Annual Report
FY 2024-2025

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NATIONAL PLANT PROTECTION CENTRE

DEPARTMENT OF AGRICULTURE

MINISTRY OF AGRICULTURE & LIVESTOCK

SEMTOKHA

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FOREWORD

The National Plant Protection Centre is pleased to present this annual report, detailing activities and accomplishments for the fiscal year 2024-2025. As a principal institute for plant protection services, the Centre remains committed to strengthening research, diagnostics, surveillance, and technology promotion to enable data-driven pest management. All interventions were guided by Integrated Pest Management (IPM) principles, which aim to reduce pest impacts while promoting sustainable, environmentally responsible, and economically viable agricultural practices.

During the reporting period, the Centre's seven technical programs, supported by a professional administrative team, implemented targeted actions to reduce crop losses and enhance resilience across farming communities. Key outputs included strengthened laboratory diagnostics, structured field research, and systematic pest surveys that refined the understanding of pest distribution and population dynamics. In parallel, on-farm technology demonstrations, farmer awareness initiatives, and capacity-building for extension personnel were scaled up to accelerate the adoption of integrated pest management practices nationwide.

The activities carried out in the 2024-2025 fiscal year delivered measurable advances in both routine and emergency pest management, contributing directly to national objectives for food and nutrition security. Notable efforts focused on priority threats such as fall armyworm, wheat rust, potato brown rot, invasive and problematic agricultural weeds, and human-wildlife conflict. These interventions have helped safeguard yields and livelihoods while generating data and recommendations to inform policymakers, farmers, and extension officers.

This report documents those achievements and the underlying evidence, reflecting the Centre's continued dedication to serving Bhutan's agricultural sector. The National Plant Protection Centre remains focused on advancing plant protection science and delivering practical, scalable solutions that strengthen national food security and agricultural development. The Centre looks forward to building on this year's progress in the seasons ahead.

Yeshey Dema

Program Director

EXECUTIVE SUMMARY

The National Plant Protection Centre (NPPC) serves as the national referral and coordinating body for plant protection services, overseeing information, policies, and related activities. Additionally, the Centre is mandated to develop and promote Integrated Pest Management (IPM) strategies to manage pests in agricultural cropping systems. In this 2024-2025 Annual Report, the NPPC highlights its key activities and accomplishments for the fiscal year. These initiatives were carried out by the Centre's seven technical Programs: Pathology, Entomology, Weeds, Vertebrate Pest Management, Pest Surveillance, Biocontrol, and Plant Protection Products Program. Below are some of the notable achievements of these programs.

Pathology Program

1. Surveyed the Emergence, Spread, and Management of Potato Bacterial Wilt (*Ralstonia solanacearum*) in Bhutan
2. Assessed the efficacy of Potassium Phosphite to manage Potato Brown Rot (*Ralstonia solanacearum*)
3. Assessed the efficacy of Potassium Phosphite to manage Garlic Rust (*Puccinia allii*)
4. Detection and preservation of Huanglongbing (HLB)-Positive Citrus Samples for Diagnostic Calibration
5. Awareness of Brown Rot Disease and its management
6. Maintenance of native *Trichoderma* cultures

Besides the above targets, the program carried out *ad-hoc* services, including laboratory diagnostics and recommendations on:

7. Paddy yellowing issue at Kharibji and Kharphu, Maedwang gewog, Thimphu dzongkhag
8. Ginger rhizome rot at Loggchina gewog, Chhukha dzongkhag.
9. Infected pear tree samples from Naemjog, Loong-nyi gewog, Paro dzongkhag
10. Potato rot issue from Kanglung gewog, Trashigang dzongkhag
11. Yellowing and wilting of paddy plant: from Gase Tshogom gewog, Wangdue Phodrang dzongkhag
12. Infected pea and zucchini samples from the Regional Seed Centre, Trashi Yangtse dzongkhag
13. Wheat yellowing samples from Yebisa, Chhubu gewog, Punakha dzongkhag
14. Cardamom rotting from Pungtenchhu Gewog, Tsirang dzongkhag
15. Infected coffee samples from major coffee-growing regions (Umling gewog, Sarpang, Serzhong gewog, Sarpang, Pemaling gewog, Samtse, and Ngangla gewog, Zhemgang)
16. Yellowing of leaves and dieback of paddy seedlings from Barp Gewog, Punakha
17. Infected onion samples from Domkhar, Tsaenkhar Gewog, Lhuentse.

Entomology Program

1. Assessed the farmers' knowledge, perception, and management practices of Fall Armyworm (*Spodoptera frugiperda*) in Bhutan
2. Assessed Fall Armyworm incidence and population dynamics using sex pheromone traps in Rilangthang, Sergithang gewog, Tsirang
3. Identification of natural enemies of Fall Armyworm (*Spodoptera frugiperda*) in Bhutan by Laboratory Rearing
4. Monitoring of planthoppers and leafhoppers in paddy fields using light traps
5. Monitoring of Planthoppers and leaf hoppers in paddy using the yellow sticky trap

Weed Science Program

1. Efficacy evaluation of synthetic weedicides for the management of wetland weeds in transplanted paddy cultivation
2. Evaluating the efficacy of a synthetic weedicide on diverse weed populations (broadleaved and grassy) in maize
3. Farmers' awareness and perceptions of Glyphosate use in agricultural production and its impacts
4. Awareness campaign on safe & efficient use of pesticides at Gangteng Gewog and Phobji Gewog in Wangdue Phodrang, Chhapchha Gewog in Chhukha, and Kar-tshog Gewog in Haa
5. Developing Weed Herbarium

Pest Surveillance Program

1. Barberry survey
2. Wheat Rust Sentinel plot establishment
3. Wheat disease surveillance
4. Strengthening the capacity of agriculture extension officers on Wheat Diseases
5. Conducting awareness campaigns on important wheat diseases and their management practices for farmers in major wheat-growing areas
6. Capacity building of plant protection officials on barberry surveys and cereal rust diagnostics
7. Establishment and capacity building on MARPLE Diagnostics for Plant Protection and Research Officials
8. Publication and Dissemination of Wheat Disease Extension Materials
9. Launch of WISER-AP Phase II and Stakeholder Consultation

Plant Protection Product Program

1. The program has initiated the implementation of the online PPP-IMS system to manage the collection and distribution of Plant Protection Products

2. A total of 7,699.04 litres of insecticide, 11,626.35 kg of fungicides, 4,98,735.05 g of herbicides and 5,430.75 litres of biopesticides, and 35,769 numbers of traps/lures have been distributed across the country.

Biocontrol Program

1. Isolation and identification of entomopathogenic fungi from Fall Armyworm (*Spodoptera frugiperda*) in Bhutan

Vertebrate Pest Management Program

1. Portable Electric Fencing (PEF) monitoring
2. Technical Cooperation Project (TCP) with JICA – *Project for Community-based Human–Wildlife Conflict Management (WiCoMP)*

A more detailed information on the activities and targets is presented under each Program in the ensuing report.

Table of Contents

1. Pathology Program

1.1 The Emergence, Spread, and Management of Potato Bacterial Wilt in Bhutan

1.1.1 Introduction

Potato bacterial wilt, also known as brown rot, caused by *Ralstonia solanacearum*, is initially indicated by wilting, yellowing, and bronzing of young leaves, and later by internal stem discoloration (Charkowski et al., 2020). This wilting can be localized or systemic. Stems often display brown discoloration within the xylem vessels. A characteristic symptom is a creamy, slimy bacterial ooze that exudes from cut stems or vascular bundles. In infected tubers, the vascular ring exhibits browning and necrosis, and a creamy fluid may ooze from the cut surface (Charkowski et al., 2020). Brown rot is a significant agricultural problem worldwide due to its high genetic diversity and severe crop losses (Ababa, 2024). Extensive strain characterization has revealed significant genetic diversity within *Ralstonia solanacearum*, leading to its classification as a "species complex" (Jones & Harmon, 2010). This group of pathogenic strains spreads through the use of infected seed potato, soils, water run-offs, plant debris, or contaminated farm equipment (Okiro et al., 2024). The persistence of *R. solanacearum* in soil, water, and plant debris makes it challenging to control.

Globally, brown rot caused significant crop losses and economic disruptions. Economic studies reveal that annual losses due to brown rot exceed billions of dollars globally, with substantial social impacts such as reduced farmer incomes and food insecurity (Elphinstone, 2005). In Asia, brown rot has been documented as a critical threat to potato production in countries like India (Sagar et al., 2014), Bangladesh (Karim et al., 2018), and Nepal (Dhital, S.P., Thaveechai, N., Shrestha, 2001). In Bhutan, the confirmation of *R. solanacearum* in Kanglung (Yonphoogla chiwog) under the Trashigang dzongkhag in July 2024 raised alarms about the disease's potential spread across major potato-growing areas.

Initial field observations in Kanglung revealed symptoms consistent with brown rot, such as grey-brown discoloration, skin rot, and necrosis in potato tubers. Molecular analysis using qPCR confirmed the presence of *R. solanacearum*. Following this detection, the centre conducted surveys to identify the spread and impacts, and proposed integrated management strategies to safeguard potato farming.

1.1.2 Materials and methods

Farmers' Awareness, Impacts, and Practices at Kanglung

Sampling and survey design: A semi-structured questionnaire survey was conducted with 113 households in Kanglung to explore the history of brown rot outbreaks, farmers' awareness, the disease's impacts, potential sources of the pathogen, and farm management practices. The survey focused on Kanglung as it was the first area where the disease was identified. Households were selected from five chiwogs, spanning elevations of 1125 to 2444 meters above sea level. The target sample was 187 households, but 113 were reached due to factors such as empty households (*Gungtongs*) and some farmers participating in community obligations.

Table 1. Survey coverage in different Chiwogs of Kanglung

Chiwog	Households covered	Elevation range (masl)
Yonphula	28	1125-2444
Pangthang_Ritsangdoong	28	1440-1852
Mertsham-Thragom	23	1889-2162
Rongthoong_Shingchhen	19	1560-2400
Maanthong	15	1475-1928
Total	**113**	

Farm Size and Potato Cultivation: Farm sizes and areas dedicated to potato cultivation were recorded across all chiwogs to understand the crop's significance within each community.

Disease Prevalence and First Incidence Reports: The historical prevalence of brown rot in Kanglung was assessed through a farmer survey, using typical symptoms of the disease as reference points for recollection. Respondents identified the year they first observed these symptoms, with the earliest reported incidences varying across chiwogs. These "first incidence" reports provide a timeline of the disease's emergence and spread in the region.

Awareness and Perceived Impacts: The level of awareness regarding brown rot and its impacts was recorded as the percentage of households familiar with the disease, highlighting the perceived severity of its effects on potato farming and livelihoods. The actual economic losses caused by the disease could not be quantified due to reports of other sources of potato loss, such as wildlife damage, blight diseases, potato tuber moth, and red ants.

Seed Sources and Pathogen Spread: Seed sourcing practices were traced to investigate their potential role in introducing and spreading the brown rot pathogen. The study examined the origins of seeds used in affected chiwogs, considering both formal sources (e.g., government agencies) and informal channels (e.g., farmer-to-farmer exchanges).

Farm Management Practices: The study also identified farm management practices that could influence the spread and management of the disease. Key practices examined include irrigation management and farm waste management through a semi-structured questionnaire administered to 113 households.

Molecular detection and pathogen load in tuber samples from different sources

Sampling: To assess and confirm the prevalence of the pathogen *Ralstonia solanacearum*, potato tuber samples were collected from various sources. A total of 118 samples were obtained through coordinated efforts. The National Plant Protection Centre (NPPC) collected 33 tuber samples from storage facilities of 33 households across different Chiwogs within Kanglung Gewog. Facilitated by the Food Corporation of Bhutan (FCB) and the Bhutan Food and Drug Authority (BFDA), the NPPC collected 33 additional samples, including 26 from the FCB Auction Yard in Phuentsholing and 7 from the BFDA Gedu base. The National Seed Centre (NSC) submitted 24 samples, of which 16 were from the Bumthang sub-station and eight from the Gangteng sub-station. In addition to this, the National Potato Program under the National Centre for Organic Agriculture (NCOA) submitted 28 samples, comprising 23 from different farmers and five from their research farm.

DNA Extraction from tuber samples and molecular detection: DNA from the tubers was extracted following the CTAB method. This involves several steps. First, plant tissue is cleaned and homogenized in CTAB extraction buffer to release DNA. The extract is then incubated at 65°C to facilitate DNA release. After phase separation with chloroform-alcohol, DNA is precipitated with isopropanol and washed with ethanol. Finally, the purified DNA is resuspended in PCR water or TE buffer and stored at -20°C for qPCR analysis.

Quantitative Polymerase Chain Reaction (qPCR) is a sensitive and reliable technique for detecting pathogens in plant DNA samples. A master mix containing primers, probes, DNA polymerase, dNTPs, $MgCl_2$, buffer, and nuclease-free water was prepared to ensure uniform reagent concentrations and consistent results across reactions. Forward primer mix (RS-I-F, B2-I-F, & COX-F), reverse primer mix (RS-I-R, B2-II-R, & COX-R) and probe mix (RS-P, B2-P, COX-P, & RSP-55T) were used for target-specific biding. The master mix components play critical roles, including DNA strand synthesis, target-specific binding, and real-time fluorescence monitoring through probe hybridization. Following preparation, the master mix was combined with DNA templates, maintaining consistent reaction volumes. qPCR amplifies target DNA sequences while measuring fluorescence emitted during each cycle to quantify the starting DNA concentration. Standard qPCR conditions include an initial 10-minute incubation at 95°C followed by 40 cycles of denaturation (95°C) and annealing/extension (60°C). This approach enables precise pathogen detection, DNA quantification, and gene expression analysis.

Pathogen detection and pathogen load: The detection results were categorized as positive or negative based on the presence of *Ralstonia solanacearum*. Ct values were used to express pathogen load, which indicates the concentration of the pathogen in the sample. Low Ct Values indicated abundant bacterial DNA, corresponding to severe or advanced infections. High Ct Values suggested a lower bacterial presence. This information provides critical data for understanding infection severity and aiding targeted disease management.

Disease spread assessment beyond Kanglung

To evaluate the spread of brown rot in Bhutan, data from molecular diagnostics and farmer surveys were analyzed and categorized into three groups: confirmed cases where *Ralstonia solanacearum* was detected through molecular testing, cases where the pathogen was not identified, and probable cases where the disease was not directly confirmed but likely due to seed movement from infected areas like Kanglung to other regions. This classification is to provide both known and potential disease distribution for guiding efforts to target surveillance and implement necessary interventions.

1.1.3 Results and Discussions

Farmers' Awareness, Impacts, and Practices:

Disease Prevalence and First Incidence Reports

The survey revealed that brown rot has been a persistent issue in Yonphula, where it was first reported in 2014 (Table 2). The disease subsequently emerged in Mertsham-Thragom in 2015 and Maanthong in 2016. Notably, the prevalence of brown rot escalated significantly between 2021 and 2023, affecting a considerable portion of the farming community in Kanglung Gewog. By 2024, 53 farmers reported encountering the disease. The implicated potato seeds were traced to various sources, including local farmers, the Agriculture Research and Development Sub-Centre (ARDSC-Khangma), the Dzongkhag Agriculture Office, and the National Seed Centre (NSC).

Table 2. First incidences of Brown rot in different Chiwogs and the seed sources for that year

First Year Observed	Chiwog	Seed Source
2014	Yonphula	NSC/Khangma
2015	Mertsham-Thragom	
2016	Maanthong	NSC/Khangma
2019	Pangthang_Ritsangdoong	Yonphula
2019	Rongthoong_Shingchhen	

Awareness and Perceived Impacts

As of 2024, most potato growers in Kanglung were aware of brown rot (Table 3). However, awareness varied across different chiwogs - Yonphula (60%), Rongthoong_Shingchhen (52%), Maanthong (46%), Mertsham-Thragom (43%), and Pangthang_Ritsangdoong (35%).

Table 3. Farmers' awareness and affected farmers

Chiwog	**Households Awareness (%)**	**Households Affected (%)**
Yonphula	60%	~39%
Rongthoong_Shingchhen	52%	~32%
Maanthong	46%	~13%
Mertsham-Thragom	43%	~52%
Pangthang_Ritsangdoong	35%	~29%

The proportion of households directly affected by brown rot was highest in Mertsham-Thragom (around 52%), followed by Yonphula (around 39%), while Maanthong had the lowest affected households (around 13%).

Sources and Pathogen Spread

Seed sourcing practices were analyzed to explore their potential role in introducing and spreading the brown rot pathogen. The study examined the origins of seeds used in affected chiwogs, considering both formal sources and informal channels.

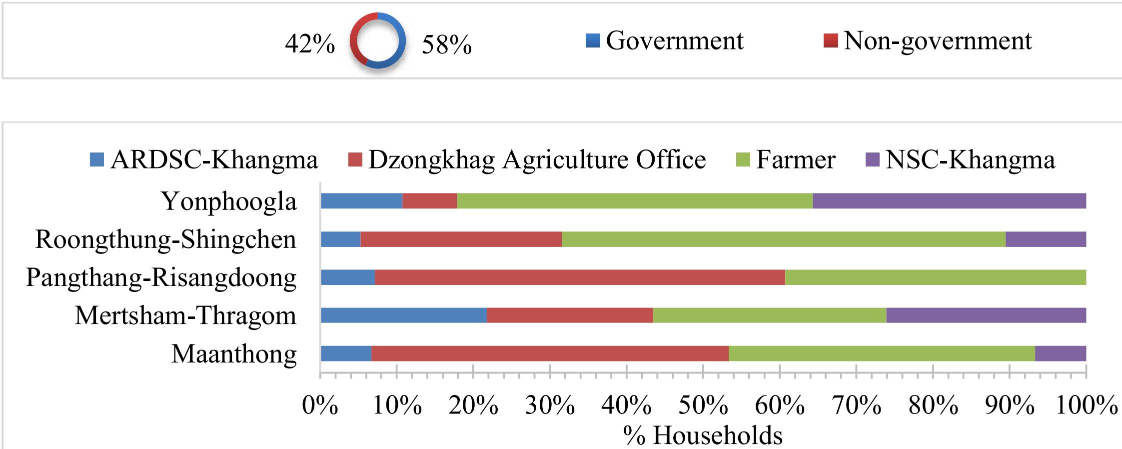

Figure 1. Seed source types and sources of seed in the last 5 years

Over the past five years, government agencies were the primary source of seeds for most farmers, accounting for 58%. In some chiwogs, particularly Rongthoong_Shingchhen, Yonphula , and Mertsham-Thragom, farmer-to-farmer seed sharing was a common practice. In contrast, Pangthang_Ritsangdoong and Maanthong received more significant seed support from the Dzongkhag Agriculture Office. All chiwogs, except Pangthang_Ritsangdoong, also received seed support from ARSC-Khangma and NSC.

The data revealed a shift in seed sourcing practices over the last five years. About 78% of seeds in the last season came from non-governmental sources, including farmer-to-farmer exchanges and reused seeds, which probably contributed to pathogen spread. This reflects trends seen in global studies where informal seed exchanges have been significant factors in brown rot spread (Abdurahman et al., 2019; Gbonamou et al., 2020; Okiro et al., 2024). Yonphula is seen as an early hotspot for brown rot and remains a significant area of concern. Similarly, the high incidence in Mertsham-Thragom highlights the urgent need for targeted interventions in this chiwog. These hotspot regions require immediate action, including intensified monitoring and rapid response systems.

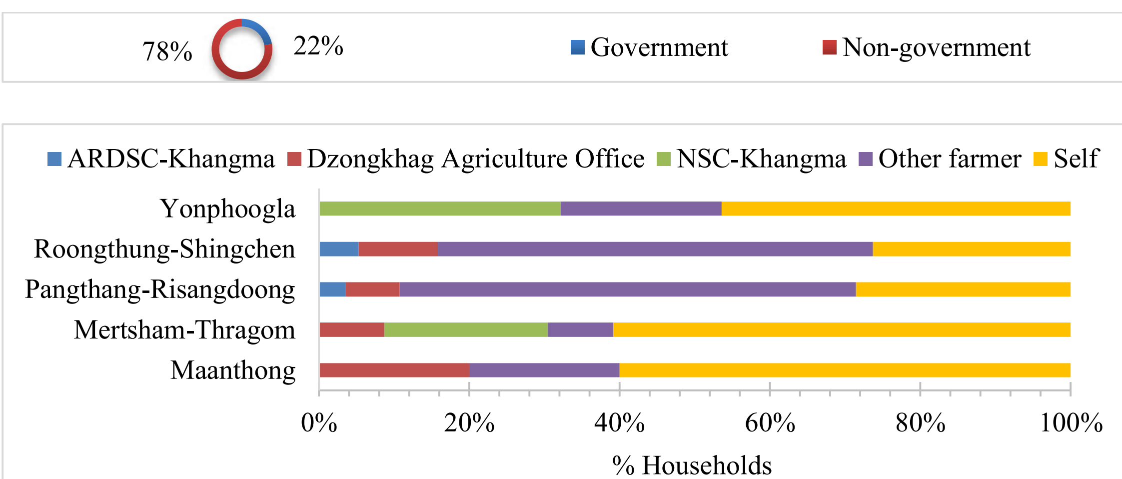

Figure 2. Seed source types and sources of seed in the last potato season

The reliance on non-certified seeds and sourcing from affected areas like Yonphula has likely facilitated the spread of the pathogen across the gewogs. To mitigate future outbreaks, it is essential to implement targeted awareness campaigns and promote improved seed management practices. Emphasizing certified seed systems and reducing informal exchanges could significantly reduce the risk of pathogen transmission.

Farm Size and Potato Cultivation

The data revealed substantial variation in farm size and potato cultivation area, offering critical insights into regional agricultural practices (Table 4).

Table 4. Farm size and potato farming

Chiwog	**Farm Size (acre)**	**Potato cultivation (Acre)**
Maanthong	1.54 ± 1.40	0.42 ± 0.31
Mertsham-Thragom	1.08 ± 1.05	0.42 ± 0.24
Pangthang_Ritsangdoong	1.04 ± 0.75	0.38 ± 0.25
Rongthoong_Shingchhen	0.87 ± 0.81	0.37 ± 0.29
Yonphula	0.95 ± 0.70	0.56 ± 0.30

Farm size distribution highlighted that Maanthong had the largest average landholding (1.54 ± 1.40 acres), while Rongthoong_Shingchhen recorded the smallest (0.87 ± 0.81 acres). Despite having a smaller average farm size, Yonphula allocated the largest proportion of its land (0.56 ± 0.30 acres) to potato cultivation, reflecting the community's strong focus on this crop. In contrast, Rongthoong_Shingchhen reported the smallest area for potato farming (0.37 ± 0.29 acres), indicating a potentially lower dependence on potato production within the community.

Farm Management Practices

Farm Waste Management and Sanitation Practices: Farm waste management practices varied in different farmers. A majority of farmers leave potato haulms and non-marketable tubers in the fields. Some utilize these materials for cattle feed or composting, while others dispose of them by dumping them at the farm's edge. Weed management practices also show diversity, with most farmers discarding weeds at the farm edges. However, a significant concern is the low rate of farm tool sanitation, with only about 54% of farmers consistently cleaning their tools with water after use.

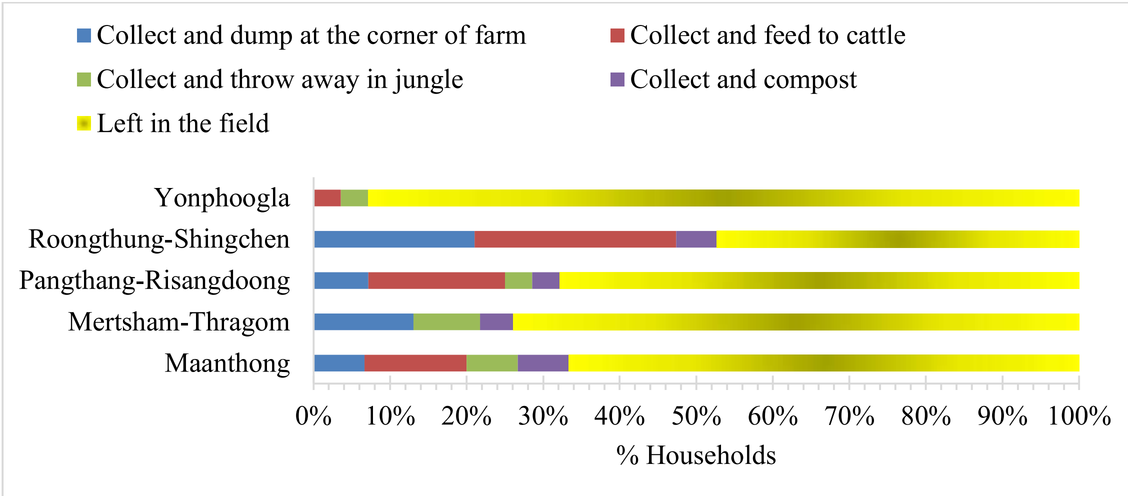

Figure 3. Management of Potato haulms and non-marketable tubers

Irrigation and Pest Management: Irrigation is a crucial practice for potato cultivation in the region, with approximately 85% of surveyed households utilizing irrigation, primarily for the second crop. Sprinkler irrigation and manual pipe systems are the most common irrigation methods. Irrigation, though essential for potato cultivation, can inadvertently facilitate the spread and severity of brown rot. The waterborne nature of *Ralstonia solanacearum* allows for easy spread through contaminated irrigation water (Elsas et al., 2001), especially with sprinkler systems. Furthermore, excessive irrigation can create waterlogged conditions, creating an ideal environment for pathogen growth. Waterlogging creates ideal conditions for *R. solanacearum* by reducing oxygen availability, increasing soil temperatures, facilitating bacterial movement, and weakening plant defences. These factors combine to significantly increase disease risk in potato crops.

