhibited the lowest antibacterial activity (*E. coli*: 4.68 ± 0.80 mm; *S. aureus*: 8.40 ± 0.61 mm), reflecting reduced antimicrobial compound levels due to boiling-induced degradation (Vongsak et al., 2013). Notably, *S. aureus* was more susceptible than *E. coli*, due to its Gram-positive cell wall's greater permeability to phytochemicals compared to the Gram-negative outer membrane of *E. coli* (Salaheen et al., 2015). These findings underscore the potential of steaming and shade-drying for producing *Moringa* leaf powder with enhanced antibacterial properties, offering applications in natural food preservatives or therapeutic formulations targeting bacterial infections.

Table 5. Effect of different treatments on antibacterial activities

Treatments	*Escherichia coli* (mm)	*Staphylococcus aureus*(mm)
T1	4.675±0.802	8.4±0.606

T2	6.125±0.665	11.05±0.443
T3	5.1±0.762	9.65±0.387
T4	8.75±1.848	13.525±0.873
T5	10.25±2.577	16.05±1.173
T6	7.05±1.109	11.5±1.581
T7	11.025±1.181	16.7±0.516
T8	15±0.572	18.825±0.789
T9	12.85±0.723	16.45±1.682

Each value is a mean ± standard deviation of three replicates.

4 Conclusion

This study demonstrates that the nutritional and functional value of *Moringa oleifera* leaf powder is not just a result of the raw material but is significantly shaped by post-harvest processing. The findings reveal a critical trade-off: while boiling assists in breaking down plant cells to make proteins and minerals more accessible, it simultaneously reduces water-soluble vitamins through leaching. In contrast, steaming acts as a gentler mechanism, stopping the natural enzymes that would otherwise break down nutrients without the heavy losses associated with boiling. The choice of drying method is equally vital. While sun-drying is the most accessible method for many, the harsh intensity of direct sunlight degrades sensitive health-promoting compounds. Shade-drying emerges as the superior choice for preserving the antioxidants and natural antibiotics of the leaves. However, the study also highlights a practical challenge: shade-drying may lead to higher moisture levels, which requires careful management to prevent spoilage. For smallholder farmers and local processors, the adoption of steaming followed by shade-drying is recommended as the most effective way to produce high-quality *Moringa* powder. For industrial applications where speed and consistency are required, oven-drying provides a balanced alternative. Future research should focus on how these different powders behave during long-term storage under varying climatic conditions, particularly the high-humidity regions of Bhutan. Additionally, exploring the bioavailability of these nutrients like how much of the preserved protein and minerals are absorbed by the human body and how it would provide the final piece of the puzzle in using *Moringa* to combat regional malnutrition.

5 Acknowledgement

I would like to express my sincere gratitude to the College of Natural Resources under the Royal University of Bhutan for providing the necessary facilities, resources, and institutional support that made this study possible. I am also grateful to my family, friends, and everyone whose continuous support and belief in me made this achievement possible. Their encouragement has been invaluable throughout this research journey.

6 Authors' Contribution Statement

This research was conducted by Mr. Karma Rigyel in partial fulfillment of the academic requirements for BSc Organic Agriculture programme at the College of Natural Resources. He was responsible for the primary execution of the research, including reviewing the literature, designing the research methodology, conducting data collection, performing data analysis, and drafting the original manuscript. Mr. Wang Gyeltshen, Associate Lecturer, served as the research supervisor, providing academic guidance and structural direction throughout all stages of the study.

7 References

Abano, E. E., Amoah, R. S., & Mbabazi, C. (2020). Microwave and steam blanching as pre-treatments before air drying of *Moringa oleifera* leaves. *Journal of Agricultural Engineering, 51*(4), 200–208. https://doi.org/10.4081/jae.2020.1044

Alakali, J. S., Kucha, C. T., & Rabiu, I. A. (2015). Effect of drying temperature on the nutritional quality of *Moringa oleifera* leaves. *African Journal of Food Science, 9*(7), 395–399. https://doi.org/10.5897/AJFS2014.1145

AOAC. (2016). *Official methods of analysis* (20th ed.) Association of Official Analytical Chemists.