Insect pests can increase the risk of *Ralstonia solanacearum* infection in potato crops. Potato tuber moth (68% of Households affected), grubs (46.02%), and red ants (33.63%) are the primary insect pests affecting potato tubers in Kanglung. These insect pests, will pose significant challenges to brown rot management in potato cultivation. Insect damage weakens plant defences, creating entry points for the *Ralstonia solanacearum* bacterium. Additionally, insect feeding can mask initial brown rot symptoms, hindering timely diagnosis and treatment.

Pathogen detection and pathogen load

Molecular detection: The study analyzed the presence as well as levels of the pathogen within the infected samples. Overall, about 75% of the 118 samples tested positive for the pathogen. Ct values, which serve as an indicator of pathogen load, varied significantly. Samples collected from Kanglung by the NPPC exhibited high levels of the pathogen with Ct values ranging from 12.49 to 35.82. Samples from the FCF and the BFDA showed lower prevalence and a wider range of Ct values (11.07 to 44.6). Samples from the NSC demonstrated moderate prevalence with Ct values between 21.04 and 40.59. Notably, all samples collected by the NPP/NCOA tested positive with low Ct values (10.42 to 14.99), indicating a high level of pathogen presence.

Table 5. Ct range of samples from different sources

Sampling	Pathogen load	Ct Range	Key observation
NPPC	High	12.49 to 35.82	Significant pathogen load
FCB & BFDA	Low	11.07 to 44.6	Wide variability in pathogen load
NSC	Moderate	21.04 to 40.59	Intermediate pathogen load
NPP/NCOA	High (all +ve)	10.42 to 14.99	Significant pathogen load

The high Ct values from certain regions, like NPP/NCOA, indicate concentrated pathogen loads in specific sources. This underscores the need for rigorous seed certification and enhanced biosecurity measures at seed production and distribution centres.

Disease spread

The molecular diagnosis detected the pathogen in several Gewogs (Table 5) within Bumthang, Wangdue Phodrang, Thimphu, Trongsa, Chhukha, and Trashigang, which are indicated as 'Detected' in Table 5. In contrast, no brown rot pathogen was found in samples from Uesu (Haa), Nagya (Paro), and Ura (Bumthang). The molecular analyses detected the pathogen in samples from farms of 36 potato seed growers associated with the NSC and NPP/NCOA, as well as research farms at NPP/NCOA, NSC-Bumthang, and NSC- Gangteng. Although no study to trace the movement of the pathogen was conducted, it is highly probable that pathogen dissemination takes place from these farms with limited pathogen screening facilities.

Table 6. Current (known) status of *Ralstonia solanacearum* presence in Bhutan[1]

Dzongkhag	Gewog	Status
Haa	Uesu	Not detected
Paro	Nagya	Not detected
Bumthang	Ura	Not detected
	Chhoekhor	Detected
	Chhumig	Detected
	Tang	Detected
Wangdue Phodrang	Gangteng	Detected
	Phobji	Detected
Thimphu	Chang	Detected
Trongsa	Tangsibji	Detected
Chhukha	Chhapchha	Detected
Trashigang	Kanglung	Detected
	Khaling	Probable
	Samkhar	Probable
	Udzorong	Probable
Monggar	Dramedtse	Probable
	Narang	Probable
	Yadi	Probable

[1] Status of brown rot spread mapped from molecular tests and suspicion from informal seed exchanges.

Further, the spread of the disease may extend beyond these Gewogs due to informal seed exchanges. In the survey conducted in Kanglung, 17% of respondents reported supplying potato seeds to their neighbours, relatives, and friends. Seeds from Yonphula and Mertsham-Thragom Chiwogs were distributed to Trashigang and Monggar (Table 6), although none of the respondents were registered seed growers. Further, the majority of day-to-day farming activities are still predominantly performed manually with labour exchange arrangements (Tshotsho et al., 2023) and sharing of farming tools, including power tillers.

These findings highlight the geographical spread of brown rot, with the potential for further expansion if unaddressed. Immediate actions are required to delimit non-affected areas and prevent pathogen introduction.

Table 7. Potato seed sourced from Kanglung in 2023-2024

Source	Supplied to	Incidences
Yonphula	Samkhar, Trashigang	4
Mertsham-Thragom	Khaling, Trashigang	2
Mertsham-Thragom	Udzorong, Trashigang	1
Mertsham-Thragom	Dramedtse, Monggar	1
Mertsham-Thragom	Narang, Monggar	1
Mertsham-Thragom	Yadi, Monggar	1

Limitations of the study

The study's limited geographical scope, focusing on a few gewogs in Bhutan, does not capture the broader context of brown rot prevalence and farm management across the country. This highlights the need for future research to cover more regions for a comprehensive understanding of disease dynamics. Additionally, reliance on farmers' recollections introduces bias, affecting the accuracy of disease timelines.

Although the study captured farmers' perceptions of brown rot's impacts, it did not quantify the economic losses, which would limit resource prioritization and understanding of the disease's full impact. Key environmental factors, such as soil moisture, temperature, humidity, and precipitation, which influence pathogen survival and spread were not analyzed for this study.

Proposed Way-forward

Seed certification, farm sanitation, crop rotation, robust disease surveillance, resistant cultivars, biological control and irrigation water management are key practices recommended by the researchers (EFSA, 2019; Aguk et al., 2018; Cardenas Gomez et al., 2024; Guchi, 2015; Moussa & Hozayn, 2018; Nion & Toyota, 2015; Njau et al., 2021; Norman et al., 2020) for the management of brown rot.

Effective management of bacterial wilt requires the use of certified, disease-free seed tubers and the reduction of reliance on informal seed systems, which are often sources of contamination. Crop rotation with non-host crops, such as maize or beans, over 4-7 seasons is essential, along with the removal of volunteer plants and planting in well-drained, non-waterlogged fields.

Farm sanitation practices, including tool disinfection and proper disposal of infected plants through burial or burning, are critical to controlling the disease. Farmers must be trained to recognize symptoms, adopt proper hygiene measures, and implement positive selection to identify healthy seed sources.

Policy support plays a key role in management, emphasizing affordable access to certified seeds, legalization of quality-declared seeds, and the establishment of robust surveillance systems. Research efforts should focus on breeding resistant potato varieties and investigating biological control agents.

Preventive actions, such as avoiding previously contaminated fields for at least three years, controlling runoff water, and ensuring compost is fully decomposed, are necessary to reduce the spread of the pathogen. These measures collectively promote sustainable potato production and effective disease management.

Drawing on established disease management frameworks, Bhutan can adopt a phased, multi-sectoral approach to address brown rot and bacterial wilt through policy reforms, field-level interventions, and institutional capacity building. By integrating immediate, medium-term, and long-term strategies, the country can establish a resilient and sustainable system to mitigate these diseases' agricultural and economic impacts.

Immediate Actions

Immediate priorities must focus on rapid data collection, containment, and stakeholder mobilization. *Information gathering* is critical, led by agencies such as the National Plant Protection Centre (NPPC), National Centre for Organic Agriculture (NCOA), and Agriculture Research and Development Centres (ARDCs). Molecular diagnostics and geospatial mapping of disease incidence will enable precise pathogen identification, delineation of hotspots, and assessment of contributing factors such as climate and soil conditions. Concurrently, evaluating potato varieties for resistance and alternative weed hosts under controlled conditions (NPPC and NPP) will inform long-term breeding strategies. To enhance field-level responsiveness, the Department of Agriculture (DoA) must procure loop-mediated isothermal amplification (LAMP) assay kits for rapid pathogen detection, coupled with training programs for personnel from NPPC and NPP. Parallel trials (NPPC/ARDCs) to assess potassium phosphite efficacy could reduce reliance on conventional pesticides. Economic analyses quantifying yield losses and trade restrictions will guide resource prioritization.

Field hygiene protocols, enforced by Dzongkhag Agriculture Offices and NPPC, must emphasize decontamination of tools, soil, and irrigation water to curb pathogen transmission. Nationwide awareness campaigns, leveraging community leaders and extension workers, will improve farmer adherence to sanitation and quarantine practices. Sentinel surveillance systems

(NPPC/ARDCs) using molecular tools will enable real-time disease tracking, while stringent seed import screening and certification enforcement (BFDA) will prevent cross-border pathogen (different strains) spread. Reducing informal seed systems through certified seed subsidies (NSC/NPP) will further mitigate risks.

Medium-Term Actions

Building on immediate measures, medium-term strategies must emphasize systemic resilience. A national *Integrated Disease Management (IDM)* framework (NPPC/BFDA/NSC) combining cultural, biological, and chemical controls will standardize coordinated responses. Farmer training programs and demonstration farms (by NPPC/ARDCs) will disseminate best practices, including crop rotation to disrupt pathogen cycles and adoption of resistant varieties. Early warning systems integrating meteorological data and mobile alerts (for NPPC/Agromet) will enable proactive interventions. Capacity building for relevant agencies, including international exposure visits, will strengthen expertise in diagnostics and adaptive management.

Long-Term Actions

Sustainable disease control requires forward-looking investments. A national *seed certification program* (monitored collaboratively by BFDA and DoA) with standardized protocols and distribution monitoring will ensure pathogen-free planting materials. Research initiatives must prioritize breeding resistant varieties with marker-assisted selection (by NPP/ARDCs) and development of biological control agents using native microbes (by NPPC/ARDCs). Advanced diagnostic tools (for NPPC/BFDA) will enhance scalable pathogen detection, while infrastructure upgrades—composting facilities and drip irrigation (NSSC/DoA)—will reduce environmental contamination.

1.1.4 Conclusions

The emergence and spread of potato bacterial wilt caused by *Ralstonia solanacearum* in Kanglung, Trashigang, poses a serious threat to the potato production in Bhutan. This study confirmed the pathogen's presence through molecular diagnostics, traced its likely spread through informal seed exchanges, and highlighted gaps in farmer awareness and farm management practices that facilitate disease transmission. High pathogen loads in Kanglung and detections in multiple dzongkhags indicate that brown rot has already moved beyond its initial hotspot. Addressing this challenge requires integrated and coordinated efforts, including the use of certified disease-free seeds, improved farm sanitation, crop rotation with non-host species, strengthened surveillance, and targeted awareness campaigns. Policy support, research on resistant varieties, and capacity building for rapid diagnostics are also essential to contain the disease and safeguard potato farming livelihoods across the country.

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1.2 Assessing the efficacy of Potassium Phosphite to manage Potato Brown Rot

1.2.1 Introduction

Potato (*Solanum tuberosum* L.) is a globally significant staple crop, particularly in developing countries, providing essential carbohydrates, vitamins, and minerals (Devaux et al., 2021; Morales-Morales et al., 2022; Tolessa, 2018; Zaheer & Akhtar, 2016). Asia, a major potato producer and consumer (Devaux et al., 2014; Nasir & Toth, 2022; Wijesinha-Bettoni & Mouillé, 2019), is particularly vulnerable to bacterial wilt outbreaks (Jiang et al., 2017; Kurabachew & Ayana, 2017). In Bhutan, potato is a vital component of agriculture and the economy (Lepcha et al., 2021; Rai et al., 2022), but recent detections of bacterial wilt, a devastating disease (Osdaghi, 2021), caused by *Ralstonia solanacearum* in eastern Bhutan (National Plant Protection Centre, 2024) highlight the urgent need for effective management strategies.

Ralstonia solanacearum, a soil-borne bacterium with a wide host range (Guo et al., 2021; Manda et al., 2020; Wang et al., 2023), invades plant vascular systems, leading to wilting, necrosis, and ultimately plant death (Charkowski et al., 2020; Uwamahoro et al., 2020). The pathogen's adaptability, including diverse races and biovars, coupled with its ability to survive in various environmental conditions, contributes to its persistence in the soil (Ahmed et al., 2022; Farnham, 2022; Geng et al., 2022; Umrao et al., 2021). The disease causes substantial yield losses, leading to food shortages, economic instability, and food security concerns (Hayward, 1985; Waals & Krüger, 2020).

Integrated disease management (IDM) is essential for controlling bacterial wilt in potatoes. This approach combines various strategies, including resistant varieties, crop rotation, soil disinfection, hygiene, certified seed potatoes, and biological control (Tessema & Seid, 2023; Tiwari et al., 2021). Potassium phosphite has emerged as a promising tool in managing bacterial wilt (Bellini et al., 2023; Su, Feng, et al., 2022; Su, Qiu, et al., 2022). This compound stimulates plant immunity, producing antimicrobial compounds and strengthening cell walls (Feldman et al., 2020; Mohammadi et al., 2021; Su, Qiu, et al., 2022; Trejo-Téllez & Gómez-Merino, 2018) enhancing plant health and vigour. Potassium phosphite at a concentration of 0.05% (wt/vol) could significantly inhibit the growth of *R. solanacearum* (Su, Qiu, et al., 2022). Therefore, NPPC conducted this study to evaluate the efficacy of potassium phosphite as a potential tool in controlling bacterial wilt on potato under field conditions.

1.2.2 Materials and methods

This demonstration research engages the potato growers of the community by involving them in the experiment and the field days.

Experimental Site and Design: The demonstration trial was established at Kanglung since it has a history of bacterial wilt outbreaks. The study was established in eight sites within Kanglung at different elevations. At each site, two treatments (treated and non-treated control) were replicated four times randomly.

The eight sites were;

- Yonphula (2 sites) and Rongthoong_Shingchhen (1 site) for higher altitudes
- Mertsham-Thragom (1) and Maanthong (1) for mid-altitude
- Pangthang_Ritsangdoong (1) for mid-altitudes
- Pangthang_Ritsangdoong (2) for low-altitudes

Cultivation practice for the experiment follows horizontal planting as rows and ridge. Irrigation water for the experimental fields follows farmers' management; from chlorine treated tap water.

Figure 4. Field setup at Rongthoong, Shingchhen and Merthsam Thragom, Thragom

Treatment applications: Potassium phosphite was applied at (2g/L of water) through foliar sprays. Application of the treatments was carried out at the vegetative growth stage, tuber initiation stage and tuber bulking stage.

Data Collection: Disease incidence and severity were assessed: i) Before the first spray, ii) Before second spray, and iii) 2 weeks after the second spray, using a standard disease rating scale of 0 to 5, where 0: No symptoms, 1: Initial wilting of one or two leaves, 2: Wilting of 25-50% of leaves, 3: Wilting of 51-75% of leaves, 4: Wilting of 76-100% of leaves, 5: Plant death. Late blight was also assessed using this standard scale. Plant growth parameters were recorded. Yield components were measured at harvest. Tuber samples were collected at harvest to detect latent infections in tubers and roots. This links final pathogen levels to yield outcomes.

Data Analysis: Statistical analysis compares treatment effects on disease control, plant growth, yield, and pathogen load. Area Under the Disease Progress Curve (AUDPC) was used to compare the disease pressure between treatments over time.

Community involvement: Farmers, Local Government members, Gewog and Dzongkhag Agriculture Officers, and Regional Plant Protection Focal Officer/s were involved in the demonstration program. Field days will be organized to show the difference between the treated and non-treated fields in one of the trial sites during the final harvest (in the third-year trial). This activity was aimed to create awareness of the benefits of Potassium Phosphite application.

1.2.3 Results and Discussions

Bacterial Wilt Symptoms (Visual Scores)

No significant differences ($p > 0.05$) were observed in bacterial wilt symptoms, as assessed by visual ratings, between phosphonate-treated and non-treated plots across all seven study sites.

Pathogen load

Quantitative PCR (qPCR) analysis was conducted to compare bacterial DNA levels in potato tuber samples from six sites (viz. Shingchhen, Rithung, Dophung, Pangthang, Manthong and Ritsangdoong) following phosphonate treatment. The pathogen load was estimated using ΔCt values (RalsCt – potatoCt), where higher ΔCt indicates lower bacterial load. Across all sites, differences between treated and untreated samples were statistically assessed using the non-parametric Mann–Whitney U test due to non-normal distribution of ΔCt values.

Shingchhen; There was no significant difference in pathogen load between treated and non-treated samples (U = 10.000, p = 0.686). However, the treated group showed a higher median ΔCt (7.09) compared to the non-treated group (5.02), suggesting a lower bacterial load in treated samples.

Rithung; No statistically significant difference was observed between the groups (U = 8.000, p = 1.000). The median ΔCt was higher in the treated group (8.825) than the non-treated group (4.950) confirming the positive reaction.

Dophung; Treated samples showed a higher median ΔCt (10.67) than non-treated samples (4.82), indicating reduced pathogen load in treated plants, confirming the positive reaction of phosphonate treatment. However, the difference was not statistically significant (U = 12.000, p = 0.343).

Pangthang; The difference between treated and control groups was not statistically significant (U = 11.000, p = 0.486). Treated samples had a higher median ΔCt (10.5) compared to non-treated samples (8.24), again indicating a trend toward lower bacterial load with treatment.

Manthong; No significant difference was detected (U = 6.000, p = 0.686), although treated samples had a slightly higher median ΔCt (6.620) than the non-treated group (4.870).

Ritsangdoog; Unlike other sites, treated samples showed a lower median ΔCt (0.8) than non-treated samples (6.36), indicating higher pathogen load following treatment. This trend, although not statistically significant (U = 1.000, p = 0.200), suggests a possible negative effect or treatment failure at this site.

Late Blight

Significant differences ($p < 0.001$) in late blight visual scores were recorded at Ristangdoong (Duptho's farm). Non-treated plots exhibited significantly higher late blight severity (Medians: treated = 37, non-treated = 75), suggesting a potential suppressive effect of phosphonate application on late blight. However, no significant differences were observed between treatments at the other study sites.

Yield and Growth Parameters

Total Yield: A significant difference (p = 0.023) in total tuber weight was observed at Ritsangdoong site 2, with treated plots yielding a higher (T1 = 667g) compared to non-treated plots (T2 = 500g).

Marketable Yield: Significant differences (p = 0.01) in marketable yield were detected at Rithung Goenpa, where treated plots demonstrated a higher yield (Medians: T1 = 412g, T2 = 245g) than non-treated plots.

Non-Marketable Yield: For non-marketable tuber weight, a significant difference ($p < 0.001$) was observed at Ritsangdoong, with non-treated plots exhibiting a higher non-marketable yield (Medians: T1 = 13.5g, T2 = 61.5g).

While bacterial wilt symptoms remained unaffected by phosphonate treatment, a positive reaction from the pathogen load was observed. Similarly, a notable reduction in late blight severity was observed at one specific location (i.e., Ristangdoong). This site-specific efficacy against late blight suggests that environmental factors, pathogen pressure, or soil characteristics may modulate the effectiveness of phosphonate. Further investigation into these interacting variables could elucidate the conditions under which phosphonate provides optimal disease suppression.

Regarding yield parameters, the positive impact of phosphonate was evident in increased total tuber weight at the lower Ritsangdoong site 2 and enhanced marketable yield at Rithung Goenpa. Conversely, non-marketable yield was significantly lower in treated plots at Ritsangdoong, further supporting the beneficial effects of phosphonate on crop quality. These findings suggest that phosphonate may contribute to improved overall productivity and a greater proportion of saleable tubers.

The observed inconsistencies in treatment efficacy across sites for late blight and yield parameters highlight the complexity of phosphonate's action and the influence of local environmental and agricultural practices. Future research should focus on understanding the mechanisms behind these site-specific responses, potentially through detailed soil analyses, pathogen population dynamics, and assessment of plant physiological responses to phosphonate under varying conditions. This would help in developing more targeted and effective phosphonate application strategies for potato cultivation.

1.2.4 Conclusions

The field evaluation of potassium phosphite demonstrated that while the treatment did not significantly reduce bacterial wilt symptoms across sites, there were consistent trends toward reduced pathogen load in treated plants, as indicated by higher median ΔCt values at most locations. Additionally, potassium phosphite showed site-specific efficacy in suppressing late blight and positively influenced yield and tuber quality at certain sites, particularly at Rithung Goenpa and Ritsangdoong. These results suggest that potassium phosphite has potential as a complementary component within an integrated disease management strategy, contributing to improved crop health and productivity under specific environmental and management conditions. However, the variability in response among sites emphasizes the need for further research to optimize application strategies and to better understand the environmental and biological factors influencing its efficacy. Long-term, multi-site trials incorporating detailed soil, pathogen, and plant analyses will be essential to refine recommendations for effective use of potassium phosphite in potato disease management in Bhutan.

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1.3 Assessing the efficacy of Potassium Phosphite to manage Garlic Rust
1.3.1 Introduction

Garlic (*Allium sativum* L.), a vital crop worldwide, is renowned for its culinary, medicinal, and economic value (Saif et al., 2020). It is native to Central Asia and is cultivated in temperate climates around the globe. Annually, approximately 28 million tons of garlic are produced on about 1.6 million hectares of land (FAO, 2023). China and India are the leading producers, contributing to 80% of the world's garlic production.

Bhutan's garlic production is vital for its culture, economy, and nutrition. It's essential in Bhutanese cuisine, supports farmers' livelihoods, and holds export potential. The production of green garlic in Bhutan experienced significant fluctuations over the period 2018-2022. The highest production was recorded in 2019 (760.89 tonnes), followed by a sharp decline in 2020. Gasa Dzongkhag, Bhutan's first organic region, leads in growing garlic, boosting local incomes. Farmers are transitioning from subsistence to commercial production, driven by ease and profitability from garlic cultivation. Khatoed gewog expanded garlic cultivation to about 20 acres in 2021, up from 15 acres in 2020 (Dorji (BBS), 2021).

However, its cultivation faces significant challenges due to fungal diseases, particularly garlic rust caused by the fungus *Puccinia allii* (Merga et al., 2019). The disease manifests as orange pustules on the leaves and stems of garlic plants, disrupting photosynthetic activity and weakening the plant (Pandey et al., 2017). This disease severely impacts garlic yield and quality, leading to substantial economic losses for farmers (Koike et al., 2001). This disease thrives in high humidity and low rainfall environments. The fungus is considered autoecious and completes its life cycle within species of the Allium genus and does not require an unrelated alternate host plant for survival or reproduction. Severe outbreaks, typically observed during bulb formation, can lead to a significant reduction in bulb weight (25-60%) and a decline in overall quality (Timila et al., 2005).

Synthetic fungicides are commonly used to control plant diseases, but their widespread use has raised concerns about cost, environmental impact, and the development of resistant fungal strains (Kumar et al., 2023). Higher doses of fungicides may be needed to address resistance, but this can increase the risk of toxic residues in food (Hahn, 2014). Public awareness of these issues has led to a demand for alternatives to synthetic fungicides, and potassium phosphite is a promising option for sustainable plant disease management (Ribeiro Chagas et al., 2020). Potassium phosphite, an inorganic salt derived from phosphoric acid, has emerged as a promising alternative for managing various plant diseases, including rusts. (Guest and Grant, 1991). Phosphonates, including potassium phosphite, have demonstrated fungicidal properties against soilborne pathogens like *Pythium* and *Phytophthora*. Its mode of action involves not only direct fungicidal effects but also the stimulation of the plant's natural defense mechanisms, offering a dual approach to disease control (Kowata et al., 2012). Additionally, they can be effective against powdery mildew and certain bacterial leaf spot diseases (Singh et al., 2021). Integrated disease management strategies, combining physical, chemical, and biological approaches, have shown promise in reducing disease incidence (Lelana et al., 2021).

The study on the efficacy of phosphonate to control Phytophthora blight in chili was previously conducted by NPPC over three consecutive years, demonstrating the product's effectiveness in managing the disease. While potassium phosphite has been studied against various fungal and soil-borne diseases, its efficacy against rust diseases, including garlic rust, remains limited. This research aimed to evaluate the effectiveness of potassium phosphite in managing garlic rust through a demonstration trial. It also aims to promote the adoption of potassium phosphonate as a sustainable and environmentally friendly alternative to synthetic fungicides for garlic rust control.