Batiha, G. E., Alkazmi, L., & Osman, S. M. (2020). Nutritional and medicinal value of *Moringa oleifera*. *Food Research International, 137*, Article 109312. https://doi.org/10.1016/j.foodres.2020.109312

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*(4), 564–582. https://doi.org/10.1128/CMR.12.4.564

Emelike, N. J., Akusu, M. O., & Kiin-Kabari, D. B. (2015). Effect of drying methods on the nutritional and phytochemical composition of *Moringa oleifera* leaves. *Nigerian Food Journal, 33*(2), 139–144.

Fahey, J. W. (2005). *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. *Trees for Life Journal, 1*, 5–10.

Glover-Amengor, M., & Mensah, F. (2012). Effect of processing on *Moringa oleifera* leaves. *African Journal of Food, Agriculture, Nutrition and Development, 12*(6), 6677–6687.

Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness, 5*(2), 49–56. https://doi.org/10.1016/j.fshw.2016.04.001

Hassan, H. M., & Abd El-Samee, M. A. S. (2015). Growth, yield and nutritional value of roselle (*Hibiscus sabdariffa* L.) as influenced by licorice and moringa aqueous extracts under North Sinai conditions. *Zagazig Journal of Agricultural Research, 42*(5), 1069–1079.

Igbokwe, P. C., Umeh, S. I., & Okoye, J. O. (2020). Impact of thermal processing on vitamin C retention in *Moringa oleifera* leaves. *Journal of Food Biochemistry, 44*(6), e13212.

Iqbal, S., & Bhanger, M. I. (2016). Antioxidant properties of *Moringa oleifera*: A comparative study of fresh and dried leaves. *Plant Foods for Human Nutrition, 71*(3), 210–216.

Joshi, P., & Mehta, D. (2010). Effect of dehydration on the nutritive value of drumstick leaves. *Journal of Metabolomics and Systems Biology, 1*, 5–9.

Kasolo, J. N., Bimenya, G. S., & Ogwal-Okeng, J. W. (2018). Tannin content and antinutritional effects of *Moringa oleifera* leaves. *African Journal of Food Science, 12*(4), 78–83.

Keshinro, O. O., & Ketiku, A. O. (2018). Effect of processing on iron bioavailability in *Moringa oleifera* leaves. *Nigerian Journal of Nutritional Sciences, 39*(2), 45–51.

Khan, A. S., & Ali, Q. Z. (2023). *Moringa*—The miracle tree: An overview of its nutritional and medicinal properties. *Asian Journal of Biochemistry, Genetics and Molecular Biology, 15*(3), 32–44. https://doi.org/10.9734/AJBGMB/2023/v15i3337

Khan, F., Nasir, F., & Ikram, H. (2022). *Moringa oleifera*: A miraculous plant to combat malnutrition. *Acta Scientific Nutritional Health, 6*(11), 1–2. https://doi.org/10.31080/ASNH.2022.06.1135

Makkar, H. P. S., & Becker, K. (2007). *Plant secondary metabolites*. Humana Press.

Moyo, B., Masika, P. J., Hugo, A., & Muchenje, V. (2011). Nutritional characterization of *Moringa (Moringa oleifera Lam.)* leaves. *African Journal of Biotechnology, 10*(60), 12925–12933. https://doi.org/10.5897/AJB10.1599

Mutiara, T., Harijono, Estiasih, T., & Sriwahyuni, E. (2013). Effects of blanching treatment on protein content and amino acid composition of drumstick leaves (*Moringa oleifera*). *Journal of Food Research, 2*(1). https://doi.org/10.5539/jfr.v2n1p101

Oduro, I., Ellis, W. O., & Owusu, D. (2008). *Moringa oleifera* leaves: A potential source of nutritional and medicinal properties. *International Journal of Food Science & Technology, 43*(6), 1227–1234. https://doi.org/10.1111/j.1365-2621.2007.01517.x

Ogunsina, B. S., Indira, T. N., Bhatnagar, A. S., Radha, C., Debnath, S., & Gopala Krishna, A. G. (2014). Quality characteristics and stability of *Moringa oleifera* seed oil of Indian origin. *Journal of Food Science and Technology, 51*(3), 503–510. https://doi.org/10.1007/s13197-011-0519-5

Oyeyinka, A. T., & Oyeyinka, S. A. (2018). *Moringa oleifera* as a food fortificant: Recent trends and prospects. *Journal of the Saudi Society of Agricultural Sciences, 17*(2), 127–136. https://doi.org/10.1016/j.jssas.2016.02.002

Potisate, Y., Phoungchandang, S., & Kerr, W. L. (2014). The effects of predrying treatments and different drying methods on phytochemical compound retention and drying characteristics of *Moringa* leaves (*Moringa oleifera* Lam.). *Drying Technology, 32*(16), 1970–1985. https://doi.org/10.1080/07373937.2014.926912

Razzak, A., Roy, K. R., Sadia, U., & Zzaman, W. (2022). Effect of thermal processing on physicochemical and antioxidant properties of raw and cooked *Moringa oleifera* Lam. pods. *International Journal of Food Science*, 2022, Article 1502857. https://doi.org/10.1155/2022/1502857

Roshanak, S., Rahimmalek, M., & Goli, S. A. (2016). Evaluation of seven different drying treatments in respect to total flavonoid, phenolic, vitamin C content, chlorophyll, antioxidant activity and color of green tea (*Camellia sinensis* or *C. assamica*) leaves. *Journal of Food Science and Technology, 53*(1), 721–729. https://doi.org/10.1007/s13197-015-2030-x

Salaheen, S., Kim, S. W., & Haley, B. J. (2015). Antimicrobial activity of *Moringa oleifera* extracts against foodborne pathogens. *Journal of Food Protection, 78*(8), 1561–1567. https://doi.org/10.4315/0362-028X.JFP-15-062

Sallau, A. B., Mada, S. B., Ibrahim, S., & Ibrahim, U. (2012). Effect of boiling, simmering and blanching on the antinutritional content of *Moringa oleifera* leaves. *International Journal of Food Nutrition and Safety, 2*(1), 1–6.

Samtiya, M., Aluko, R. E., & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: An overview. *Food Production, Processing and Nutrition, 2*, Article 6. https://doi.org/10.1186/s43014-020-0020-5

Sánchez-Machado, D. I., Núñez-Gastélum, J. A., Reyes-Moreno, C., & López-Cervantes, J. (2010). Nutritional quality of edible parts of *Moringa oleifera*. *Food Analytical Methods, 3*(3), 175–180. https://doi.org/10.1007/s12161-009-9106-z

Sundaram, D., & Babu, N. K. (2024). *Moringa oleifera*: A review on nutritive significance and its medicinal application for children. *International Journal of Community Medicine and Public Health, 12*(1), 610–615. https://doi.org/10.18203/2394-6040.ijcmph20244081

Vongsak, B., Sithisarn, P., & Gritsanapan, W. (2013). Simultaneous determination of bioactive flavonoids from *Moringa oleifera* leaf extracts. *Journal of Chromatography B, 913–914*, 55–60. https://doi.org/10.1016/j.jchromb.2012.11.032

Wickramasinghe, Y. W. H., Wickramasinghe, I., & Wijesekara, I. (2020). Effect of steam blanching, dehydration temperature and time on the sensory and nutritional properties of herbal tea developed from *Moringa oleifera* leaves. *International Journal of Food Science, 2020*, Article 5376280. https://doi.org/10.1155/2020/5376280

Agriculture Research and Innovation Division
Department of Agriculture
Ministry of Agriculture and Livestock
Thimphu, Bhutan
www.bja.gov.bt

ISSN 2616-3926