1.3.2 Materials and methods

A demonstration trial was established at four locations in Gasa Dzongkhag, specifically two sites each in Khamae and Khatoe. These sites were chosen based on their previous history of garlic rust outbreaks and the significance of garlic as a key cash crop in the region. The aim was to evaluate the effectiveness of potassium phosphite applications in managing garlic rust under field conditions.

Experimental Site and Design: At each site, three treatments were implemented in a randomized design with three replications. The treatments included: a single application of potassium phosphite at 95 days after sowing (Treatment 1), two applications at 95 and 150 days after sowing (Treatment 2), and a control treatment where an equivalent volume of water was applied (Treatment 3). The four trial sites were designated as Khatoe 1, Khatoe 2, Khamae 1, and Khamae 2.

The trials were established on existing garlic fields, and standard agronomic practices were followed throughout the cultivation period. This included recommended farmer practices for planting spacing, irrigation, fertilization, and weed management to ensure consistency and relevance to local farming conditions.

Figure 5: Experimental design and data collection

Treatment application: Potassium phosphite was applied at a concentration of 2 grams per liter of water in two separate sprays. The first application was carried out at 95 days after sowing (DAS), during the leaf development stage, as a preventive measure against garlic rust. A second application was made at 150 DAS, based on the anticipated risk of disease progression.

Data Collection: Disease incidence and severity were assessed at three critical stages of garlic development to monitor the progression of rust infection in relation to treatment applications. Evaluations were conducted at: (i) the initial appearance of symptoms (95 days after sowing (DAS)), (ii) during active disease progression (150 DAS), and (iii) at crop maturity (240 DAS). Disease severity was rated using a standardized 0–5 rust severity scale, where 0 indicated no visible symptoms, and scores 1 to 5 corresponded to increasing levels of infection based on the percentage of leaf area covered with pustules (1 = 1–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100%). Disease incidence was calculated accordingly. Plant growth data, including the number of leaves per plant, were recorded, while bulb weight and diameter were measured at harvest.

Data Analysis: Statistical analysis using a non-parametric Kruskal-Wallis test was done to compare the effect of the treatment on disease control, plant growth, and yield.

1.3.3 Results and Discussions

This study evaluated the efficacy of two potassium phosphite treatments (T1: single application, T2: two applications, T3: untreated control) on garlic rust severity, plant growth, and yield across four distinct sites: Khamae 1, Khamae 2, Khatoe 1, and Khatoe 2. Non-parametric Kruskal-Wallis tests, followed by Dunn's post-hoc tests, were used to determine statistical significance, accounting for the inherent variability attributed to differing climatic conditions and elevations across the sites.

Garlic rust severity

Garlic rust severity, assessed visually at various critical growth stages, exhibited site-specific responses to the applied treatments.

At Khamae 1, no significant differences ($p>0.05$) in rust severity were observed among treatments at any assessment time point. This suggests that under the environmental conditions present at Khamae 1, neither single nor double applications of potassium phosphite significantly impacted rust development.

In contrast, at Khamae 2, a significant difference in rust severity ($p<0.001$) was recorded at the maturity stage. The one-time application (T1) resulted in a significantly higher median rust severity (3.5%) compared to the two-time application (T2), which showed a median severity of 0.010%. This indicates that a double application of potassium phosphite was more effective in reducing rust severity at this site.

At Khatoe 1, a significant difference ($p<0.001$) in rust severity was observed at maturity. Both T1 (median = 1.5% severity) and T2 (median = 4% severity) demonstrated significantly lower rust severity compared to the untreated control (T3, median = 27.5%). There was no significant difference between T1 and T2 at this site, suggesting that both single and double applications were effective in suppressing rust.

Similarly, at Khatoe 2, a significant difference ($p=0.001$) in rust severity was found at the harvest stage. The two-time application (T2) recorded the lowest median severity (5%), which was significantly lower than the one-time application (T1) with a median severity of 85%. This further supports the superior efficacy of two applications of potassium phosphite for disease suppression at Khatoe 2, highlighting the benefit of increased spraying frequency for enhanced rust control.

Effect on garlic height and yield parameters

The impact of potassium phosphite applications on garlic growth and yield varied considerably depending on the site and treatment.

Plant Height

Significant treatment effects on plant height were observed at Khamae 1 ($p=0.001$) and Khatoe 2 ($p<0.001$). At Khamae 1, T1 (median = 31 cm) resulted in significantly greater plant height compared to both T2 and T3. No significant difference was observed between T2 and T3. This indicates that a single application of potassium phosphite (T1) was most effective in promoting plant height at Khamae 1, with no additional benefit from a double application (T2).

At Khatoe 2, a highly significant difference ($p<0.001$) was found among treatments for plant height. T1 (median = 33.5 cm) significantly enhanced plant height compared to both T2 (median = 29.5 cm) and T3 (median = 28 cm). There was no significant difference between T2 and T3. Consistent with Khamae 1, T1 consistently promoted the greatest plant height at Khatoe 2.

Bulb Weight

A significant difference ($p<0.001$) in bulb weight was observed at Khamae 1. Both T1 (median = 57 g) and T2 (median = 50 g) performed similarly and were not significantly different from each other. However, both treatments significantly outperformed the untreated control (T3), which exhibited the poorest bulb weight. This suggests that both single and double applications of potassium phosphite positively influenced bulb weight at Khamae 1.

Bulb Diameter

At Khamae 2, a significant difference ($p=0.002$) was noted in bulb diameter. T2 (median = 5.5 cm) resulted in the highest bulb diameter, being significantly different from both T1 and T3 (both with a median of 5 cm). This indicates that two applications of potassium phosphite (T2) were most effective in increasing bulb diameter at Khamae 2.

Our study shows potassium phosphite's effectiveness on garlic rust, growth, and yield varies significantly by location, emphasizing the need for site-specific disease management.
While treatments had no significant effect on rust at Khamae 1, they significantly reduced rust at Khamae 2, Khatoe 1, and Khatoe 2. A double application (T2) proved superior in controlling rust at Khamae 2 and Khatoe 2, especially under higher disease pressure, suggesting increased application frequency enhances control.

Regarding plant growth and yield, a single application (T1) consistently promoted greater plant height at Khamae 1 and Khatoe 2. However, this didn't always translate to improved yield components. Both T1 and T2 enhanced bulb weight at Khamae 1, but T2 was most effective for bulb diameter at Khamae 2, indicating a dose-dependent effect on specific yield parameters.

The discrepancies between disease control and growth suggest a complex interaction with the environment. Potassium phosphite effectively manages rust, but its impact on growth and yield is more nuanced and site-dependent. Further research is needed to understand the underlying mechanisms of these variations.

1.3.4 Conclusions

Potassium phosphite showed promising potential for managing garlic rust, with two applications providing superior disease control at Khamae 2 and Khatoe 2. Its effects on plant growth and yield were site-specific, with single applications enhancing plant height at some locations and both single and double applications improving bulb traits at others. These results highlight potassium phosphite as a sustainable alternative for rust management, though its effectiveness depends on local conditions and application frequency.

The fungus survives and overwinters on volunteer allium crops or wild Allium hosts as it infects various Allium species exclusively, such as garlic, onion, leek, chives, shallots, and wild alliums. This specificity makes rotation and removal of volunteer/wild alliums an essential management practice for controlling garlic rust.

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1.4 Detection and Preservation of Huanlongbing (HLB)-Positive Citrus Samples for Diagnostic Calibration

1.4.1 Introduction

Huanglongbing (HLB), or citrus greening, caused by the phloem-limited bacterium *Candidatus* Liberibacter asiaticus (CLas), represents one of the most devastating diseases affecting global citrus production (Garnier & Bové, 1993). Characterized by symptoms such as yellowing shoots, lopsided fruit, and bitter juice, HLB has caused significant economic losses in major citrus-growing regions, including Asia, the Americas, and Africa (Tipu et al., 2021). In Bhutan, where citrus cultivation supports rural livelihoods and contributes to food security, the detection of HLB in recent years has raised concerns about its potential impact on orchards and the National Citrus Repository (NCR). Effective management of this disease relies heavily on early detection, robust surveillance, and the availability of reliable diagnostic tools.

The 2024 HLB survey was conducted by the Centre, with the primary objective of establishing a repository of CLas-positive samples for future assay calibration and validation. A total of 35 citrus plants were sampled from key locations, including farmer fields in Tsirang Dzongkhag, the NCR in Tsirang, and the Royal Orchard at Baychu in Wangdue Phodrang. Samples comprising of leaves and bark tissues were collected following standardized protocols, and were analyzed using real-time PCR with CLas-specific primers and probes. This initiative addresses a critical gap in Bhutan's HLB management framework: the lack of locally relevant positive controls, which are essential for maintaining diagnostic accuracy as existing controls degrade over time. The findings from this study will inform surveillance strategies, support quarantine decisions, and contribute to the development of integrated HLB management protocols tailored to Bhutan's agroecological conditions.

1.4.2 Materials and methods

Sample Collection and Preparation

Symptomatic citrus plants showing characteristic HLB symptoms (leaf yellowing, blotchy mottling, and lopsided fruit) were selected for sampling across three key locations in Bhutan during July 2024. From each plant, the followings were collected:

- Leaf samples: 10-16 leaves (size-dependent) from multiple branches, focusing on symptomatic tissue.
- Bark samples: 10-12 phloem-rich strips (1-2 × 3-5 cm) from main stems.

Samples were processed as follows:

1. Leaves were divided into 3-6 subsamples to increase detection probability.
2. Bark tissues were prepared as 1-2 subsamples per plant.

3. All samples were immediately stored in sterile bags with desiccant and transported to NPPC laboratories.

Sample Details and Locations
A total of 132 samples were collected from 35 citrus plants representing:

- Farmers' fields (*n=68* samples): Tsirang Dzongkhag (major citrus-growing area)
- National Citrus Repository (*n=20*): Tsirang Dzongkhag
- Royal Orchard (*n=44*): Baychu, Wangdue Phodrang Dzongkhag

Molecular Detection
DNA extraction was performed from all samples between September 24-27, 2024 using the CTAB method. Real-time PCR was conducted using:

- HLB-specific primers/probes (Li et al., 2006):
    - HLBas: 5'-GTCGAGCGCGTATGCAA-3'
    - HLBr: 5'-CTACCTTTTTCTACGGGATAACGC-3'
    - HLB probe: 5'-AGACGGGTGAGTAACGCG-3'
- COX internal control (plant mitochondrial DNA):
    - COXf: 5'-GGTATGCCACGTCGCATTCCAGA-3'
    - COXr: 5'-GAATGCCCTTAGCAGTTTTGGC-3'
    - COX probe: 5'-ATCCAGATGCTTACGCTGG-3'

Results Interpretation:
Results were interpreted using Ct values: a Ct value of 0 or greater than 36 indicated 'not detected', while a Ct greater than 0 but less than or equal to 36 was considered 'positive'. Annually, citrus plant samples from the NCR germplasm are typically tested twice before being cleared for mass production or destruction. However, due to a lack of departmental approval for HLB testing, the Pathology Program at NPPC could not conduct these routine tests this year.

Despite this, the program collected 132 citrus samples from 35 plants of regions previously identified with HLB. These samples were subjected to qPCR analysis to confirm positive samples, which will serve as new positive controls for future HLB detection work in citrus. This proactive measure is crucial as existing positive controls are prone to degradation, necessitating the maintenance of fresh controls. For plants with contradictory test results (positive in one test, negative in another, or vice versa), re-sampling and re-testing were recommended. If subsequent (third) tests still yielded positive Ct values, these plants were designated for future test calibration.

1.4.3. Results and Discussions
As shown in Table 8, a total of 132 samples were collected from 35 citrus plants across seven previously identified HLB-positive locations. Sample collection varied by location (1-10 plants) and per plant (2-10 samples), including both leaf and bark tissues. From these 35 plants, at least one sample per plant was analyzed using qPCR. The results were mixed: some samples tested positive for HLB, others showed no detection, and some produced invalid results requiring future re-sampling and re-testing. In total, 91 of the 132 collected samples were

analyzed, with outcomes including positive detections, no detections, and invalid results.

Table 8: Total number of samples collected for positive sample calibration

Location	Plant no.	No. of samples
Pemathang	1	5
	2	5
	3	2
	4	5
	5	5
	6	5
Phuensoomgang	7	5
	8	5
	9	5
Barshong Maed	10	2
	11	3
	12	3
	13	3
	14	3
	15	3
Salleri	16	3
Pemashong	17	3
	18	3
ARDSC, Maenchhana	19	2
	20	2
	21	2
	22	2
	23	2
	24	2
	25	2
	26	2
	27	2
	28	2
Baychu, Wangdue Phodrang	29	5
	30	5
	31	5
	32	5
	33	6
	34	10
	35	8
Total	**35**	**132**

From the analyzed samples, 14 positive HLB samples were detected from 12 distinct plants across five locations. Specifically, Plant 1 from Pemathang and Plant 7 from ARDSC, Maen chhana, each had two positive samples, while all other positive plants yielded one. Pemathang recorded the highest number of positive samples (6), whereas Barshong Maed and ARDSC, Maenchhana, had the fewest (1 each).

Table 9 details the Ct values for these positive samples, ranging from 22.43 to 34.3. These higher Ct values indicate a relatively lower pathogen load, leading to detection in later PCR cycles. Nevertheless, as Ct values between 0 and 36 are considered positive, all 14 samples are confirmed for future HLB work. The centre will continue analyzing the remaining samples to identify additional positive controls. Furthermore, to ensure a sustained supply of positive controls for future laboratory use, more samples will be collected from previously identified positive locations.

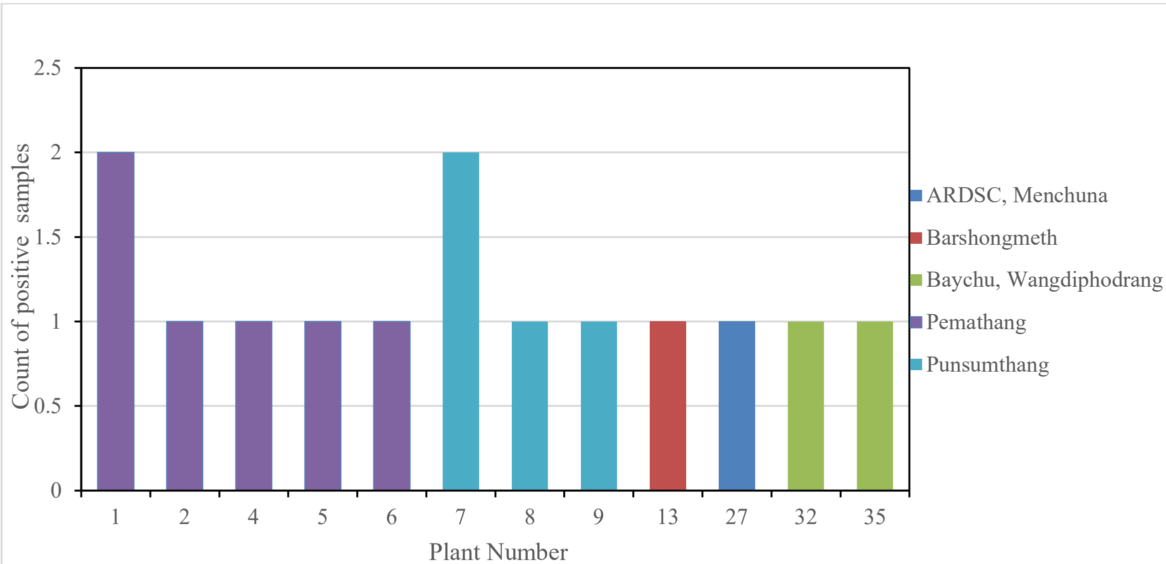

Figure 6: Number of positive plants from different locations

Table 9: Ct value of positive samples from each positive plant

Location	Plant no	Sample No	Ct value
Pemathang	1	1-24	27.84
Pemathang	1	4-24	28.62
Pemathang	2	6-24	25.69
Pemathang	4	13-24	26.75
Pemathang	5	18-24	28.46
Pemathang	6	23-24	22.43
Phuensoomgang	7	28-24	27.71
Phuensoomgang	7	32-24	29.6
Phuensoomgang	8	33-24	26.76
Phuensoomgang	9	38-24	26.71
Barshong Maed	13	51-24	27.06
ARDSC, Maenchhana	27	85-24	29.01
Baychu, Wangdiphodrang	32	104-24	34.3
Baychu, Wangdiphodrang	35	125-24	30.03

The HLB testing successfully identified 14 PCR-positive samples from 12 citrus plants across five locations in Bhutan, confirming the persistent presence of *Candidatus* Liberibacter asiaticus in the country's citrus-growing regions. This detection of HLB-positive samples, despite the absence of routine testing due to administrative constraints, reinforces the urgency of sustained monitoring and proactive management to mitigate the spread of this devastating disease.

1.1. Key Findings and Implications

1.1.1. *Geographical Distribution and Pathogen Load*

The highest number of positive samples (6) originated from Pemathang, suggesting localized hotspots of HLB infection, while lower detections in Barshong Maed and ARDSC, Maenchhana (1 each) may indicate early-stage infections or uneven vector distribution. The Ct values of positive samples ranged from 22.43 to 34.3, reflecting variable pathogen titers. Higher Ct values (e.g., 34.3 at Baychu) likely indicate low bacterial loads.

1.1.2. *Diagnostic Challenges and Sampling Strategies*

The mixed results (positives, negatives, and invalids) obtained from the test highlight the challenges of HLB detection, particularly in asymptomatic or early-infected trees. Bark and leaf sampling, while effective for PCR, may miss low-titer infections (Chen et al., 2018). Re-testing contradictory results, as done here, is critical to avoid false negatives. Future efforts should prioritize:

- Multi-tissue sampling (phloem, roots) to improve sensitivity.
- Seasonal testing to account for CLas titer variability (Lopes et al., 2016).

1.1.3. *Importance of Fresh Positive Controls*

The collection of 132 samples for future calibration addresses a critical gap: degradation of existing controls. Fresh controls from field-positive samples (e.g., Pemathang's high-titer detections, Ct = 22.43) will enhance assay reliability. This aligns with global practices where localized controls improve diagnostic accuracy (Ding et al., 2024).

1.2. Management Recommendations

- Immediate Actions:
    - Roguing: Remove PCR-positive trees (Ct ≤ 36) to reduce inoculum, especially in high-density areas like Pemathang.
    - Vector Control: Target *Diaphorina citri* with insecticidal sprays or biological agents, as vector suppression is pivotal to curb spread.
- Long-term Strategies:
    - Resistant Varieties: Screen NCR germplasm for HLB-tolerant cultivars.
    - Farmer Engagement: Train growers on symptom recognition and reporting, leveraging Bhutan's extension network.

1.4.4 Conclusion

The detection and preservation of 14 HLB-positive citrus samples from 12 plants across five locations confirmed the continued presence of *Candidatus* Liberibacter asiaticus in key citrus-growing regions of Bhutan. These newly identified positive samples address the critical need for fresh diagnostic controls, ensuring the accuracy of future surveillance and testing. The variable Ct values highlight differences in pathogen load, highlights the importance of strategic sampling and repeated testing to improve detection reliability. These findings emphasize the need for sustained monitoring, timely roguing of infected trees, and strengthened vector management to prevent further spread of HLB.

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1.5 Awareness on Brown Rot Disease and Its Management

1.5.1 Introduction

Potato is a crucial crop for Bhutan's agriculture and economy, supporting a significant portion of the population. However, its production is threatened by various pests and diseases, including potato late blight, early blight, potato tuber moth, and bacterial wilt. Bacterial wilt is an emerging disease in potato, caused by *Ralstonia solanacearum*, and is a serious threat, capable of causing substantial yield losses. This disease, first reported in Kanglung, Trashigang in 2022, has the potential to significantly impact Bhutan's potato industry. To address this threat, the NPPC conducted a one-day awareness training program on the disease identification and management options in Kanglung with fund support from Bhutan for Life (BFL) Project. Taking advantage of the gathering, safe pesticide uses and integrated pest management for other pests were highlighted. The primary objectives of the training were:

- To educate farmers about the identification and management of brown rot, other pests affecting potato and maize.
- To promote safe and efficient pesticide use, emphasizing safety protocols and minimizing environmental impact.

The training was held on November 29, 2024, at Kanglung Gewog Centre with participants including Gewog staff (Mangmi, Gewog Administrative Officer, and 5 Tshogpas), the Assistant Dzongkhag Agriculture Officer, the Regional Focal Officer for the National Potato Program from the Agriculture Research and Development Sub-Centre, Khangma, Gewog Extension Officers from Kanglung, Kangpar, Yangnyer, and Udzorong, and 151 farmers from Kanglung Gewog.

Figure 7. Participants and trainers for the Brown Rot Awareness Program

1.5.2 Training Methodology

The training program was designed to raise awareness among farmers about the various pests and diseases affecting potato and maize crops, which are of significant importance to the region's agricultural economy. The training methodology primarily involved traditional lectures complemented by PowerPoint presentations. These presentations delivered foundational knowledge on pest identification, integrated pest management strategies for the major pests affecting potato and maize, and safe pesticide use.

To enhance participant engagement, the trainers incorporated interactive elements into the training sessions. Questions were posed throughout the presentations, encouraging active participation from the audience. The participants demonstrated enthusiasm by promptly answering the questions. Additionally, open discussion and interactive question-and-answer sessions were held after each topic, providing an opportunity for participants to seek clarification and delve deeper into specific areas of interest. The trainers actively facilitated these discussions, providing thorough explanations and addressing any queries raised by the participants. This dynamic learning environment fostered a deeper understanding of the subject matter and encouraged knowledge sharing among participants.

Training topics and activities

The training program covered the following topics or session;

A. Brown Rot Awareness and Survey and Laboratory Reports

The training session focused on the serious threat posed by Brown rot, a disease caused by *Ralstonia solanacearum*, to potato crops. Participants were introduced to the disease symptoms, causal organism, and management options for this disease. A survey conducted from October 10 to 16, 2024, revealed a history of brown rot outbreaks in all chiwogs (Yonphula, Mertsham-Thragom, Pangthang_Ritsangdoong, and Rongthoong-Shingchhen) of

Kanglung gewog since 2014. The participants were made aware with their current practices of farming in their field affecting the disease based on the survey data collected. Accordingly, emphasis was placed on the causes of brown rot, including poor soil health, improper water management, use of infected seeds, and contaminated tools. Management strategies discussed during the session included the adoption of crop rotation, use of disease-free seeds, and the maintenance of soil and crop sanitation. Participants were informed about the importance of

cleaning farming equipment, safe disposal of infected plant materials, and implementing biocontrol measures. Participants were also informed on the sustainable and eco-friendly management strategy that will be tested by establishing a demonstration trial on efficacy of potassium phosphite. Additionally, laboratory findings were shared, confirming pathogen presence through advanced testing (qPCR) and recommending resistant potato varieties for improved disease control. This comprehensive approach aimed to equip participants with the knowledge and tools needed to effectively mitigate the impact of brown rot in their gewog.

Figure 8. The presenter is highlighting the potential alternative hosts of the brown rot pathogen

B. Major Diseases of Potato and Its Management

The session on the major diseases of potato and their management provided an in-depth understanding of various diseases affecting potato crops, including their symptoms, causes, and control measures. Participants gained insights into fungal diseases like Early Blight, caused by *Alternaria solani,* and Late Blight, caused by *Phytophthora infestans*, which thrive in warm and humid conditions. Bacterial diseases, such as Bacterial Wilt and Fusarium Dry Rot, were discussed alongside viral issues like Potato Leaf Roll Virus, which causes leaf curling and stunted growth. The session also addressed problems like Verticillium wilt, Common Scab and Potato Wart Disease. The importance of understanding the factors contributing to disease spread, such as poor drainage, contaminated tools, and the use of infected seeds, were emphasized. Effective management strategies were discussed, emphasizing the need for regular field inspections, timely application of fungicides like Mancozeb and Potassium Phosphite, and implementing sanitation protocols like removing infected plant materials and improving water drainage. The importance of sustainable agriculture, including integrated pest management and the use of resistant varieties, was also emphasized to ensure long-term crop health.

Figure 9. Presentation on the major diseases of potato and its management.

C. Potato Tuber Moth (PTM) Management

The training session delved into the significant threat posed by the Potato Tuber Moth (PTM) to potato crops. Participants gained a comprehensive understanding of the pest's life cycle and its detrimental impact on both field-grown and stored potatoes. The economic consequences of PTM infestations were highlighted, emphasizing the potential for substantial yield losses and reduced market value. Storage facilities were identified as critical areas prone to PTM infestation due to poor maintenance, which allows pests to thrive and spread. Contaminated potatoes lose their marketability, leading to significant financial losses. Similarly, in-field infestations, particularly in soil and growing crops, can cause diseases that reduce overall yield such as the brown rot and late blight. These challenges underline the importance of implementing effective pest management practices.

Figure 10. Presentation on the management of PTM

Participants were introduced to practical control measures, including maintaining cleanliness and proper ventilation in storage areas to minimize pest proliferation. Infected potatoes should be isolated to prevent the spread of infestation. Chemical control methods were also discussed, emphasizing safety protocols and the proper use of personal protective equipment (PPE) during application. Integrated Pest Management (IPM), which combines cultural, biological, and chemical control methods, was highlighted as an effective strategy for long-term PTM management. This session provided a comprehensive overview of Potato Tuber Moth management, equipping participants with the knowledge and tools to effectively address infestations.

D. Fall Armyworm (FAW): Biology, Impact, and Management Strategies

The session provided a comprehensive overview of the Fall Armyworm (FAW), a destructive pest that poses a significant threat to global maize production, including in Bhutan. Originating in the Americas, FAW has rapidly spread across continents, causing substantial economic losses and food security challenges.

The session delved into the invasive nature of FAW and its rapid reproductive capabilities, highlighting its potential to devastate crops. The larval stage, in particular, is highly destructive, feeding on both leaf surfaces and boring into plant tissues, leading to significant yield losses and reduced crop quality.

Participants were also informed about the broader impact of FAW infestations, which extends beyond direct crop damage. By creating wounds on plants, FAW can make them more susceptible to secondary infections, further exacerbating crop losses.

Figure 11. Presentation on the Fall Armyworm

To effectively manage FAW, an integrated pest management (IPM) approach was emphasized. This approach combines various strategies, including cultural control (crop rotation, timely planting, and sanitation), physical control (manual removal of larvae and pheromone traps), biological control (natural enemies), and chemical control (pesticides used judiciously). Regular monitoring and early detection are crucial for timely intervention and minimizing the impact of FAW infestations.

E. Vertebrate Pest Management

Participants delved into the significant impact of vertebrate pests, including monkeys, elephants, and deer on Bhutan's agricultural productivity. Traditional methods of pest deterrence, such as scarecrows, live fencing, and bamboo barriers, were explored alongside modern solutions, particularly electric fencing systems (EFS).

The evolution of EFS in Bhutan was detailed, from its early research and subsequent legalization in 2013 to its widespread adoption. With over 6,484 kilometers of EFS installed, the system has benefited more than 30,000 households and protected 60,646 acres of valuable farmland. The session also highlighted the introduction of portable electric fencing in 2021, emphasizing its portability, durability, and environmentally friendly nature.

Figure 12. Explaining on portable electric fencing and its advantages.

In addition to EFS, other mitigation strategies were also discussed, such as chain-link fencing, bioacoustics technology (utilizing predator and distress calls), and ultrasonic devices for monkey repellence. The advantages and challenges associated with each of these methods were explored, providing a comprehensive understanding of the diverse approaches available for addressing vertebrate pest issues in Bhutan.

F. Safe and Efficient Pesticide Management

This segment focused on the safe and efficient handling, application, and storage of pesticides to minimize risks to both human health and the environment. Participants were introduced to the diverse range of pesticides, including insecticides, fungicides, herbicides, and bio-pesticides, and their crucial role in enhancing crop yield and quality. However, the session also highlighted the potential risks associated with pesticide use, such as acute health hazards (skin irritation, respiratory problems) and chronic illnesses, as well as environmental issues like water, soil, and air pollution.

To mitigate these risks, the session emphasized the importance of adhering to strict safety measures, including the consistent use of protective gear, careful reading of pesticide labels, and proper disposal of containers. Innovative practices, such as designating specific storage areas for pesticides and utilizing approved spraying equipment, were recommended to further minimize exposure. Additionally, the session provided detailed first aid protocols to be followed in case of accidental pesticide exposure.

Figure 13. Presenting on the safe use of pesticides.

To emphasize the importance of safe pesticide practices, the participants were shown a case study on the misuse of Endosulfan in cashew plantations in Kerala, India. The presentation detailed the catastrophic consequences of neglecting proper personal protective equipment (PPE) and safety measures during pesticide application. Participants learned how such unsafe practices led to severe health and environmental crises. The widespread misuse of Endosulfan resulted in chronic illnesses, genetic disorders, and a high incidence of birth defects among the local population. Additionally, the ecological impact was profound, with soil and water contamination causing long-term damage to the region's biodiversity. This example served as a cautionary tale, underscoring the critical need for stringent safety protocols and responsible pesticide management to prevent similar tragedies.

A session on the illegal usage of pesticides from across the border was addressed with emphasis on its toxicity, expiry and the legal issues associated with such usage.

Additionally, the training introduced a new pest management strategy: the use of super grain bags to protect stored crops from pests. Participants were shown how to use these bags effectively and were informed about their significance in maintaining crop quality and preventing losses.

1.5.4 Conclusion

The one-day awareness training program on safe pesticide use and integrated pest management, conducted by the NPPC in Kanglung, provided a comprehensive overview of the challenges faced by potato farmers in the region. The training focused on identifying and managing major pests and diseases affecting potato crops, with a particular emphasis on the emerging bacterial disease, a serious threat to potato production.

Through a combination of lectures, discussions, and practical demonstrations, participants gained a deeper understanding of the biology, ecology, and management of major pests and diseases of potato and maize. The training highlighted the importance of integrated pest management (IPM) as a sustainable and environmentally friendly approach to pest control. IPM strategies emphasized the use of a variety of techniques, including cultural control, biological control, and chemical control, to minimize the reliance on chemical pesticides and reduce their potential negative impacts.

By promoting safe pesticide use and IPM practices, the NPPC aims to enhance agricultural productivity, reduce environmental pollution, and protect human health. The training also encouraged participants to adopt sustainable farming practices, such as crop rotation, intercropping, and the use of organic fertilizers, to improve soil health and reduce the need for chemical inputs.

The success of this training program will depend on the continued implementation of the knowledge and skills acquired by participants. The NPPC will continue to provide technical support and extension services to farmers to ensure the long-term sustainability of potato production in Bhutan.

1.6 Maintenance of native Trichoderma cultures

1.6.1 Introduction

The genus *Trichoderma* comprises a diverse group of rhizosphere-competent filamentous fungi that are ubiquitous in various ecosystems worldwide. These fungi are particularly abundant in forest and agricultural soils across all latitudes and can be readily cultured in vitro (Brotman et al., 2010). *Trichoderma* species have gained significant attention as effective biocontrol agents against economically important plant pathogens, including *Fusarium*, *Rhizoctonia*, and *Pythium* (Harman et al., 2004). Their antagonistic activity is mediated through multiple mechanisms: direct mycoparasitism, production of antifungal metabolites (e.g., peptaibols, gliotoxin), competition for nutrients and space, and induction of systemic resistance in host plants (Naher et al., 2014).

Additionally, *Trichoderma* species promote plant growth through phosphate solubilization, production of phytohormones, and enhancement of nutrient uptake (Contreras-Cornejo et al., 2009). Due to their multifunctional benefits and adaptability to large-scale production, *Trichoderma* species have become among the most commercially successful biological control agents in modern agriculture (Samuels, 1996). The extensive agricultural potential of Trichoderma has driven decades of laboratory cultivation, with preservation techniques critical for maintaining strain viability for research and application. Given this agricultural importance, this work focused on reviving *Trichoderma* cultures previously preserved by the pathology program to mass-produce it for future applications.

1.6.2 Materials and methods

Strain source

The *Trichoderma* strains revived in this study were originally isolated from rhizosphere soil samples collected from an agricultural field in Jiminang, Thimphu, during the 2018-2019 FY.

Table 9. List of *Trichoderma* strains revived

SI.	ID	Genus	Species	Place of origin
1	TC2a	*Trichoderma*	*asperellum*	Jiminang, Thimphu
2	TC5b	*Trichoderma*	*asperelloides*	Jiminang, Thimphu
3	TC6a	*Trichoderma*	*harzianum*	Jiminang, Thimphu
4	TC6	*Trichoderma*	*harzianum*	Jiminang, Thimphu
5	TC7a	*Trichoderma*	*afroharzianum*	Jiminang, Thimphu

Sub-culturing process:

The revival of *Trichoderma* strains from agar slant preservation was systematically executed using Potato Dextrose Agar (PDA) as the primary growth medium. Five preserved *Trichoderma* strains were revived from agar slants stored at 4°C. Prior to transfer, slants were acclimated in a laminar flow hood at 25°C for 20-30 minutes to minimize condensation. Necessary tools (inoculation loops, forceps) were sterilized by flaming until red-hot in a Bunsen burner and then cooled for 10-15 seconds inside the hood to avoid thermal damage to the culture. And a Bunsen burner created an upward air current for aseptic conditions.

A sterile cork borer (5mm diameter) was flame-sterilized and allowed to cool for 10-15 seconds. For each mother culture, ten uniform agar plugs were carefully excised from the most actively growing regions of the slant, avoiding the older central portion. These plugs were then transferred to fresh Potato Dextrose Agar (PDA) plates, with each strain receiving ten replicate plates (totaling 50 plates across all strains). Plates were then labeled with strain code and preparation date.

The inoculated plates were incubated under controlled conditions at 26°C to promote optimal growth. Daily observations were conducted to track mycelial expansion and conidiation patterns. Healthy colonies typically developed within 3 to 5 days, showing the distinctive white growth of mycelium.

Spore suspension preparation
All tools and containers underwent thorough sterilization procedures. Glass funnel bottles (500mL capacity) were autoclaved at 121°C for 15 minutes. The work area was meticulously disinfected with 70% ethanol before commencing operations.

Upon reaching full sporulation (typically 5-7 days post-inoculation), *Trichoderma* material was carefully harvested from the PDA plates. Using sterile spatulas, the upper layer of mycelium and spores was gently scraped from the agar surface. The harvested biomass was transferred to prepared funnel bottles containing 100mL of sterile distilled water. The mixture was then homogenized using flame-sterilized glass rods, with gentle agitation for approximately 2 minutes to achieve uniform dispersion.

Storage and Preservation
Suspensions were aseptically distributed into pre-sterilized 50mL polypropylene centrifuge tubes, filling each to 80% capacity to allow for thermal expansion. Each container received a dual sealing system: primary closure with screw-caps featuring Polytetrafluoroethylene (PTFE) liners, followed by secondary sealing with laboratory-grade Parafilm to prevent evaporation and contamination.

The aliquots were stored under controlled environmental conditions at 4°C in dedicated refrigeration units. To maintain spore viability, the storage area was maintained in complete darkness with minimal temperature fluctuation. Storage racks were organized to allow for proper air circulation around each container.

1.3.3 Conclusions

Way Forward: Research, Monitoring, and Long-Term Maintenance
To make the most of these revived *Trichoderma* strains, the focus should be on two key areas: research and regular monitoring. First, the need to study how effective these strains are at controlling common crop diseases like *Fusarium* and *Rhizoctonia*. Laboratory and small-scale field trials help to evaluate their practical effectiveness. Simultaneously, an analysis of the antifungal compounds they produce needs to be planned, which could contribute to the

development of innovative biocontrol methods

To ensure these strains remain viable for future use, there is a need for a maintenance plan. That means regularly checking spore viability (every 3 months), testing their disease-fighting ability (twice a year), and confirming genetic stability (every 2 years). To ensure long-term culture viability, it should be implemented in a phased revival schedule: refreshing active working stocks annually, renewing frozen master stocks every three years, and transitioning high-priority strains to lyophilization for permanent preservation.

1.6.4 References

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1.7 Ad-Hoc activities of the Pathology Program: Disease diagnostics

In the FY 2024-2025, the program has carried out 11 disease diagnostics at the pathology laboratory. Key findings include that paddy yellowing (Maedwang, Gase Tshogom) and wheat yellowing (Chhubu) were largely due to abiotic factors, with no significant fungal pathogens found. Conversely, ginger rhizome rot (Loggchina) was attributed to *Fusarium* and *Pythium*, and potato tuber rot (Kanglung) showed strong bacterial presence with some *Fusarium*. Coffee samples revealed common leaf spot diseases caused by *Cercospora, Colletotrichum, and Alternaria,* while pear damage (Naemjog) showed no harmful pathogens, and cardamom rotting (Tsirang) was linked to insect larvae. Overall, the report emphasizes the varied causes of plant issues, from specific pathogens to environmental stressors, underscoring the need for tailored management strategies. The detailed reports are as below;

1.7.1 Paddy Yellowing issue at Maedwang gewog (Report No: PLR-1)

Background

This report summarizes the findings from the field visit conducted by a team from the National Plant Protection Centre (NPPC) in response to a report from the Extension Agent (EA) of Maedwang Gewog, Thimphu, Bhutan. The report highlights yellowing of paddy plants in the paddy fields of Kharibji and Kharphu.

Examination method: Instant sample observation and Incubation of samples in growth chamber.

Figure 14. Sample cultures after 4 days of incubation; leaf samples (left) and root sample (right)

Incubation under growth chamber. The samples were processed on wet filter paper for fungal growth and placed under a growth chamber at 28ºC for about five days, monitoring regularly for any fungal growth. The samples were categorized as leaf and root samples (Figure 1). On the 4th day (15/07/2024) of incubation, no growth of any fungal pathogens was observed visually as well as under a microscope. (Note: Fungal growth takes two to three days on average under the growth chamber at standard parameters).

Recommendations

Paddy plants exhibiting yellowing without detectable pathogens present a complex challenge. A thorough understanding of potential abiotic factors, nutrient analysis, and physical damage is essential. NPPC suggested seeking help from relevant agencies for testing of soil for nutrient content, testing for seeds, or variety qualities. Also, NPPC recommended regular monitoring and surveillance of crops.

1.7.2 Ginger Rhizome Rot at Loggchina, Chhukha (Report No. PLR-2)

Background

This report examines the causative agent of ginger rhizome rot; a significant issue observed in Lobneykha Gewog under Chhukha Dzongkhag. Rhizome samples were collected from the affected fields and submitted for diagnosis by the National Centre of Organic Agriculture (NCOA).

Sample summary:

The NCOA submitted seven samples for analysis: three leaf samples and four rhizome samples. These samples were subjected to various diagnostic methods, including wet tissue culture and cultivation on Potato Dextrose Agar (PDA) and Carrot Agar. To ensure accurate identification, some cultures were sub-cultured for further confirmation. Consistent results were observed across all media and sample types, with *Fusarium spp*. identified as the primary pathogen in all cases.

Methodology

Wet filter paper incubation

Both leaf and rhizome samples, including both symptomatic and asymptomatic portions, were cut into small pieces (approximately 4-6 per sample). These pieces were placed on moistened filter paper within 15 cm diameter Petri dishes. The prepared samples were incubated at 30°C until fungal or bacterial growth was observed on the plant tissues. Microscopic examination of the samples was conducted daily, starting 72 hours post-inoculation.

Isolation on artificial growth media

Ginger leaf and rhizome tissues were cut into 1 cm pieces and three pieces were placed on each of two media: Carrot Agar (CA) and Potato Dextrose Agar (PDA). CA was amended with antibiotics to suppress bacterial growth, allowing for the selective isolation of fungi. Both media were used to investigate potential fungal and bacterial pathogens. The inoculated plates were incubated at 30°C until microbial growth was observed on the plant tissue. As microbial growth typically occurs within 3-5 days, observations were conducted for five days.

Subculture of the isolate for further identification.

After 5 days of incubation, the cultures and plant tissues on wet tissue culture were examined. Due to ambiguity in some pathogen identifications, sub-culturing was performed. Small amounts of fungal isolates were transferred from the original growth regions on PDA or CA plates to fresh culture media. These sub-cultures were then incubated at 30°C until microbial growth was observed. The growth of the isolates was monitored regularly for signs of contamination. Once microbial growth was evident on the new media, microscopic examination was conducted.

Results

Fusarium spp. was identified as the predominant pathogen in both ginger rhizomes and leaves. Microscopic observations revealed the presence of characteristic sickle-shaped, multi-celled macroconidia on both wet tissue cultures and artificial media. Additionally, coenocytic and irregularly branched hyphae were observed, suggestive of *Pythium* infection. Subsequent sub-culturing confirmed the presence of *Pythium*. Microscopic examination of the sub-culture revealed coenocytic, irregularly branched hyphae bearing ovoid or spherical sporangia.

Wet tissue method incubation

Pinkish-colored mycelial growth was observed on samples incubated on wet tissue paper. Microscopic examination revealed the presence of branched, septate hyphae (Figure 15, Left). Notably, large, multi-celled (3-5 septa) macroconidia with a characteristic banana or sickle shape were also observed, confirming the pathogen as *Fusarium sp.* (Figure 16: Right).

Isolation on growth media (Carrot Agar and Potato Dextrose Agar)

Microscopic examination of samples incubated on Potato Dextrose Agar (PDA) and Carrot Agar (CA) revealed fungal growth. This fungal growth exhibited characteristics similar to those observed on wet tissue cultures, with *Fusarium sp*. being the most prominent fungus. *Fusarium sp.* was characterized by the presence of septate, boat-shaped macroconidia, suggesting its involvement in rhizome rot. Additionally, coenocytic and irregularly branched hyphae were observed on PDA and CA, posing a challenge for direct pathogen identification. Sub-culturing of the isolates exhibiting these coenocytic hyphae confirmed the presence of *Pythium*. Microscopic examination of the sub-cultures revealed coenocytic hyphae along with ovoid-shaped spores, identified as globose sporangia characteristic of *Pythium* (Fig 15).

Recommendations

Laboratory analysis of ginger samples collected from Lobneykha, Chhukha, has identified the soil-borne fungi *Fusarium spp*. and *Pythium spp*. as the causative agents of ginger rot. Based on these findings, the NPPC recommended the following field-level measures to prevent further disease spread and mitigate its impact.

Immediate measures:

Monitor regularly: Keep a close eye on the plants for any signs of disease. Early detection can help prevent further spread.

Hygiene: Maintain good hygiene practices in the field to prevent the spread of the disease. Eliminate infected plants using appropriate methods.

Proper Drainage: Ensure good drainage to prevent waterlogging, which favours the growth of *Pythium*.

Balanced Fertilization: Maintain a balanced nutrient supply to promote plant health and vigor, which can enhance disease resistance.

Fungicides: Apply fungicides like carbendazim and mancozeb at NPPC recommended rates.

Soil Drenching: Apply fungicides as a soil drench to control soil-borne pathogens.

Long-term measures:

Resistant Varieties: Plant disease-resistant ginger varieties if available. This is the most sustainable and effective long-term strategy.

Crop Rotation: Avoid planting ginger in the same field for consecutive seasons. Rotate with non-host crops like cereals or legumes to reduce pathogen populations.

Seed Treatment: Treat ginger seeds (rhizomes) with fungicides like carbendazim or mancozeb before planting.

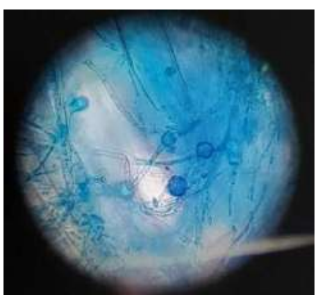

Figure 15. Globose sporangia of *Pythium* under microscopic examination.

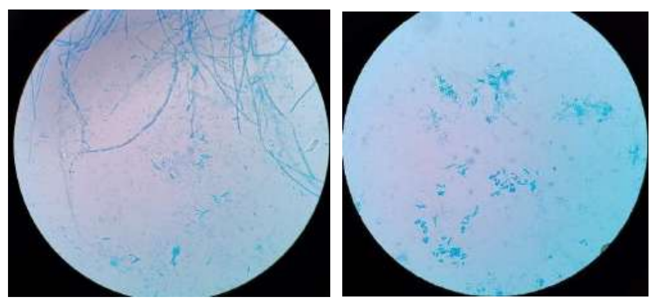

Figure 16. Hyphae of *Fusarium sp.* (Left) and Macroconidia of *Fusarium sp.* (Right).

1.7.3 Pear samples from Naemjog, Loong-nyi gewog, Paro (Report No: PLR-3)

Introduction

Pear trees in Naemjog, Paro, were reported to have damage indicative of possible disease. A sample from these trees was collected to analyze the presence of any pathogens. This report detailed the process of sample collection, preparation, incubation, and analysis to confirm the presence or absence of pathogens, specifically investigating potential fungal growth.

Materials and Methods

Sample Collection

Location: Naemjog, Paro

Sample: Pear tree components, including bark and stem.

Sample Preparation

The collected sample was cleaned and cut into small pieces.

The pieces were separated into bark and stem sections.

Artificial Incubation

Media Preparation: Two types of artificial fungal growth media were prepared:

- Potato Dextrose Agar (PDA)
- Carrot Agar

Inoculation: Both bark and stem pieces were placed into the prepared media.

Incubation: The inoculated media were incubated at a controlled temperature for fungal growth.

Microscopic Analysis

Observation Period: After 2-3 days, growth was observed in the media.

Slide Preparation: Samples from the media were prepared on microscope slides.

Microscopic Observation: The slides were examined under a microscope to identify the type of fungal growth.

Results

Growth Observation: Greenish fungal growth was observed in both PDA and carrot agar media after the incubation period.

Microscopic Analysis: The microscopic examination confirmed the growth to be Trichoderma, a green-colored fungus.

Discussion

Identification: The growth observed was identified as Trichoderma, a common soil fungus known to be beneficial and not harmful to pear trees. Trichoderma controls soil-borne and some leaf and panicle diseases, promotes plant growth, enhances nutrient uptake and plant resistance, and helps mitigate agrochemical pollution (Yao et al., 2023).

Pathogen Presence: No harmful pathogens were detected in the sample, indicating that the observed damage to the pear trees was not caused by a disease or any pathogenic organism.

Conclusion

The laboratory analysis concludes that the pear tree damage in Naemjog, Paro, was not caused by any harmful pathogen. The fungal growth detected in the sample was identified as Trichoderma, a non-pathogenic and beneficial fungus commonly found in soil. Hence, the damage observed in the pear trees is unlikely to be disease-related.

References

Yao, X., Guo, H., Zhang, K., Zhao, M., Ruan, J., & Chen, J. (2023). Trichoderma and its role in biological control of plant fungal and nematode disease. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1160551

Figure 17: Fungus growth from the bark and the stem of the sample in artificial media.

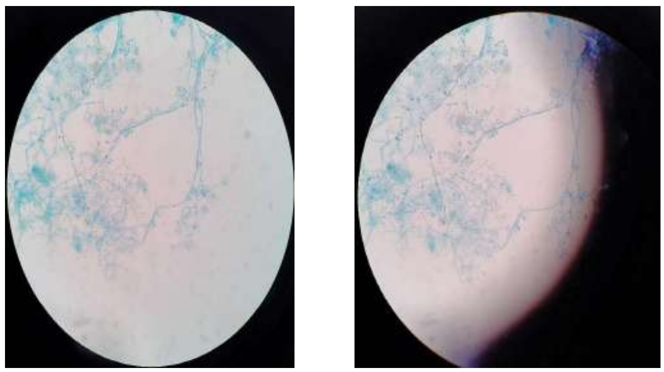

Figure 18. Fungus observation under the microscope (*Trichoderma)*

1.7.4 Potato rot issue: Tuber samples from Kanglung, Trashigang (Report No: PLR-4)

Background

This report investigates the potato disease outbreak affecting potato tubers in Kanglung, Trashigang, in recent years.

Sample summary:

The team brought potato tubers from 18 sites (1 sample with stem) and samples were processed for each method mentioned. Considering the sites as several samples and processed for four methods, a total of 96 laboratory samples were examined.

Methodology

Bacterial streaming test

The potato tubers were cut into halves (inclusive of both infected and healthy portions) and were immersed vertically in the beaker with sterilized distilled water. The cut tuber was pierced with a sterilized nail at the end to support the tuber on the top of the beaker. After observing for few seconds to one minute, a milky thread-like bacterial ooze flowed from the cut tubers into the water (Figure 19). *(Caution: the immediate cloudy exudates released into the water* *were excluded as it is due to starch particles released from the cut).*

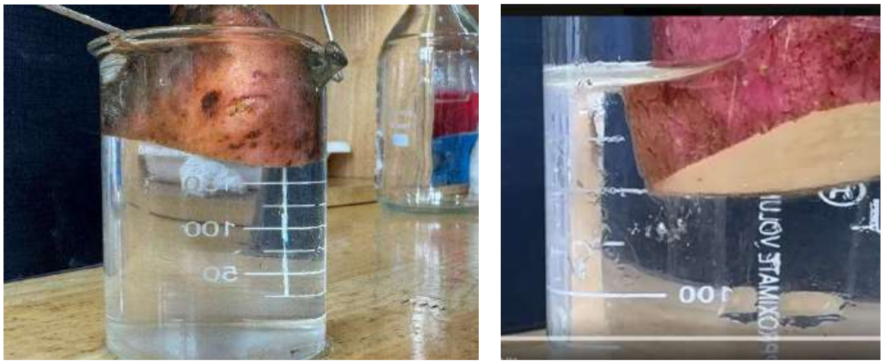

Figure 19: Bacterial streaming test set up (left) and exodus of ooze after immersion into water (right)

Wet filter paper incubation

The samples (tubers) were cut into smaller pieces (inclusive of both symptomatic and asymptomatic) and placed at around 4 to 6 pieces on the wet filter paper into the petri dish of 15cm diameter. The processed samples were incubated in the incubator at 30 ºC till the growth of bacteria or fungi was observed on the plant tissue (tubers). The processed samples were examined under a microscope for any fungal and bacterial growth after 24 hours and the examination was completed by 3rd day of inoculation.

Isolation on growth media

The plant tissue (tubers) was cut into pieces of around 1cm and placed three pieces on the growth media CA and PDA). Both the media were used to rule out bacteria and fungi as the causal agents were multiple on the tubers. The CA were amended with antibiotics to suppress the growth of bacteria. The processed samples were incubated in the incubator at 30 ºC till the growth of bacteria or fungi was observed on the plant tissue (tubers). The micro-organism growth on media takes 3 to 5 days, so the team observed growth till 5th day of incubation.

DNA extraction and Molecular analysis (PCR)

The DNA extraction for PCR analyses was done both from plant tissue and bacterium colonies grown on PDA. From the plant tissue (tuber), the CTAB extraction method was followed as per the *NPPC protocol on DNA extraction from plant tissue*. DNA extraction from colonies was done by suspending one colony in 100 µL PCR water that was grown on PDA for around 7 days and following the CTAB method. Only six bacterial cultures were able to grow in sub-culture and were used for molecular detection. Subsequently, the samples were subjected to a Real-time PCR (qPCR) test to detect the presence of the bacterium. RT PCR was performed using the assay of EPPO, 2022. The primers and probe for the '*Ralstonia solanacearum* real-time PCR specified in Table 10.

The 25 µl reaction mixture consists of a final concentration of 1× TaqMan real-time PCR master mix, 100 nM of each primer, 50 nM probe and 2 µl DNA template were used for the assay. The amplification conditions were an initial 10-minute incubation at 95°C followed by 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C.

The Cycle threshold (Ct) values were used to interpret the test results. A Ct value of zero (Ct= 0 or Ct > 36) indicates that the pathogen is 'non-reactive or not detected'. A Ct value greater than zero but less than or equal to 36 (Ct > 0 or Ct ≤ 36) is considered 'reactive or positive'.

Table 10. Primers and probes for TaqMan real-time PCR.

Species/strain	Primer pair	Primer Sequence
The primer probes target specific 16S rRNA genes within *R. solanacearum, R. pseudosolanacearum,* and *R. syzygii* strains.	RS-I-F	5′-GCATGCCTTACACATGCAAGTC-3′
	RS-II-R	5′-GGCACGTTCCGATGTATTACTCA-3′
	B2-I-F	5′-TGGCGCACTGCACTCAAC-3′
	B2-II-R	5′-AATCACATGCAATTCGCCTACG-3′
	COX-F	5′-CGTCGCATTCCAGATTATCCA-3′
	COX-R	5′-CAACTACGGATATATAAGAGCCAAAACTG-3′ (COX F/R and COX P target plant cytochrome oxidase if plant samples are used).
	RS-P (probe)	5′-[FAM]-AGCTTGCTACCTGCCGGCGAGTG-[TAMRA]-3″
	B2-P (probe)	5′-[VIC]-TTCAAGCCGAACACCTGCTGCAAG-[TAMRA]-3′
	COX-P (probe)	5′-[VIC]-TGCTTACGCTGGATGGAATGCCCT-[TAMRA]-3′

Results

The bacterial streaming test identified bacteria in eight tuber samples. Wet tissue incubation revealed bacterial growth in 15 samples and fungal growth in four. Isolation on growth media confirmed similar bacterial growth and the presence of Fusarium sp. The PCR analysis showed strong pathogen gene expression in all DNA samples, confirming pathogen presence.

Bacterial streaming test

When cut tubers were immersed in the sterilized distilled water, eight samples produced a milky thread-ooze, indicating the presence of bacteria (Annexure 1).

Wet tissue method incubation

Both fungi and bacteria were observed under different microscopes from the samples incubated on wet tissue culture. A foul smell with watery bacterial ooze growth was observed on incubated tuber tissues, indicating the growth of bacteria. Bacterial cells with rod-shaped (bacilli) and a few spherical (cocci) shapes were able to be observed under a compound microscope with 1000X magnification. About 15 samples were detected with bacteria grown on wet tissue methods, and from four samples, the fungus was detected (Annexure 1).

Isolation on growth media (Carrot Agar and Potato Dextrose Agar)

The team observed the samples incubated on PDA and CA under the compound microscope and found mostly bacterial (Figure 20) and fungal growth on CA. Similar bacterial growth was observed when grown on wet tissue methods, and the prominent fungus observed was *Fusarium* sp. (septated, boat-shaped macroconidium), which might have caused bacterial dry rot on potatoes (Figure 20). Details are reflected in Annexure 1.

Figure 20. Bacterial growth on PDA (left) & macroconidium of *Fusarium* sp. at 400x (right).

DNA extraction and Molecular analysis (PCR)

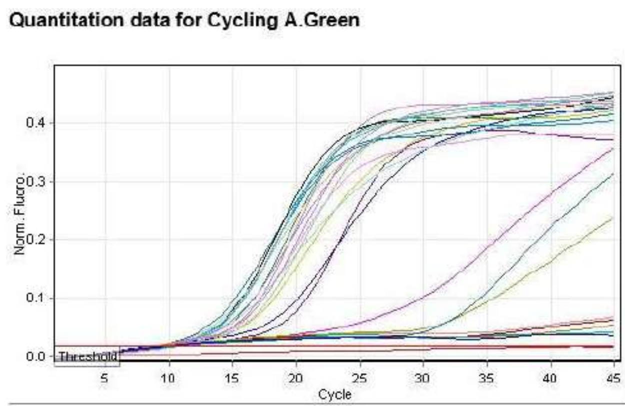

Figure 21. qPCR result of samples mostly curved from cycle 9, representing the presence of the pathogen.

In both the channels (green and yellow), the NTC (not control template), which used sterilized distilled water, showed no curve, indicating the run procedure was accurate. All the samples (DNA template) showed an increase in both channels. The threshold cycle (Ct) value for samples ranges from 9 to 11, depicting that the gene of the pathogen is highly expressed and reactive (positive) with curved starts from cycle around 9 (Figure 21). Detailed qPCR results are presented in Annexure 1.

1.7.5 Yellowing and wilting of paddy plant: From Gase Tshogom, Wangdue Phodrang (Report No: PLR-5)

Following a report from the Sr. Extension supervisor, Ms. Deki Pem of Gase Tshogom gewog, Wangdue Phodrang, regarding the yellowing and wilting of paddy plants at Shingkhey village, NPPC, Pathology Program received the paddy samples on 21/08/2024.

Sample summary:

The received samples were a bunch of paddy plants consisting of whole plant parts. The observed symptoms on the received samples were sheath browning, mycelium growth above the root zone.

Methodology

Wet filter paper incubation

The samples (shoots and roots) were cut longitudinally (inclusive of both symptomatic and asymptomatic) and placed at around 2 to 3 pieces on the wet filter paper into the petri dish of 15cm diameter. The processed samples were incubated in the incubator at 30 ºC till the growth of bacteria or fungi was observed on the plant tissue (tubers). The processed samples were examined under a microscope for any fungal and bacterial growth after 24 hours, and the examination was completed by the 4th day of inoculation (Figure 22).

Figure 22. Plant culture showing no symptoms of any pathogen growth even on the fourth day of incubation

Isolation on growth media

The plant tissues (roots and shoots) were cut into pieces of around 1cm, and three pieces were placed on the growth media (CA and PDA). Both the media were used to rule out bacteria and fungi as the causal agents may be multiple on the infected plants. The CA was amended with antibiotics to suppress the growth of bacteria. The processed samples were incubated in the incubator at 30 ºC till the growth of bacteria or fungi was observed on the plant tissue. The micro-organism growth on media takes 3 to 5 days, so the team observed growth till the 5th day of incubation.

Results

The samples were observed after 24 hours for any fungal or any other pathogen growth. Usually, it takes 3-4 days for any growth in the incubator. There was no fungal or any other growth being observed in the wet tissue paper method, even on the 4th day. However, there was *Fusarium* growth being observed from the samples placed in the artificial media (PDA). The *Fusarium* may be a saprophyte on the paddy plant. Therefore, there was no distinctive causal agent identified, which suggests that the issue could be due to other abiotic factors.

Inferences

The absence of identifiable pathogens in laboratory tests suggests several alternative causes for the observed symptoms in the field of paddy as reported by the AEO of Gase Tshogom Gewog. Based on the symptom observation and laboratory examination, also taking into account the report submitted by AEO, NPPC is considering the following factors that could have led to the current situation.

1. Abiotic stress factors such as nutrient deficiencies, water stress (both drought and waterlogging lead to symptoms of wilting and yellowing, soil PH and toxicities, and environmental extremes).

Recommendation

Paddy plants exhibiting disease symptoms without detectable pathogens present a complex challenge. A thorough understanding of potential abiotic factors, chemical, and physical damage is essential. NPPC suggested seeking help from relevant agencies for testing of soil for nutrient content, testing for seeds, or variety qualities. Also, we suggest regular monitoring and surveillance of crops for easy generation of a full report consisting of the history of crop management and weather factors of the past few weeks.

1.7.6 Pea and zucchini samples from National Seed Centre, Trashi Yangtse (Report No: PLR-6)

Background

The pea and zucchini in the National Seed Centre, Trashi Yangtse, were reported to have damage indicative of possible disease. The samples of pea and zucchini were collected and sent by the centre to analyze the presence of any pathogens. This report details the process of sample preparation, incubation, and analysis to confirm the presence or absence of pathogens, specifically investigating potential fungal growth.

Sample summary:

The received samples for both pea and zucchini plants consisted of leaves and stems. The observed symptoms were yellowing and the presence of rust spores on a pea leaf. The leaves and the stems of the zucchini samples were almost rotten as the samples were packed in the bottle.

Direct visual observation

The samples of pea and zucchini were examined with the naked eye

Result

The spores of rust were found on the leaves of the pea (Figure 23), and for further confirmation, the samples were observed under a microscope, and it was confirmed that the leaves of the pea were infected by rust (Figure 24). The pea stem does not show any growth of a pathogen, and it is yellow in colour.

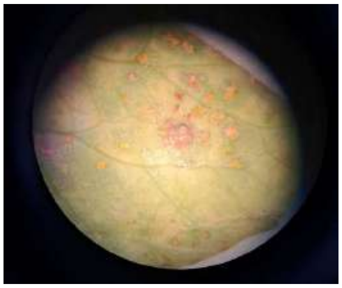

Figure 23: Rust spores on leaves under direct observation

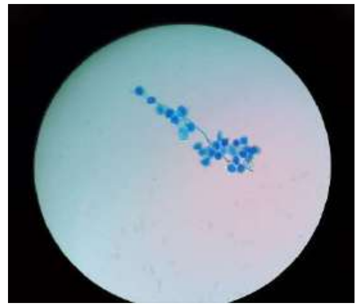

Figure 24: Rust spores on leaves under the microscope

The leaves and the stems of the zucchini samples were almost rotten as the samples were packed in the bottle. Therefore, it was observed that the samples were water soaked like symptoms.

Wet filter paper incubation

The samples (stem and leave) were cut and placed at around 2 to 3 pieces on the wet filter paper in the petri dish of 15cm diameter. The processed samples were incubated in the incubator at 30 ºC till the growth of bacteria or fungi was observed on the samples. The processed samples were examined under a microscope for any fungal and bacterial growth after 24 hours and the examination was completed by the 4th day of inoculation.

Result

There was no fungal or any other growth being observed in the wet tissue paper method even after fortnight incubation.

Figure 25. Plant culture showing no symptom of any pathogen growth even at fourth day of incubation

Isolation on growth media

The pea and zucchini leaves and stems were cut into pieces of around 1 cm and placed three pieces on the growth media (CA and PDA). Both the media were used to rule out bacteria and fungi as the causal agents may be multiple on the infected plants. The processed samples were incubated in the incubator at 30 ºC till the growth of bacteria or fungi. The micro-organism growth on media takes 3 to 4 days, so the team observed growth till the 5th day of incubation.

Figure 26: Growth of Fusarium on CA and bacteria on PDA

Results

The samples were observed after every 24 hours for any fungal or any other pathogen growth. Usually, it takes 3-4 days for any growth in the incubator but there was no fungal or any other growth being observed even on the 4th day. However, there was Fusarium growth being observed from the pea samples placed in the artificial media (PDA) only after 8 days of incubation. The Fusarium may be a saprophyte on the pea plants. Therefore, there was no distinctive causal agent identified which concludes that the issue could be due to rust infection or other abiotic factors.

Whereas, the zucchini samples placed on the media did not show any growth of pathogens. This could be due to the damaged samples.

Figure 27: Fusarium under microscopic examination

1.7.7 Wheat yellowing samples from Yebisa, Chhubu gewog, Punakha (Report No:PLR-7)

Background

Following a report from the Agriculture Sector, Jola Enterprise regarding wheat crops at Yebisa, Chubu Gewog, Punakha Dzongkhag, exhibiting symptoms of leaf-tip drying and yellowing despite good germination, samples were collected and submitted to ARDC-Bajo for diagnosis. The Pathology Program received the samples on 4th February.

Examination Method: Instant sample observation and incubation of samples in wet tissue and artificial growth media (PDA).

Observation in samples:

- Yellowing symptoms were more visible in older leaves of the wheat compared to younger ones (Figure 28)
- Roots were observed to be healthy
- No visible symptoms of any major wheat disease were observed.
- No other insect pest damage.

Figure 28: Wheat Sample

Wet filter paper incubation

Both leaf and root samples, including both symptomatic and asymptomatic portions, were cut into small pieces (approximately 4-6 per sample). These pieces were placed on moistened filter paper within 15 cm diameter Petri dishes. The prepared samples were incubated at 28°C until fungal or bacterial growth was observed on the plant tissues. Microscopic examination of the samples was conducted daily.

Isolation on artificial growth media

Wheat leaves and roots were cut into 1 cm pieces, and three pieces were placed on PDA. The samples were categorized as leaf samples and root samples. The inoculated plates were incubated at 28°C until microbial growth was observed on the plant tissue. As microbial growth typically occurs within 3-5 days, observations were conducted regularly.


Figure 29: Wet tissue culture

Figure 30: Root and leaf sample on PDA

Results

On the third day of incubation (7/02/2025), no fungal pathogen growth was observed. The samples were kept in the incubation chamber for a few more days. By the sixth day, no fungal growth was detected visually or under the microscope in the wet tissue culture and leaf samples on PDA. Given that the yellowing symptoms were observed on the leaves, fungal growth was expected on the leaf samples, but none was detected.

(Note: *Fungal growth takes two to three days on average under the growth chamber at standard parameters*.)

Recommendations

Wheat seedlings exhibiting yellowing without detectable pathogens present a complex challenge. A comprehensive assessment of abiotic factors, including nutrient analysis and physical damage, is crucial. NPPC recommends seeking assistance from relevant agencies to test soil nutrient content. Proper irrigation management is advised to prevent waterlogging and excessive drying. Nutrient deficiencies and root damage from faulty irrigation might present as yellowing and tip drying. Additionally, regular monitoring and surveillance of crops are essential to identify and address potential issues early.

Immediate measures:

Monitor regularly: Keep a close eye on your plants for any signs of disease and look out for hoppers. Early detection can help prevent further spread.

Balanced Fertilization followed by proper irrigation: Maintain a balanced nutrient supply to promote plant health and vigor, which can enhance disease resistance. Monitor soil moisture to avoid waterlogging and drying.

1.7.8 Cardamom rotting issue from Tsirang. (Report No: PLR-8)

Background

Following a report from the Dzongkhag Agriculture Sector, Tsirang Dzongkhag, regarding drying of Cardamom plants in Pungtenchhu gewog, Tsirang Dzongkhag, exhibiting symptoms of leaves turning white and decaying stem heart, samples were collected and submitted by ARDC-Bajo for diagnosis. The Pathology Program received the samples on 10th February, 2025.

Examination Method: Instant sample observation and incubation of samples in wet tissue and artificial growth media (PDA).

Observation in samples:

- Yellowing leaves with a healthy stem before dissection
- Observed larvae feeding on the heart of the cardamom stem during conducting dissection for sample culture.

Wet filter paper incubation

Both leaf and stem samples, including both symptomatic and asymptomatic portions, were cut into small pieces (approximately 4-6 per sample). These pieces were placed on moistened filter paper within 15 cm diameter Petri dishes. The prepared samples were incubated at 28°C until fungal or bacterial growth was observed on the plant tissues. Microscopic examination of the samples was conducted daily.

Isolation on artificial growth media

Cardamom leaves and stems were cut into 1 cm pieces, and three pieces were placed on PDA. The samples were categorized as leaf samples and stem samples. The inoculated plates were incubated at 28°C until microbial growth was observed on the plant tissue. As microbial growth typically occurs within 3-5 days, observations were conducted regularly.

Figure 31. Cardamom sample with an insect inside the stem

Results

On the third day of incubation (7/02/2025), no fungal pathogen growth was observed. The samples were kept in the incubation chamber for a few more days. By the sixth day, no fungal growth was detected visually or under the microscope in the wet tissue culture and leaf samples on PDA. Given that the yellowing symptoms were observed on the leaves, fungal growth was expected on the leaf samples, but none was detected.

(Note: *Fungal growth takes two to three days on average under the growth chamber at standard parameters)*

Recommendations

Regular Monitoring and surveillance of crops are essential to identify and address potential issues early.

Immediate measures:

Monitor regularly: Keep a close eye on your plants for any signs of disease and look out for insect pests. Early detection can help prevent further spread.

1.7.9 Coffee samples from major coffee-growing regions (Report No: PLR-9)

Sample Location: Major coffee-growing regions of sarpang (Umling, Sarpang and Serzhong gewogs), Samtse (Pemaliong gewog) and Zhemgang (Ngangla gewog)

Date: 14/03/2025

Background

Coffee plants from various nurseries and plantations were reported to exhibit leaf spot symptoms. Samples were examined for common foliar pathogens affecting coffee in Bhutan.

Examination Method: Instant sample observation and incubation of samples in wet tissue and artificial growth media (PDA)

Identified Pathogens and Symptoms

1. ***Cercospora spp.*** (*Cercospora* Leaf Spot)

Symptoms: Brown spots with grey centers and brown margins surrounded by yellow halos (Figure 32). Lesions spread across the leaf; dark blotches may form on berries, leading to premature drop.

Figure 32. Infection by *Cercospora* spp. (brown spots)

Distribution & Impact: Observed in a few plants with low impact at:

i. Sarpang dzongkhag-
   a. Umling gewog (Linggar)
   b. Serzhong gewog
ii. Samtse dzongkhag
   a. Pemaling gewog (Tsajangsa).

While nurseries in Ngangla (Panbang) and Pemaling (Tsajangsa) showed moderate and low impact, respectively.

Spread & Favorable Conditions: Airborne and rain-splashed spores; infection favored at 20–28°C with 36–72 hours of leaf wetness. Poor nutrition (low N & K), drought, and sunlight stress increase susceptibility.

2. ***Colletotrichum* spp.** (Anthracnose)

Symptoms: Brown to black necrotic lesions with yellow halos; affects leaves, berries, and twigs, causing dieback (Figure 33).

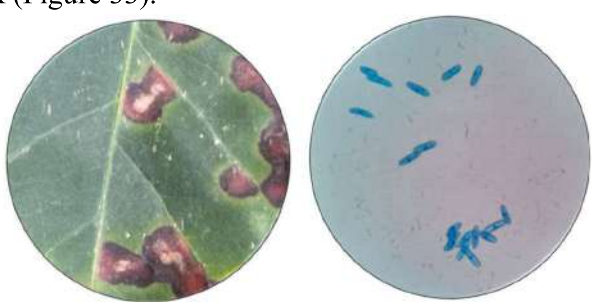

Figure 33. *Colletotrichum* leaf spot in coffee leaves (left) and spores (right)

Spread: Airborne spores, water splashes, and infected debris.

3. ***Alternaria* spp.**

Symptoms: Brown, irregular leaf spots with concentric rings and dark margins surrounded by yellow halos. Affects leaves and stems of seedlings (Figure 34).

Figure 34. Alternaria leaf spots in coffee (left) nursery and spores (right)

Distribution & Impact: Found in Ngangla (Panbang) and Pemaling (Tsajangsa) nurseries with low impact.

Spread & Conditions: Both airborne and soil-borne; thrives in warm, humid climates.

Management Recommendations

Cultural and Agronomic Practices:

- Maintain balanced nutrition (adequate N & K); conduct regular soil/tissue tests.
- Prune regularly and remove infected debris.
- Avoid planting in high-risk areas (high elevation, poorly drained).
- Provide 35–65% shade and maintain proper spacing to improve air flow.
- Timely harvesting and irrigation to reduce plant stress.

Chemical Control (if needed):

- **Fungicide:** Copper-based fungicides like Copper Oxychloride
- **Dose:** 2 g/L of water
- **Application:** Three times per season, starting at flowering
- **Note:** Use a spreader/sticker for better coverage; spray in dry, calm conditions.

1.7.10 Disease diagnosis for Paddy seedlings from Barp Gewog, Punakha (Report PLR-10)

Background

The Agriculture Extension Officer of Barp gewog under Punakha dzongkhag had submitted a paddy seedling sample collected from Laphuna village under Barp gewog, Punakha Dzongkhag. The samples exhibited symptoms such as yellowing of leaves and dieback. Additionally, white mycelia growth on the root and brown lesion on the crown were observed. The Pathology Program received the samples on 9th May 2025.

Examination Method: The samples were subjected to instant visual observation, wet-tissue incubation, and isolation on artificial growth media, Potato Dextrose Agar (PDA).

Observation on samples

- Yellowing and dieback of leave
- Brown lesion on crown
- Presences of white mycelia growth on root
- No insect pest damage observed.

Figure 35. Paddy seedling sample

Wet filter paper incubation

Both symptomatic and asymptomatic parts of leaves and roots were cut into pieces (approximately four samples) and placed on moistened filter paper in 15 cm diameter Petri dishes. The samples were incubated at 28°C, and observations were made daily for fungal or bacterial growth.

Isolation on artificial growth media

Leaf and root tissues were cut into approximately 1 cm pieces and inoculated onto PDA. Three pieces from each sample were placed on separate plates. The plates were incubated at 28°C and monitored daily for microbial growth, typically appearing within 3–5 days.

Results

Wet tissue method incubation

- Light mycelia growth was observed after 5 days of culture
- No bacterial growth was observed.

Figure 36. Light mycelia growth on wet tissue method

Isolation on growth media (Potato dextrose agar)

Both fungal and bacterial growth were observed on the sample. The fungal structures were consistent with *Rhizoctonia* sp. and *Fusarium* sp. The morphology observed was similar to that in the wet-tissue method. The dominant fungus identified was *Rhizoctonia* sp.**,** which is likely responsible for the drying up of paddy seedling.

Figure 37. Fungal and bacterial growth on PDA
(**Note:** *Fungal growth takes two to three days on average under the growth chamber at standard parameters*).

Rhizoctonia and *Fusarium* are two major fungal pathogens that cause distinct but serious diseases in paddy seedlings. Here's what each cause:

A. *Rhizoctonia solani*-disease: Sheath Blight

Figure 38. Hyphae with a septum of Rhizoctonia

Symptoms:

- Initial lesions appear as small, circular or oval, water-soaked spots on the leaf sheath near the water line.
- Lesions enlarge, turn brown with grayish-white centers.
- In seedlings, it can cause crown rot — the crown tissue becomes brown and necrotic, leading to wilting and seedling death.

Conditions Favoring Disease:

- High humidity and temperatures (25–30°C)
- Dense planting

- Excessive nitrogen fertilizer
- Poor drainage

B. *Fusarium* spp.-disease: Seedling Blight / Crown Rot / Root Rot

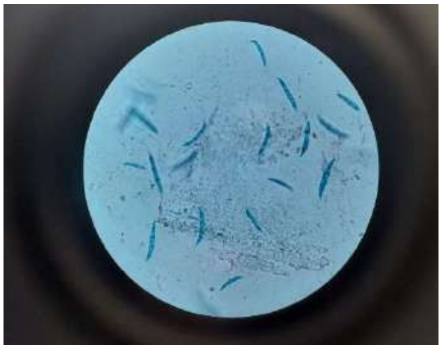

Figure 39. Septated, boat-shaped macro conidia of *Fusarium* sp.

Symptoms:

- Poor or patchy germination
- Browning and dry rot of roots, coleoptile, or crown
- Seedlings turn yellow, wilt, and die
- In some cases, you may see a pink or white fungal growth on affected tissues

Conditions Favoring Disease:

- High soil moisture
- Low soil temperature during germination
- Use of infected seed
- Poor nursery hygiene

Recommendations

1. Management Tips for both *Rhizoctonia and Fusarium.*
    - Use certified, disease-free seeds
    - Ensure good nursery drainage
    - Avoid overuse of nitrogen fertilizers
    - Rotate nursery areas or solarize nursery soil
2. Chemical control/ Recommended Fungicides:
    - ❖ Carbendazim 50% WP
        - Dose: 1–1.5 g/liter (or 2 g/kg seed for seed treatment)
        - Action: Systemic fungicide effective for seedling diseases and seed treatment.
    - ❖ Captan 50% WP
        - Dose: 2 g/kg seed
        - Action: Non-systemic seed protectant against soil-borne fungi.
    - ❖ Mancozeb 75% WP
        - Dose: 2–2.5 g/liter
        - Action: Broad-spectrum protectant, often used as a foliar spray.

Integrated Application Tips: Soil/Nursery Drench (For Infected Beds):

- Apply Hexaconazole to nursery beds where crown rot or sheath blight is observed.

Precautions:

- Always follow label instructions and use PPE during application.
- Rotate fungicides to avoid resistance buildup.
- Ensure good drainage and spacing to reduce humidity and fungal spread.

1.7.11 Disease diagnosis for Onion samples from Domkhar, Tsaenkhar gewog, Lhuentse dzongkhag (Report PLR-11)

Background

The Agriculture Research and Development Centre (ARDC), Wengkhar, submitted an onion sample collected from Domkhar village under Tsaenkhar gewog, Lhuentse dzongkhag. The samples exhibited symptoms such as yellowing of leaves, dieback, wilting, and water-soaked spots on leaves. Additionally, white mycelial growth and rotting bulbs with foul smell were observed. The Pathology Program received the samples on 18th April 2025.

Examination Method: The samples were subjected to instant visual observation wet-tissue incubation and isolation on artificial growth media, Potato Dextrose Agar (PDA).

Observation on samples

- Yellowing of leaves, dieback, wilting and water-soaked spots on leaves
- Presences of white mycelial growth and rotten bulbs with a foul smell
- No insect pest damage observed.

Figure 40: Onion Sample

Wet filter paper incubation

Both symptomatic and asymptomatic parts of leaves and bulb were cut into small pieces (approximately 4-6 per sample) and placed on moistened filter paper in 15 cm diameter Petri dishes. The samples were incubated at 28°C, and observations were made daily for fungal or bacterial growth.

Isolation on artificial growth media

Leaf and bulb tissues were cut into approximately 1 cm pieces and inoculated onto PDA. Three pieces from each sample were placed on separate plates. The plates were incubated at 28°C and monitored daily for microbial growth, typically appearing within 3–5 days.

Results

Wet tissue method incubation

- Fungal (*Fusarium* sp.) growth was observed on bulb samples (Figure 41 right).
- No fungal or bacterial growth was detected in leaf sample.

Figure 41. Fungal growth on bulb sample (left) and macro conidia of *Fusarium* sp. at 400x magnification (right).

Isolation on growth media (Potato dextrose agar)

Fungal growth was observed in both leaf and bulb samples. The fungal structures were consistent with *Fusarium* species (Figure 42). The morphology observed was similar to that in the wet-tissue method. The dominant fungus identified was *Fusarium* sp., which is likely responsible for the bulb rot.

Fusarium basal rot is a fungal disease caused by *Fusarium* sp. It mainly affects the roots and basal plate (bottom part of the bulb where roots grow) of the onion plant. The fungus lives in the soil and infects the plant through the roots, especially when conditions are favorable with warm temperatures and moist soil.

Figure 42. Fungal growth on PDA on both leaf and bulb samples (left) and septated, boat-shaped macro conidia of *Fusarium* sp. at 400x magnification (right).

Recommendations

Fusarium basal rot is primarily a soil-borne disease, meaning the fungus (Fusarium sp.) lives and survives in the soil, even in the absence of the onion crop. Once a field is infected, the fungus can persist for many years, making management extremely challenging. So, the following are a few management practices:

Immediate recommendations;

1. Remove Infected Plants and Debris

   Regularly monitor the field and uproot any infected plants. After harvest, clear and destroy plant debris to reduce the pathogen load in the field.

2. Apply Organic Amendments

   Incorporate well-rotted compost or farmyard manure to enhance beneficial soil microflora, which can help suppress Fusarium.

3. Proper Post-Harvest Storage

   After harvest, cure the onions properly in a dry, well-ventilated place before storage. Store in cool, dry conditions to prevent fungal growth.

Recommendations for next season;

1. Practice Crop Rotation

   Avoid planting onions or other Allium crops (e.g., garlic, leeks) in the same field for at least 3–4 years. Rotate with non-host crops like maize, beans, or cabbage to reduce soil-borne inoculum.

2. Use Disease-Free Seed and Sets

   Ensure that onion seeds or planting sets are certified and disease-free. Avoid replanting bulbs from infected fields.

3. Select Resistant Varieties (if available)

   Where possible, grow Fusarium-tolerant or resistant onion varieties.

4. Improve Field Drainage

   Fusarium thrives in poorly drained, waterlogged soils. Use raised beds or contouring to ensure proper water drainage.

5. Avoid Overwatering

   Maintain optimal soil moisture. Avoid excessive irrigation, especially during bulb development and late growth stages.

6. Solarize Soil (for small plots)

   Before planting, solarize the soil using transparent plastic sheets for 4–6 weeks during hot months to kill Fusarium spores near the surface if it is applicable.

7. Use Fungicidal Seed Treatment

Treat seeds with Carbendazim before sowing to reduce early infection.

2. Entomology Program

2.1 Assessing the Farmers' Knowledge, Perception, and Management Practices of Fall Armyworm (*Spodoptera frugiperda*) in Bhutan

2.1.1 Introduction

The fall armyworm (FAW), *Spodoptera frugiperda*, is a highly destructive pest that feeds on over 350 plant species due to its polyphagous nature (Holy, 2019), including major food crops such as maize, sorghum, rice, and soybean, as well as fiber crops like cotton (Prasanna, Huesing, Peshchke, & Eddy, 2021). Originally native to tropical and subtropical regions of the Americas, FAW is a strong migratory pest and has rapidly expanded its range worldwide since its first detection in West Africa in 2016 (Rwonushana, 2019). It typically occurs in tropical regions with an annual temperature ranging from 10 to 26 degrees Celsius and 500 to 700 mm annual precipitation (Early, Gonzalez-Moreno, Murphy, & Day, 2018).

In Bhutan, the Fall Armyworm was first identified in 2019 through morphological and molecular analysis, and has been reported in several dzongkhags, including Punakha, Chhukha, and Sarpang (A Guide for Fall Armyworm Identification and Management , 2021). In 2020, FAW was reported in the country as plausibly C-strain (Pashley, 1986) that infests maize. It can suddenly become a major pest (De Groote, et al., 202) due to its short development period and high fecundity (Navesero & Navasero, 2020) posing a significant threat to the country's food security. The high reproductive capacity of the FAW with females laying approximately 1,000 eggs (Murua, et al., 2015) each, combined with its ability to fly more than 100 km in a single night, allows for rapid and widespread infestations. FAW inflicts damage at various maize growth stages by defoliating leaves, damaging whorls, and feeding on ears, leading to significant yield losses and increased costs for pest management.

FAW poses a significant threat to maize production and food security in Bhutan; however, there is a limited understanding of how farmers perceive and manage this pest. This lack of insight hampers the development of effective, locally appropriate pest control strategies. To address this, it is essential to assess farmers' ability to identify FAW, their understanding of its lifecycle and damage, perceptions of its severity and causes, the control methods they use, and the challenges they face in implementing these methods.

This study aimed to fill the knowledge gaps by evaluating farmers' awareness, analyzing the effectiveness of existing practices, and identifying key barriers to effective FAW management. The findings will offer valuable insights into the realities of FAW management at the farm level, informing the design of more targeted and sustainable interventions. Ultimately, the research seeks to support improved pest management, enhance maize productivity, and contribute to greater agricultural resilience and food security in Bhutan. These insights will be crucial for guiding future extension services and policy decisions tailored to farmers' specific needs.

2.1.2 Study Site

The survey was conducted in Chhukha (27° 00′ 00″ N, 89° 29′ 60″ E, 1600 masl), Monggar (27° 15′ 42″ N, 91° 17′ 21″ E, 1800 masl), and Tsirang (26° 54′ 60″ N, 90° 04′ 60″ E, 1100 masl) Dzongkhags. The research focused on gewogs and chiwogs with the highest maize cultivation in target areas most affected by FAW, using purposive sampling to ensure relevant and reliable results.

Figure 1. Maize fields of farmers in Chhukha (left) and Tsirang (right)

Sampling Approach and Sample Size

Purpose sampling was used to select participants with relevant maize farming and FAW experience, fitting the qualitative research design aimed at understanding local challenges and informing effective interventions. To determine the appropriate sample size, a 5 % margin of error with a 90% confidence level was applied using a standard formulation for large or unknown populations:

$n = (Z^2 \times p \times (1 - p)) / E^2$

Where:

n = required sample size

Z = z-score for 90% confidence level (1.645)

p = estimated population proportion (0.5, assuming maximum variability)

E = margin of error (0.05)

A calculation yielded an estimated sample size of approximately 271 respondents. To allow flexibility during the data collection, a range of ±10 respondents was accepted, setting the sample size between 261 and 281, thereby maintaining the desired confidence level and margin of error while accommodating practical constraints.

Data Collection and Analysis

Primary data was collected using a semi-structured questionnaire covering demographics, FAW knowledge, perceptions, management practices, and government support. A pilot test with maize farmers ensured clarity and relevance. Face-to-face interviews encouraged detailed responses, with data recorded via KoboToolBox and Epicollect. After data cleaning in Excel, qualitative data were thematically coded, and quantitative data were analysed using descriptive and inferential statistics in JASP and R.

Figure 2. NPPC staff surveying farmers on their knowledge, perceptions, and management practices related to Fall Armyworm

2.1.3 Results and Discussions

Demographic Profile: Gender, Age, and Education Distribution

In Chhukha, most respondents were male (64.42%), while females were the majority in Monggar (56.6%) and Tsirang (54.71%), underscoring the need for gender-responsive outreach. The farming population was largely older, with over 46 years dominating across regions, and Chhukha having the highest proportion above 56 years (51.93%). Education levels were lowest in Chhukha and Monggar, where over 83% had no formal education, in contrast to a more educated farmer base in Tsirang. These differences highlight the importance of tailoring FAW programs using visual, community-based approaches in less-educated regions and more technical strategies in areas like Tsirang.

Maize Varieties Cultivated

Both improved and local maize varieties are widely cultivated across the three dzongkhags, with regional differences. Yangtsepa, an improved variety, dominated in Tsirang (69.81%) and Monggar (60.38%), but was less common in Chhukha (49.03%), where local varieties remained prominent (59.61%). This reflects ongoing adoption of improved seed, especially where access and extension support are stronger, while traditional varieties persist for their adaptability.

Levels of Knowledge of FAW

In Chhukha and Tsirang, around 65% of respondents correctly recognized that FAW attacks only maize, showing accurate awareness of its host specificity. However, in Monggar, 81% believed FAW attacks maize and other crops, reflecting a broader but incorrect understanding. This misconception may lead to misdirected management efforts, emphasizing the need for clear communication about FAW's actual host range to improve control practices.

Knowledge Levels on Identification of FAW Stages

Most respondents could recognize FAW larvae, with high awareness in Chhukha (98.07%) and Tsirang (92.45%), while Monggar was lower at 50.94%. Egg identification was strongest in Tsirang (75.47%) but much lower in Chhukha (20.19%) and Monggar (13.21%). Recognition of adult moths was moderate across dzongkhags, ranging from 35.57% in Chhukha to 58.49% in Monggar. These results suggest that farmers in Tsirang have a more complete understanding of FAW life stages, aiding timely management. Strengthening egg and moth identification in Chhukha and Monggar could enhance early detection and control.

FAW Damage Symptoms

In Chhukha and Tsirang, farmers commonly recognized FAW symptoms such as leaf holes (75% and 61.32%) and cob damage (75.96% and 56.6%), while these were not reported in Monggar. Whorl clipping was widely identified in Monggar (100%) and also noted in Tsirang (93.39%) and Chhukha (62.5%). Recognition of silk and tassel damage was low overall, highest in Tsirang (24.47%) and Chhukha (14.4%), and absent in Monggar. These differences suggest varying exposure or awareness of FAW damage, highlighting the need for targeted training in Monggar to improve symptom recognition and timely management.

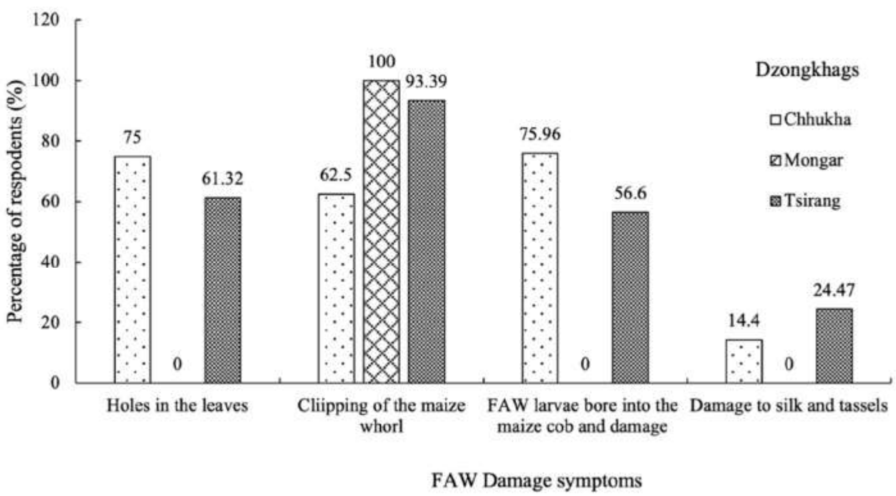

Figure 3. Percentage distribution of FAW damage symptoms across Dzongkhags

Farmers' Perception of the Causes of FAW Infestation

In Monggar, 35.85% of farmers linked FAW outbreaks to climate change, indicating greater environmental awareness compared to Tsirang (20.76%) and Chhukha (4.8%). Monocropping was mentioned only in Monggar (13.2%), while a few respondents in Monggar and Tsirang cited a lack of natural predators and poor pest management. Pest migration was noted by a small number in Chhukha (2.89%) and Tsirang (3.77%). However, the majority, especially in Chhukha (92.31%) and Tsirang (72.64%) were unsure of the causes, highlighting a significant knowledge gap and the need for targeted education on FAW biology and management.

Table 1. Farmers' perceptions of the Causes of FAW Infestation presented as percentages

Causes of Faw infestation	**Dzongkhag**		
	Chhukha	**Monggar**	**Tsirang**
Climatic change	4.8 (n=4)	35.85 (n=19)	20.76 (n=22)
Monocropping	0 (n=0)	13.208 (n=7)	0 (n=0)
Lack of natural predators	0 (n=0)	3.77 (n=3)	0 (n=0)
Poor pest management practices	0 (n=0)	1.88 (n=2)	2.83 (n=3)
Pests migrating from nearby farms	2.89 (n=3)	0 (n=0)	3.77 (n=5)
I don't know	92.31 (n=96)	45.28 (n=23)	72.64 (n=77)

Farmers' Perception of the Trend of FAW Infestation

Farmer perceptions of fall armyworm (FAW) severity varied across dzongkhags, with 60.38% in Monggar reporting increased infestation, compared to 34.61% in Chhukha and 42.45% in Tsirang. Around one-third of farmers in Chhukha and Tsirang observed a decline, while only 11.31% in Monggar reported improvement. These differences suggest variations in management practices, climate, or crop resilience, highlighting the need for localized strategies. FAW severity was highest in Chhukha and Tsirang, while Monggar saw milder impacts, likely due to earlier exposure. Peak infestations occurred in March–April in Chhukha and April–May in Monggar and Tsirang, underscoring the importance of region-specific monitoring and timely control.

Management practices of FAW

Farmers employed various management practices to control FAW, with notable differences across dzongkhags. Pesticide use was highest in Monggar (47.17%) and Chhukha (39%), while Tsirang favored handpicking (16.66%) and ash application (15.74%) with low pesticide use (8.33%). Traditional methods like cow urine were reported in Chhukha and Tsirang but were absent in Monggar. Plant removal was minimal across all areas, and pheromone traps were used only in Tsirang (1.85%). These differences reflect varying access, awareness, and cultural practices, emphasizing the need for locally adapted integrated pest management strategies.

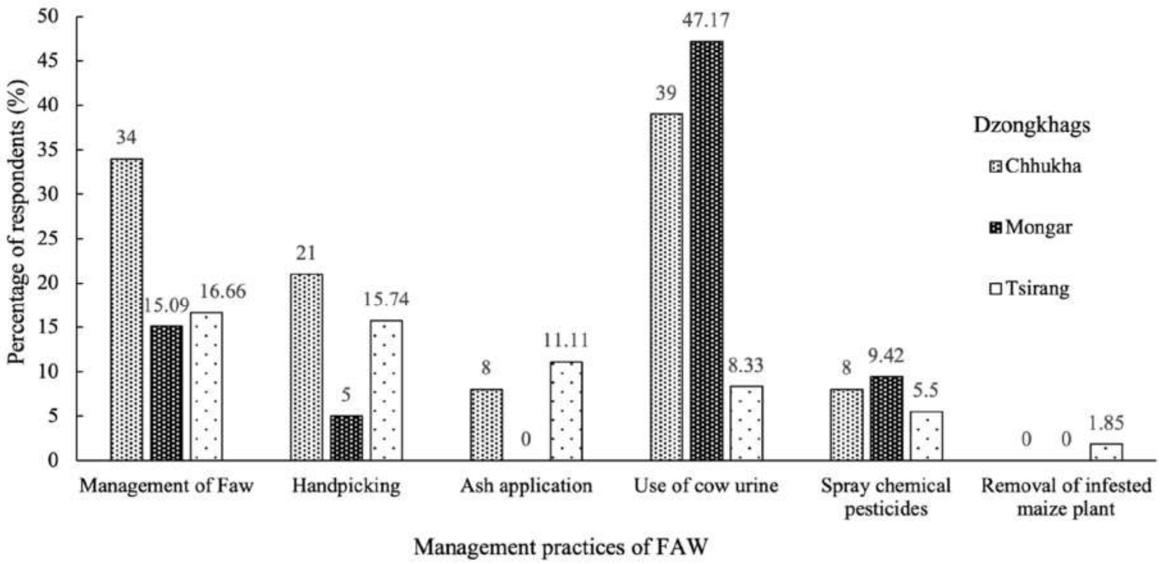

Figure 4. Management strategies deployed by farmers against FAW presented as percentages

Chemical Usage Among Farmers

Pesticide use for FAW management varied across dzongkhags. Monggar had the highest adoption (47.17%), mainly Cypermethrin 10 EC, with most farmers applying once per season (45.28%). In Chhukha, 37.5% used pesticides, though some were unsure of the products, and multiple applications were more common. Tsirang had the lowest use (9.4%) and fewer applications. Nearly all farmers sourced pesticides locally.

Challenges of FAW Infestation Faced by Farmers

Farmers across dzongkhags faced challenges managing FAW, with yield loss reported most in Tsirang (92.45%) and Monggar (84.9%), and less in Chhukha (54.81%). While pesticide access was easy in Chhukha, it remained a challenge in Monggar (9.43%) and Tsirang (19.81%). Lack of pest control knowledge was common in Monggar (47.17%) and Tsirang (29.24%), but absent in Chhukha. Limited sprayer availability affected Monggar (33.96%) and Tsirang (14.15%) farmers. These issues highlight the need for improved extension, input access, and targeted training for better FAW management.

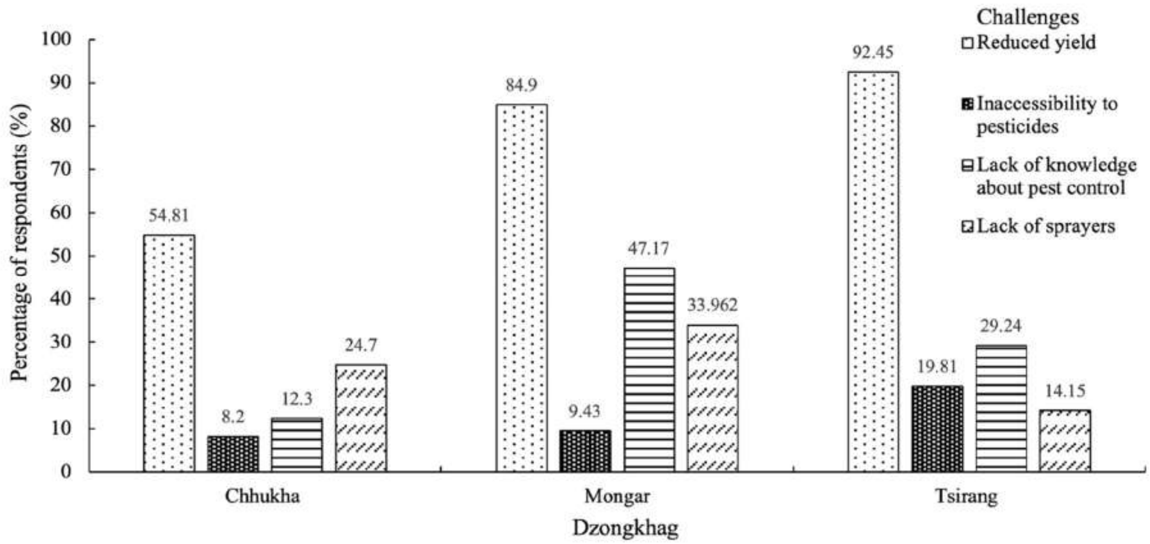

Figure 5. Challenges faced by farmers due to FAW infestation are presented as percentages

Government Support Needed by Farmers

The majority of farmers across all dzongkhags prioritized access to effective pesticides, with the highest demand in Chhukha (93.26%), highlighting the critical need for reliable control options. The need for sprayers was especially high in Monggar (49.05%) and Tsirang (29.24%), but minimal in Chhukha (2.89%). FAW management training was also a key need, cited by 62.26% of farmers in Monggar, 49.05% in Tsirang, and 44.23% in Chhukha, reflecting awareness of knowledge gaps. Additionally, access to resistant maize varieties was important for many, particularly in Monggar (28.3%) and Tsirang (17.9%).

2.1.4 Conclusion

The study found notable regional differences in demographics, knowledge, perceptions, and management of Fall Armyworm (FAW) among farmers in Chhukha, Monggar, and Tsirang. Older age groups and low education levels in Chhukha and Monggar highlight the need for visual, community-based training, while Tsirang's more educated farmers may benefit from technical approaches. Knowledge gaps in identifying FAW stages and symptoms, especially in Monggar, along with widespread uncertainty about infestation causes, point to the need for targeted awareness programs. Variations in infestation severity, peak periods, and management practices reflect the influence of local conditions, with Monggar and Chhukha relying heavily on pesticides and Tsirang favoring cultural methods. Persistent challenges such as yield loss, limited input access, and inadequate pest control knowledge reinforce the importance of integrated government support, improved access to effective control tools, and promotion of sustainable, locally adapted integrated pest management strategies.

2.1.5 Reference

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2.2 Assessing Fall Armyworm Incidence and Population Dynamics Using Sex Pheromone Traps in Rilangthang, Sergithang, Tsirang

2.2.1 Introduction

The Fall Armyworm (*Spodoptera frugiperda* J.E. Smith), a polyphagous lepidopteran pest native to the Americas, has become a serious threat to global food security following its rapid invasion of Africa, Asia, and Oceania in recent years (Goergen et al., 2016; Early et al., 2018). Since its first detection in Bhutan in 2019 (DoA, 2019), the pest has spread to most maize-growing dzongkhags, causing significant yield losses and posing challenges to subsistence farming systems that depend heavily on maize as a staple food and cash crop. Its high reproductive rate, migratory behaviour, and broad host range make its management particularly complex (Day et al., 2017).

Monitoring pest populations is a critical step in integrated pest management (IPM) as it provides data for forecasting pest outbreaks, determining optimal control timing, and reducing unnecessary pesticide applications (Ramasamy et al., 2021). Among the available monitoring tools, sex pheromone traps have been widely used for early detection and surveillance of *S. frugiperda* male moth populations worldwide (Mitchell, 1979; Prasanna et al., 2018). These traps utilise synthetic female sex pheromones to attract males, enabling the estimation of adult population dynamics and potential infestation risks in the field (Meagher & Nagoshi, 2004).

In Bhutan, studies on FAW have so far focused mainly on documenting its occurrence, host plants, and farmer perceptions (Dorji et al., 2023), with limited quantitative data on seasonal population patterns. Understanding the incidence and fluctuations of FAW populations in relation to crop phenology and local climatic conditions is vital for designing timely and cost-effective management interventions.

Rilangthang under Sergithang Gewog in Tsirang Dzongkhag represents one of Bhutan's key maize-growing pockets with a sub-tropical agro-ecological setting favourable for FAW proliferation. This study aims to assess FAW incidence and population dynamics in the area using sex pheromone traps, generating empirical data to support decision-making for localised pest management.

2.2.2 Materials and methods

Study Design and Duration

A longitudinal study was conducted to monitor the population dynamics of FAW using sex pheromone traps. The monitoring period spanned from February 2025 to May 2025, covering the entire maize-growing season.

Pheromone Traps and Lure Setup

Funnel-shaped Phero-Sensor™ traps were used to capture fall armyworm (FAW), strategically placed at both the edges and center of each field, with a minimum spacing of 50 meters to prevent interference. Fourteen traps were installed to cover approximately 14 acres. Trap height was standardized at 1.5–2 meters above ground and adjusted regularly to stay at least 20 cm above the maize canopy, following protocols by Sisay et al. (2024) and Manisha et al. (2024).

Figure 6. Field staff installing pheromone traps in a maize field to monitor FAW (right) and explaining the use of the traps local maize growers (left)

Figure 7: Monitoring sites for FAW using fourteen Phero-Sensor™ pheromone traps in maize fields. The locations of the traps are marked on the Google Earth image, showing their distribution across the study area (left); a Funnel trap

Synthetic sex pheromone lures containing Z9-14Ac (87%), Z11-16Ac (12.5%), and Z7-12Ac (0.5%)—supplied by PHEROMONE CHEMICALS, India—were used in each trap and replaced every 45 days according to manufacturer guidelines.

FAW Sample Collection and Laboratory Identification

Insect samples were collected biweekly from pheromone traps throughout the study. In the field, insects were gently removed using a soft brush or forceps and placed into labeled collection bottles marked with Trap ID and date. Bottles were kept upright in a cool box during transport. Environmental data, including temperature, wind speed, and humidity, were recorded using the AccuWeather app on each collection day. In the lab, insects were sorted into FAW and non-FAW groups, counted per trap, and FAW moths were identified under a stereomicroscope based on wing markings.

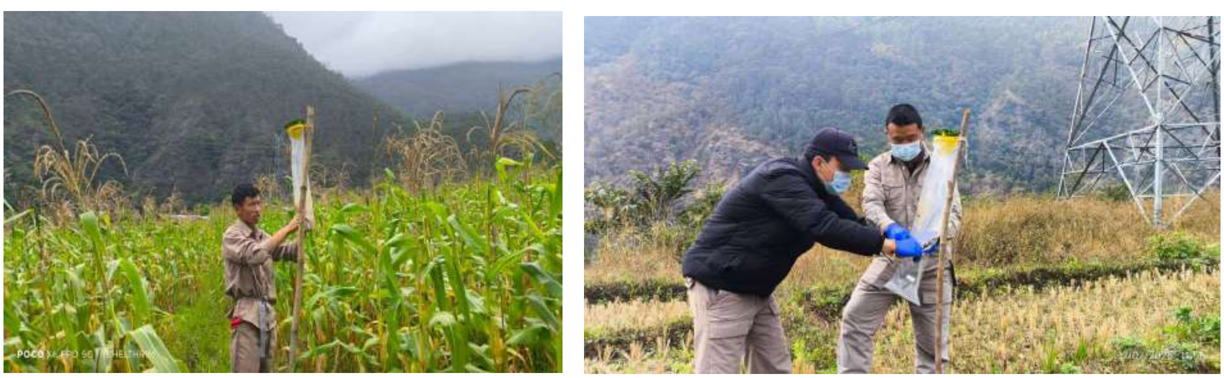

Figure 8. Field staff inspecting a pheromone trap in a maize field for FAW sample collection

2.2.3 Results and Discussions
Fall Armyworm Population Dynamics

FAW captures from pheromone traps exhibited a clear seasonal pattern, with peak activity observed in early March during the maize vegetative growth stage. The monitoring period, spanning from February to May, showed that FAW populations were highest in late February to early March, with 2, 4, and 0 individuals captured on 17 February, 3 March, and 17 March, respectively. The maximum capture occurred on 3 March, coinciding with the early vegetative stage of maize. This pattern aligns with previous studies indicating that FAW populations typically peak during the early vegetative stage of maize when young plants provide optimal conditions for oviposition and larval development (Prasanna et al., 2018; Day et al., 2017; Goergen et al., 2016).
Following the peak, FAW activity declined sharply, with no moths captured between 17 March and 26 May. Similar declines reported elsewhere may reflect natural reductions in local populations or the end of the generation's flight period (Prasanna et al., 2018; Day et al., 2017). However, other regions have shown varied FAW dynamics, including multiple peaks or prolonged activity influenced by environmental factors (Nagoshi et al., 2018; Hruska, 2019). In this study, correlation analysis showed negative associations between FAW captures and temperature ($r = –0.16$), humidity ($r = –0.38$), and wind speed ($r = –0.28$). Notably, the 3 March peak occurred under low humidity (27%), moderate temperature (25 °C), and calm winds (7 km/h), suggesting such conditions may favor moth activity. Figure 35: Population dynamics of FAW captured using funnel traps over the monitoring period

Population Dynamics of Non-target Species

During the monitoring period, non-target insect species were captured in the traps, with a total of 3,189 individuals recorded compared to only 6 FAW (Figure 3). This results in a very low FAW to non-FAW ratio of approximately 0.00188, indicating that the majority of insects attracted to the traps were non-target species. The capture of non-target species in FAW pheromone funnel traps can be attributed partly to the chemical composition of the synthetic lures used. The lures, consisting mainly of Z9-14Ac (87%), Z11-16Ac (12.5%), and Z7-12Ac (0.5%), are designed to mimic the sex pheromones of FAW (Bratovich et al., 2019). However, some of these compounds may be shared or similar to pheromone components of other noctuid or lepidopteran species (Witzgall et al., 2010b).

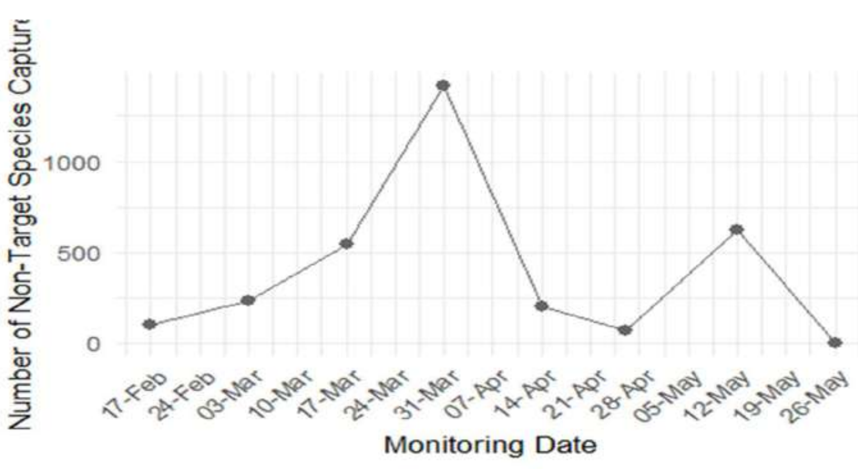

Figure 9: Population dynamics of non-target insect species captured using funnel traps over the monitoring period.

This study offers valuable insights into the seasonal patterns of fall armyworm (FAW) in Rilangthang, supporting more effective pest management strategies for local maize farmers. The key finding is that FAW activity peaks in early March, aligning with the early growth stages of maize. This period represents a critical window for timely intervention, as pest pressure is highest when plants are most vulnerable. After this peak, FAW captures declined sharply and remained low for the rest of the monitoring period. A notable challenge encountered was the high number of non-target insects caught in pheromone traps over 500 for every FAW moth, complicating accurate population assessments. These findings have practical implications: by concentrating monitoring and control efforts in early March, farmers can optimize resource use, reduce management costs, and enhance the overall effectiveness of FAW control strategies.

However, several research gaps remain that require future investigation. Long-term studies are needed to confirm whether this seasonal pattern is consistent or varies with different weather conditions. Additionally, research is needed to determine whether multiple generations of fall armyworm occur in Rilangthang. Further studies should also examine how planting dates affect fall armyworm population dynamics, as this information could help farmers choose optimal planting strategies to minimize pest pressure.

2.2.4 Conclusion

This study highlights the importance of pheromone trap monitoring for understanding the seasonal dynamics of Fall Armyworm (FAW) populations in Rilangthang, Tsirang. The results indicate a distinct population peak in early March, coinciding with the maize vegetative growth stage, which represents a critical period for targeted pest management interventions. The sharp decline in trap captures after mid-March suggests a relatively short window of high pest activity during the maize-growing season. However, the very low FAW-to-non-target capture ratio underscores the need to refine trapping techniques to improve monitoring accuracy. These findings provide practical guidance for optimizing the timing of control measures, thereby enhancing the efficiency of integrated pest management (IPM) strategies. Future research should focus on multi-season monitoring to validate these patterns, assess potential generational overlaps, and evaluate the influence of agronomic practices such as planting dates on FAW population dynamics.

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2.3 Natural Enemies Complex of Fall Armyworm (*Spodoptera frugiperda*) in Bhutan: Evidence from Laboratory Rearing

2.3.1 Introduction

The Fall Armyworm (*Spodoptera frugiperda*), an invasive lepidopteran pest native to the Americas, has rapidly established itself across multiple continents, posing significant threats to staple crops, especially maize, through its remarkable fecundity, migratory abilities, and capacity to feed on over 80 plant species (Singh, et al.). Since its initial detection in Bhutan in recent years, limited knowledge of indigenous natural enemies has necessitated the predominance of chemical control methods for pest management (Dorji, Khando, Kinley, & Jamtsho, 2023).

Biological control, leveraging naturally occurring predators, parasitoids, and entomopathogens, offers a sustainable alternative particularly suited for resource-limited agricultural settings like those in Bhutan. Globally, a diverse array of natural enemies has been identified attacking S. *frugiperda*: egg parasitoids like *Telenomus remus* and Trichogramma spp., which have achieved high parasitism rates across Latin America, Africa, and Asia (Kafle & Joshi, 2025) larval parasitoids such as *Campoletis sonorensis*, *Cotesia marginiventris*, *Chelonus texanus*, among others as well as predatory arthropods and microbial agents, including entomopathogenic fungi (e.g., *Metarhizium rileyi, Beauveria bassiana*), nematodes, viruses, and bacteria, which have demonstrated efficacy under laboratory and field conditions (Abbas, et al., 2022).

In the Himalayan region geographically and ecologically proximate to Bhutan regional investigations have noted natural predation of FAW larvae by spiders, pentatomid bugs, and wasps, alongside significant mortality from pathogens like NPV and fungi (Singh, et al.). However, systematic documentation of these biological agents in the Bhutanese context had been lacking until a recent study by Dorji *et al.* (2023), which presents a foundational inventory of local natural enemies. This study identified a total of 48 species associated with FAW-infested maize fields in west-central Bhutan. It further detailed instances of parasitism by *Chelonus formosanus*, *Trichogramma* spp., *Winthemia trinitatis*, *Linnaemya* spp., and *Campoletis sonorensis*, and predation by *Polistes olivaceus*, *Stenodynerus smilibaronii*, *Tetraponera allaborans*, *Crematogaster rogennhoferi*, and *Dolichoderus thoracicus* (Dorji, Khando, Kinley, & Jamtsho, 2023).

By integrating these initial findings with broader regional and global evidence on FAW's natural enemies, this paper aims to deepen understanding of potential biocontrol agents within the Bhutanese agro-ecological landscape. This could pave the way for developing more ecologically sound and integrated management strategies that minimize reliance on chemical inputs.

2.3.2 Materials and methods

Study area

FAW larvae were collected from major maize-growing regions in Bhutan, specifically Chhukha (four sites: Aaleg, Hatidara, Jemthang, Pagshinggang), Tsirang (five sites: Goenteg-kha, Gomsoom, Rilangthang, Soonkosh, Tsholingkhar Maed) and Samtse (one site: Sangngagchhoeling). These areas had reported significant FAW infestations and represented diverse ecological zones (ranging from ~300 m to over 2500 m altitude), making them suitable for assessing the presence and diversity of natural enemies.

Field Sampling of FAW Larvae

FAW larvae were collected through targeted sampling from infested maize plants across selected fields. In each field, 25 plants were assessed based on visible signs of FAW infestation, such as windowpane damage caused by young larvae, shot holes or irregular feeding patterns, the presence of frass (larval droppings) in the leaf whorls, and silk damage near the tasselling stage. Only infested plants were selected for sampling (Figure 1). Larvae were carefully collected using forceps to avoid injury. Larger instars (L4–L6), known for their cannibalistic behaviour, were separated and placed individually in plastic cups containing fresh maize leaves and a piece of moist tissue paper to maintain humidity. Smaller larvae (L1–L3) were kept together in group containers. All collected samples were stored in a cool box in the field and transported to the laboratory for further rearing and observation. Field-level data, including site details and collection observations, were recorded using KoboCollect.

Figure 10. Collection of FAW larvae from maize plants in the field

Laboratory Rearing and Parasitoid Observation

Collected FAW larvae were incubated at 27°C with 70% relative humidity (RH). Each larva was placed in an individual plastic cup and monitored daily for parasitoid emergence (Figure 2). Larvae were fed with clean, untreated pieces of fresh maize leaves grown in a greenhouse. Feces were removed regularly, and the cups were cleaned using cotton wool before replenishing with fresh maize leaves. Dead larvae were removed from the cups immediately after parasitoid emergence. Unparasitized larvae were allowed to complete their development to the adult stage for use in separate studies. Adult parasitoids that emerged were preserved in 99.9% ethyl alcohol and stored at 4°C for identification. Parasitoid species were identified based on morphological characteristics using standard taxonomic keys, and the number of individuals of each species emerging from parasitized larvae was recorded.

Figure 11. FAW larvae in individual plastic cups for parasitoid observation (left). Plastic cups placed inside an incubator set at controlled temperature and humidity conditions (right)

2.3.3 Results and Discussions

Parasitism Rate of FAW Larvae

A total of 140 FAW larvae were collected across 11 sites. Parasitism rates varied, with the highest observed at Pagshinggang and Jemthang in Darla, Chhukha (50%, 5/10 and 50%, 1/2 larvae parasitized, respectively). Several sites, including ARDSC, Tsirang, and Sangngagchhoeling, Samtse, showed no parasitism despite larval presence. Moderate parasitism was recorded at Soonkosh in Tsholingkhar, Tsirang (25%, 3/12), Rilangthang in Sergithang, Tsirang (17.6%, 3/17), Goenteg-kha in Pungtenchhu gewog, Tsirang (8.7%, 2/23), Tsholingkhar Maed in Tsholingkhar, Tsirang (6.2%, 1/16), and Gomsoom in Tsholingkhar, Tsirang (4.5%, 1/22). No parasitism was detected at ARDSC, Tsirang; Aaleg, Darla, Chhukha; Hatidara, Loggchina , Chhukha; and Sangngagchhoeling, Samtse. The current finding is in line with global findings; in East Africa, parasitism rates between 16% and 45% have been reported for dominant larval parasitoids like *Cotesia icipe* and *Chelonus curvimaculatus* (Abbas et al., 2022).

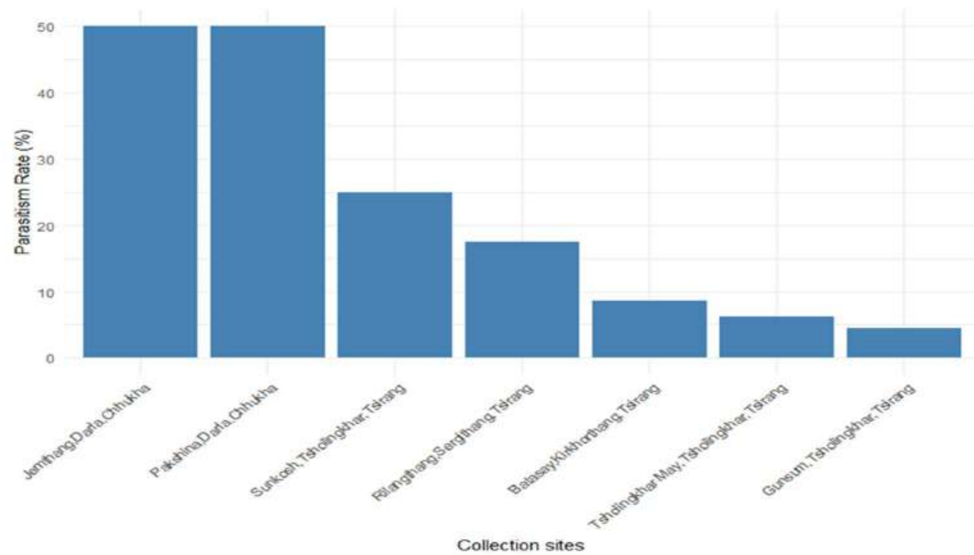

Figure 12: Parasitism rate (%) of FAW larvae collected from different collection sites in Bhutan.

Relative Abundance of Emerged Parasitoids

A total of 279 parasitoids emerged from parasitized FAW larvae across different sites. Two parasitoid taxa were identified based on morphological characteristics: *Cotesia* spp. and *Charops* spp.. Among these, *Cotesia* spp. was the most dominant, accounting for the majority of emergences at several sites. Notably, *Cotesia* spp. constituted 100% of emergence at Jemthang and Rilangthang, and over 99% at Pagshinggang, Darla, and Chhukha. In contrast, *Charops* spp. was the sole parasitoid emerging at Gomsoom and Soonkosh, Tsholingkhar, Tsirang, and contributed to a smaller proportion at Goenteg-kha and Pagshinggang. These results highlight *Cotesia* spp. as the predominant larval parasitoid of FAW in the surveyed areas. Comparable dominance of *Cotesia* has been noted in Africa, particularly *C. icipe*, which constitutes over 60% of larval parasitoid emergences. Meanwhile, *Charops* spp. have been recorded in East and West Africa at relatively lower proportions (Agboyi et al., 2020). The presence of both parasitoid genera in Bhutan shows natural enemy complexes observed in other tropical regions, suggesting a likely similar ecological role.

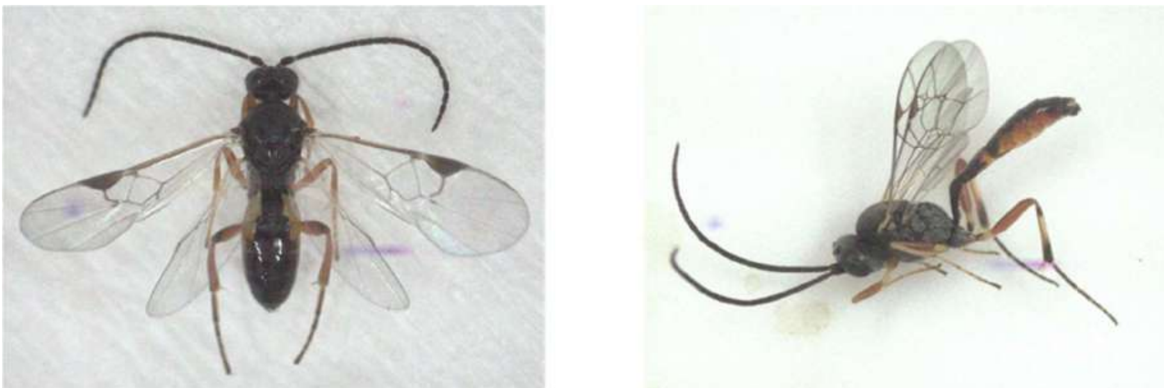

Figure 13: Adult parasitoids emerged from reared FAW larvae: (left) Cotesia spp. and (right) Charops spp., collected from maize fields in Bhutan.

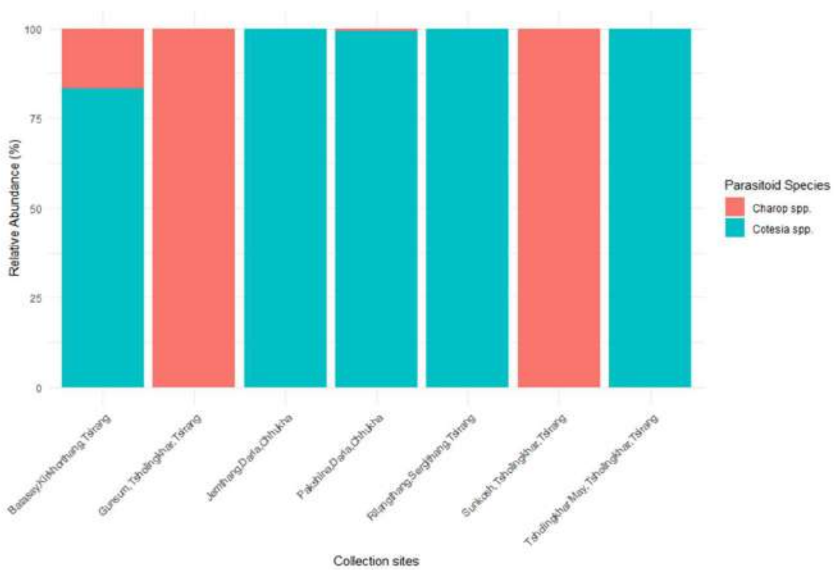

Figure 14: Relative abundance (%) of larval parasitoid species emerging from FAW across different collection sites in Bhutan. The chart shows the proportion of *Cotesia* spp. and *Charops* spp. among all emerged parasitoids at each site where parasitism was observed.

Figure 15: Two types of parasitoid cocoons observed on maize plants during field monitoring

The absence of parasitism in several sites may be attributed to environmental factors or management practices. For example, seasonal or microclimatic conditions such as rainfall, temperature significantly affect parasitoid emergence (Yan et al., 2024). Moreover, larval parasitism rates are often underestimated due to sampling biases. For example, smaller instars in whorls are less likely to be collected, even though they are the preferred target of parasitoids such as *Cotesia* spp. (Allen et al., 2021).

From a pest management standpoint, the predominance of *Cotesia* spp. positions it as a promising candidate for augmentative biological control in Bhutan. Conservation strategies, such as reducing broad-spectrum insecticide use and enhancing habitat complexity, could help boost its populations. Meanwhile, the detection of *Charops* spp. emphasizes the importance of preserving parasitoid biodiversity. These findings align with international recommendations advocating integrated pest management approaches that use native natural enemies to combat FAW outbreaks (Otim et al., 2024).

Future work should examine seasonal trends and conduct field-based parasitism assessments. Additionally, molecular identification of *Cotesia* spp. and *Charops* spp. would refine ecological understanding and improve targeting in biocontrol programs.

2.3.4 Conclusion

This study documented two key larval parasitoids of Fall Armyworm in Bhutan, namely Cotesia spp. and Charops spp., with Cotesia spp. emerging as the dominant species across most sites. Parasitism rates varied geographically, indicating localized natural enemy activity. These findings highlight the potential of native parasitoids for biological control and provide a foundation for integrating sustainable, ecologically based strategies into FAW management programs.

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2.4 Monitoring of Planthoppers and Leafhoppers in Paddy Fields Using Light Traps

2.4.1 Introduction

Rice (*Oryza sativa* L.) is one of the most important staple crops worldwide, sustaining nearly half of the global population (YAO et al., 2020). However, its production is significantly affected by more than 100 insect pest species (Mhd bookeri et al., 2021). Among these, Rice planthoppers (*Nilaparvata lugens, Sogatella furcifera*) and leafhoppers (*Nephotettix spp.)* are major threats to paddy production (Kumar et al., 2022). These pests cause damage by sucking sap from leaves and tender tissues, leading to wilting, chlorosis, and eventually "hopper burn." Additionally, they serve as vectors for major viral diseases such as rice tungro, ragged stunt, and grassy stunt viruses (Shah et al., 2023). The honeydew secreted by these insects also fosters fungal growth, compounding crop loss. Previous studies have highlighted their high migratory behavior (Hu et al., 2017), which facilitates sudden pest outbreaks in new rice-growing areas. Therefore, understanding both their movement and timely detection is essential for developing integrated pest management strategies.

One of the widely adopted methods for monitoring hoppers is the use of light traps, which exploit the insects' phototactic behavior (Mhd bookeri et al., 2021). Light traps are cost-effective and eco-friendly tools that enable farmers and researchers to observe population trends and pest dynamics over time (YAO et al., 2020). It also helps in identifying peak periods of pest activity, facilitating better timing of control interventions. Several studies have demonstrated their effectiveness in monitoring hopper populations in rice ecosystems (Shah et al., 2023).

In this context, our study was conducted under the AFACI PMP+ project, focusing on the population dynamics and abundance of rice planthoppers and leafhoppers in two sites: ARDC Bajo On-station and Wakhuna, Punakha. Hopper samples were collected from rice fields at both locations to monitor seasonal trends and fluctuations in population levels. The primary objective was to understand how hopper populations vary over time and across sites, providing insights into their temporal patterns and potential impact on rice cultivation. These findings are expected to support the development of more effective, timely, and site-specific pest management strategies within rice ecosystems.

2.4.2 Materials and methods

Monitoring sites and period

Light traps were installed in the paddy field of ARDC Bajo on-station (27°29'24.84" N, 89°53'54.69" E, 1211m asl), Thedtsho Gewog, Wangdue Phodrang, and Wakhuna (27°39'12.97" N, 89°46'27.37" E, 1338 m asl) Kabisa Gewog in Punakha Dzongkhag as shown in Figures 1 and 2. The monitoring and data collection were conducted from the vegetative stage (July) until maturity (November) in both sites.

Figure 16. Light trap at ARDC Bajo, Wangdue Phodrang (left) and light trap at Wakhuna, Punakha (right)

Light trap

The light trap was constructed from green-painted mild steel, with overall dimensions of 40 cm × 40 cm × 25 cm. Each trap featured a funnel measuring 30 cm in length and 5 cm in diameter, positioned below a 20 W incandescent bulb. The distance between the light source and the trap's roof was maintained at 20 cm to optimize insect attraction. A collection box was placed at the bottom of the funnel to hold trays for collecting the trapped hoppers. The traps were installed at the edge of the paddy fields and sheltered to prevent rain interference. The operation of the light traps, including switching them on at dusk and off at dawn, was performed manually by the regional plant protection officer and the local farmer daily.

Data collection

Insects trapped were transported to the laboratory, where hoppers were segregated and morphologically identified. The number of adult brown planthoppers (*Nilaparvata lugens*), white-backed planthoppers (*Sogatella furcifera*), green leafhoppers (*Nephotettix nigropictus* and *Nephotettix virescens*), and zigzag planthoppers (*Recilia dorsalis*) was recorded bi-weekly. Hopper collection and counting began two weeks after the installation of the light traps and continued at fortnightly intervals until the paddy crop reached maturity.

2.4.3 Results and Discussions

Hopper Population Trends

At ARDC Bajo, the population dynamics of hoppers revealed a distinct peak during the reproductive stage of the rice crop, as shown in Figure 17. All four hopper species, Green Leafhopper (GLH), Brown Planthopper (BPH), Zigzag Leafhopper (ZZLH), and White-Backed Planthopper (WBPH), recorded their highest numbers during this stage. The highest counts observed were 88 GLH, 41 ZZLH, 19 BPH, and 18 WBPH. This suggests a clear population buildup leading up to the reproductive stage, particularly noticeable in October. In other growth stages, the hopper populations remained relatively low, indicating that the reproductive stage provides the most favorable conditions for hopper proliferation. The findings from Skawsang et al. (2019) also observed an increase in incidence during the reproductive stage of the paddy.

In contrast, hopper populations at Wakhuna were generally lower across all stages of crop growth, as indicated in Figure 18. However, GLH showed an occasional spike during the booting stage, while WBPH reached a small peak of 8 individuals during the reproductive stage. Other hopper species showed minimal or no significant fluctuations at this site.

Species Distribution

Among the four hopper species, the GLH was the most dominant at both sites. At ARDC Bajo, it exhibited multiple peaks with the highest count of 88 during the reproductive stage, while at Wakhuna, the total count of GLH reached 50 individuals. BPH showed moderate abundance, peaking late in the season at both locations, with 19 hoppers at ARDC Bajo and only 2 at Wakhuna, both recorded during the reproductive stage. WBPH occurred in relatively low numbers but showed distinct peaks during the reproductive stage at ARDC Bajo (11 and 18 hoppers). ZZLH was the least abundant and sparsely distributed, recorded only at ARDC Bajo with 41 individuals during the reproductive stage and absent at Wakhuna.

Inter-Site Comparison

Overall, hopper populations were consistently higher at ARDC Bajo than at Wakhuna. This trend was particularly evident for GLH and WBPH, suggesting that ARDC Bajo offers more favorable environmental conditions or host plant suitability that promote hopper survival and reproduction.

The contrasting population trends between the two sites highlight the potential influence of site-specific ecological or agronomic factors on hopper abundance and distribution.

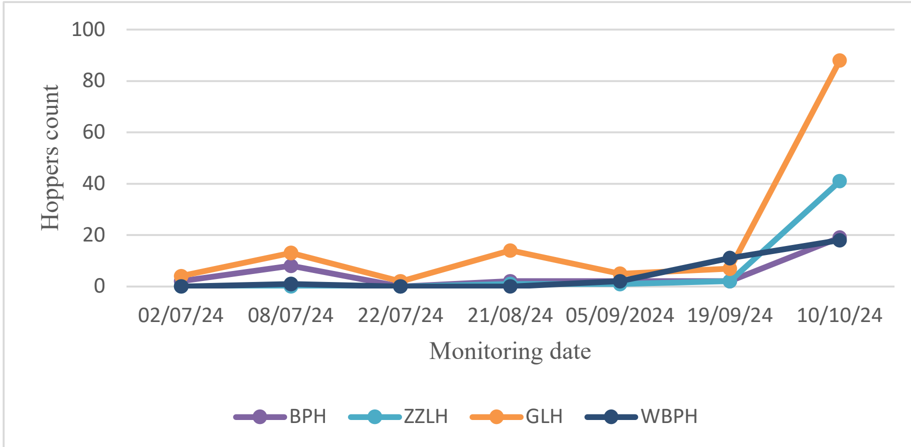

Figure 17. Hopper population dynamics at ARDC Bajo, Wangduephodrang

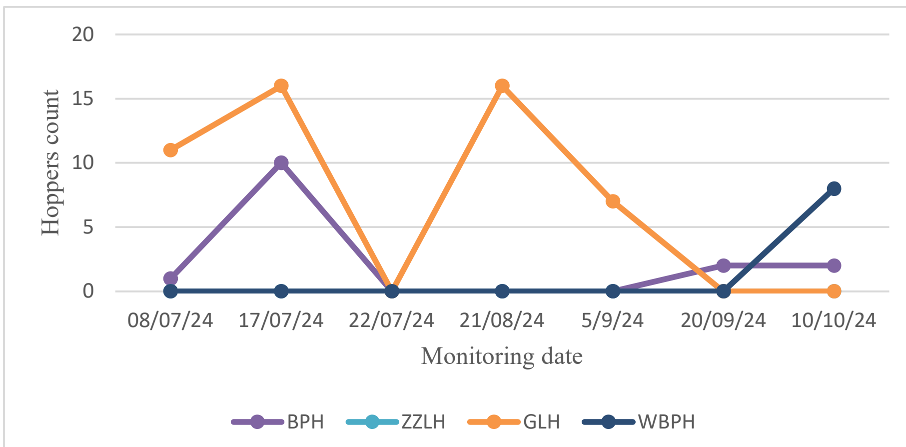

Figure 18. Hopper population dynamics at Wakhuna, Punakha

2.4.4 Conclusion

The findings from this study reveal clear temporal and spatial trends in hopper populations across the two monitored sites, ARDC Bajo and Wakhuna. Hopper densities, particularly those of the GLH, peaked significantly during the reproductive stage of the rice crop, with ARDC Bajo exhibiting notably higher populations than Wakhuna. This suggests that the reproductive stage provides favorable conditions for hopper proliferation and that site-specific factors at ARDC Bajo may enhance hopper survival and reproduction.

Among the four hopper species observed, GLH was the most dominant across both sites, followed by BPH, WBPH, and ZZLH, the latter being the least abundant and absent from Wakhuna. The marked differences in species distribution and abundance between the sites underscore the importance of localized pest monitoring and tailored management strategies.

These results emphasize the need for timely pest surveillance, particularly during the reproductive stage of the crop, to enable early intervention and minimize potential yield losses. Furthermore, the variation in hopper abundance across sites highlights the importance of integrating site-specific integrated pest management (IPM) strategies that consider both ecological conditions and pest behavior.

2.4.5 Reference

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2.5 Monitoring of hoppers in paddy using the yellow sticky trap

2.5.1 Introduction

Rice agroecosystems are highly vulnerable to insect pest infestations, which can significantly threaten both yield and quality (Hu et al., 2017). Among the most persistent threats are rice planthoppers (*Nilaparvata lugens*, *Sogatella furcifera*) and leafhoppers (*Nephotettix* spp.), which damage crops through direct feeding and by transmitting destructive viral diseases such as rice tungro, grassy stunt, and ragged stunt. Prolonged feeding by large populations causes the rice plants to wither and die in patches, a condition commonly referred to as 'hopper burn' (Kumar et al., 2022). In addition, their honeydew secretions promote secondary fungal infections, further compounding crop losses. The migratory nature of these pests allows them to rapidly colonize new rice-growing areas, leading to sudden and severe outbreaks (Kalile et al., 2022). In this context, effective monitoring is essential not only for early detection but also for timely decision-making in pest management interventions.

To support this goal, yellow sticky traps are widely used as a passive field monitoring technique that enables continuous and low-disturbance collection of flying insects (Böckmann et al., 2021). These traps are particularly effective for capturing small, mobile insects such as rice planthoppers, leafhoppers, and their natural enemies, including parasitoids and predatory flies(Se, 2017). The bright yellow color acts as a visual attractant, drawing insects toward the adhesive surface, where they are trapped upon contact (Shi et al., 2021). By deploying these traps at regular intervals, researchers can gather valuable data on the presence, abundance, and seasonal trends of target species. This method helps in identifying peak pest pressure and assessing the relative activity of beneficial arthropods, providing insights into pest–natural enemy interactions. Such data are instrumental in informing the timing and intensity of control measures, especially in strategies aimed at reducing pesticide inputs and enhancing biological control. Additionally, the use of yellow sticky traps allows for standardized sampling, making it easier to compare population dynamics across locations and time periods.

As part of a collaborative effort under the AFACI PMP+ project, this study was initiated to enhance understanding of the paddy hopper population dynamics in Bhutanese rice ecosystems. Yellow sticky traps were deployed in selected rice fields over the cropping season. The collected specimens were preserved, identified, and analyzed to assess the composition and abundance of the paddy plant and leaf hoppers. This research aims to generate baseline data on species diversity and population dynamics, which can inform ecologically based pest management strategies and contribute to the development of early warning systems. Ultimately, improving our knowledge of paddy hopper population dynamics through trap-based monitoring to support sustainable rice production.

2.5.2 Materials and Methods

Study Locations and Sampling Design

The study was conducted at three locations: ARDC Bajo (27°29'24.84" N, 89°53'54.69" E, 1211m asl), Sirigang (27°38'13"N, 89°48'21"E, 1291 m asl), and Wakhuna (27°39'12.97" N, 89°46'27.37" E, 1338 m asl). At each location, three distinct rice terraces or fields, spaced more than 10 meters apart, were selected as sampling sites. Each field was required to be at least 1,000 m² in area to ensure adequate sampling coverage.

Yellow Sticky Trap Setup

Yellow sticky traps were employed to monitor the population dynamics of leaf and plant hoppers, along with their associated natural enemies. Each sticky trap was assigned a unique code comprising four parts: the country code based on the top-level domain (e.g., bt for Bhutan), the location code representing each sampling site, L1 for ARDC Bajo On-station, Wangdue Phodrang; L2 for Sirigang, Punakha; and L3 for Wakhuna, Punakha, the sampling date in the format YYYYMMDD (e.g., 20240601 for June 1, 2024), and the replication code (R1, R2, or R3, indicating three replications per site).

Sampling Procedure

Sampling was initiated ten days after rice transplanting and conducted biweekly until the crop was harvested. During each sampling event, ten points within each field were selected either diagonally or vertically across the terrace. At each designated point, a yellow sticky trap was placed between two rice plants, and the plants adjacent to the trap were tapped three times to dislodge insects onto the sticky surface. A photograph of each trap was taken immediately after deployment to visually document insect presence. Following sampling, each trap was covered with a transparent plastic film to prevent contamination and then transported carefully to the laboratory for further examination.

Field Data Collection

In addition to insect trapping, several key field-level data were recorded during each sampling. These included photographs capturing the entire field and all three terraces to provide visual context, as well as crop-specific details such as the rice variety grown, the crop's growth stage at the time of sampling, and the transplanting date. The GPS coordinates of each sampling site were also recorded to ensure spatial accuracy and future traceability.

Laboratory Analysis

In the laboratory, insects adhered to the sticky traps were carefully identified based on their morphological characteristics. The primary insect groups of focus included planthoppers such as the Brown Planthopper (*Nilaparvata lugens*), White-Backed Planthopper (*Sogatella furcifera*), and Small Brown Planthopper (*Laodelphax striatellus*), as well as leafhoppers including the Green Leafhopper (*Nephotettix virescens*) and the Zigzag Leafhopper (*Recilia dorsalis*). Beneficial arthropods trapped on the sticky surface were also morphologically identified and recorded in the laboratory. Following identification and counting, sticky traps were preserved in refrigerators for potential future reference. For molecular analyses, selected insect specimens were preserved in ethanol-filled tubes. Each tube was labeled internally using a paper strip inscribed in pencil, indicating the sampling site, collector’s name, date of sampling, host plant or crop, and GPS coordinates. This labeling ensured proper traceability for future reference.

2.5.3 Results and Discussions

The monitoring of hoppers using the yellow sticky trap revealed notable patterns in the population dynamics of hoppers across the three sampling locations, ARDC Bajo On-station (Location 1), Sirigang (Location 2), and Wakhuna (Location 3), throughout the rice growth period.

Dominant Species

Among the hopper species captured, the GLH emerged as the dominant species across all sites, as shown in Figures 1, 2, and 3. GLH exhibited a sharp population peak at Location 1 (ARDC Bajo) during the reproductive stage of rice growth, indicating a strong association with this critical phenological phase.

Minor Species

In contrast, the BPH and SBPH were detected only in very low numbers across all locations and growth stages. Their minimal presence suggests a low pest pressure from these species during the cropping period. The WBPH, though more consistent in appearance, remained at low to moderate levels, indicating it was not a dominant pest threat but warrants occasional monitoring.

Temporal Trends

Across all sites, hopper populations generally increased gradually during the vegetative stage and reached their peak during the reproductive stage. This trend underscores the reproductive stage as a critical window for monitoring and management, especially for GLH, which showed significant activity during this period. The presence of hoppers during early stages also indicates the potential for early colonization, emphasizing the need for continuous surveillance starting from the vegetative phase.

These findings highlight the importance of stage-specific pest management strategies. Targeted interventions during the reproductive stage, particularly at locations with historically high GLH populations, may be essential to prevent economic damage. The localized peak of ZZLH at Wakhuna also suggests that site-specific ecological factors, such as microclimate or host plant variety, may influence hopper distribution and should be further studied.

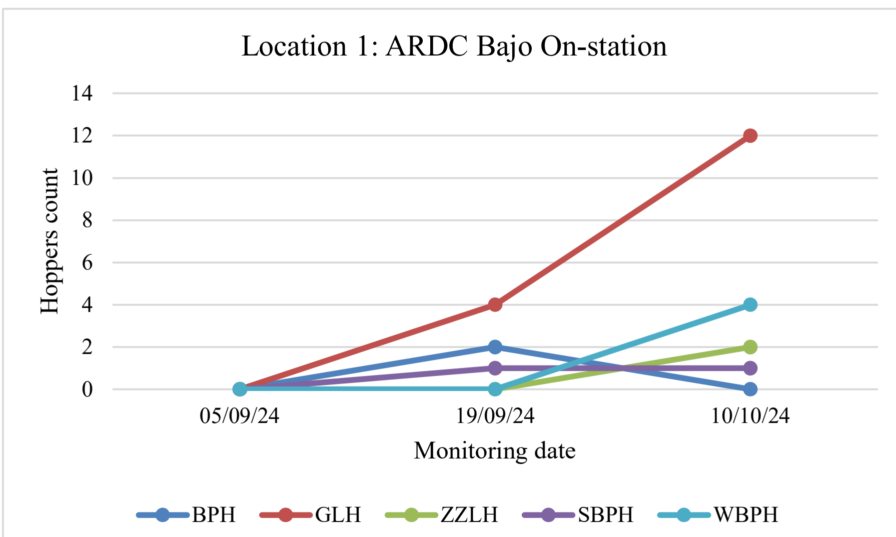

Figure 19: Hopper population dynamics at ARDC Bajo

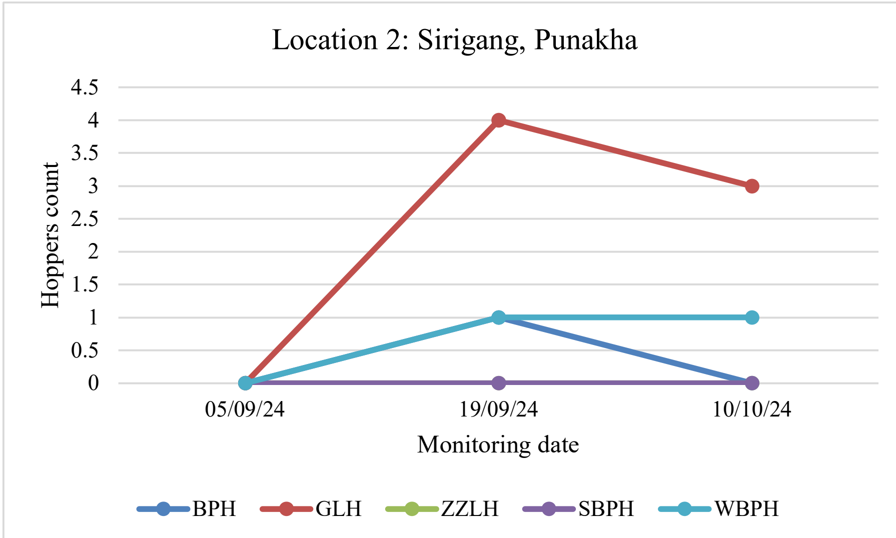

Figure 20: Hopper population dynamics at Sirigang, Punakha

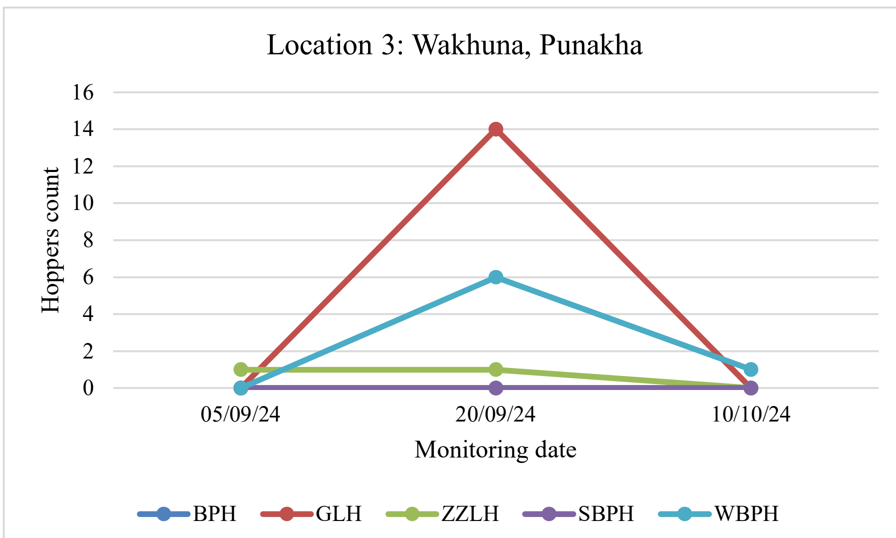

Figure 21. Hopper population dynamics at Wakhuna, Punakha

2.5.4 Conclusion

The study using the yellow sticky traps successfully elucidated the temporal distribution patterns of paddy plants and leaf hoppers across three rice-growing sites, ARDC Bajo On-station, Sirigang, and Wakhuna. The GLH is identified as the dominant species, with population peaking during the reproductive stage at ARDC Bajo. In contrast, other species such as the BPH, SBPH, and WBPH were present in negligible to moderate numbers, indicating relatively low pest pressure from these species during the cropping period.

The population dynamics indicated that hopper densities increased progressively from the vegetative stage and culminated during the reproductive stage of the rice crop. These findings underscore the reproductive stage as a critical period for intensive pest surveillance and management interventions, particularly for GLH.

Overall, this study provides crucial baseline information for developing targeted IPM strategies against rice plant and leaf hoppers in Bhutan. The distinct population peaks of GLH followed by WBPH highlight the importance of stage-specific and site-specific monitoring. To enhance pest management precision, future research should incorporate climatic factors and varietal responses to support sustainable and resilient rice production systems.

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2.6 Survey of Insect Pests of Quinoa in Bhutan

2.6.1 Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a highly nutritious pseudo-cereal from Bolivia, Chile, and Peru, cultivated for over 6,000 years (Bazile, 2015). Quinoa cultivation has spread to over 125 countries due to its high nutritional value and adaptability to various environments (Bazile, 2023). It has superior protein content and amino acids than other cereals (Silva et al., 2020), making it a nutrient-rich ingredient for livestock feed (Repo-Carrasco, Espinoza, & Jacobsen, 2003). Quinoa was introduced to Bhutan in 2015 by the Department of Agriculture with FAO support to diversify farming systems and improve nutritional security and income (Katwal, Wangdi, & Giri, 2019).

Quinoa cultivation presents potential challenges, particularly in managing insect pests. Quinoa is vulnerable to damage from various insect pests, especially leaf miners, borers, sap-sucking, and leaf cutters during the growing period (Dube, Mudada, Gama, & Muziri, 2022); (Szczepaniec & Alnajjar, 2023); (Rasmussen, Lagnaoui, & Esbjerg, 2003). In South America, approximately 80 species of phytophagous arthropods infest quinoa (Cruces, de la Peña, & De Clercq, 2024), with the quinoa moths *Eurysacca melanocampta* and *Eurysacca quinoae* being major pests, while thrips and aphids are of minor importance (Cruces, de la Peña, & De Clercq, 2020).

In Bhutan, the limited research on insect pests affecting quinoa presents significant challenges. While detailed knowledge of the pests is lacking, sporadic observations of aphids and leaf miner damage have occurred. Developing effective pest management strategies is difficult without a detailed understanding of the pests, which risks reducing quinoa yields and quality. To address this issue, this comprehensive survey of insect pests infesting quinoa in Bhutan was conducted to provide the essential groundwork for developing targeted and effective pest management practices.

2.6.2 Materials and Methods

Survey sites

The survey was conducted in the dzongkhags of Chhukha (Bongo, Chhapchha, Sampheling, and Darla), Wangdue Phodrang (Darkar, Thedtsho, Gangteng, and Phobji), Dagana (Khebisa and Tshendengang), and Tsirang (Kilkhorthang and Sergithang).

Methodology

The method described by Cruces et al., 2020 was followed. At each survey location, the field was divided into five sectors (based on the acreage of the field). In each sector, five quinoa plants were observed for insect pests and disease according to the plant’s growth stage. Insects and diseases were examined at various stages of plant development. During the germination stage, the entire plant was inspected. In the vegetative stage, the stem and leaves were examined. At the reproductive stage, insects were inspected on the panicles containing clusters of flowers. Border plants were avoided during sampling.

Insects were collected in labeled glass vials containing 75% v/v ethanol for identification. Additionally, damaged plant parts, particularly in cases where insects were not visible or were suspected to be hiding, such as with leaf miners and stem borers, were also collected to ensure thorough examination and identification of concealed pests. At each sampling event, different random plants were selected and sampled to maintain a comprehensive overview of pest presence across the field.

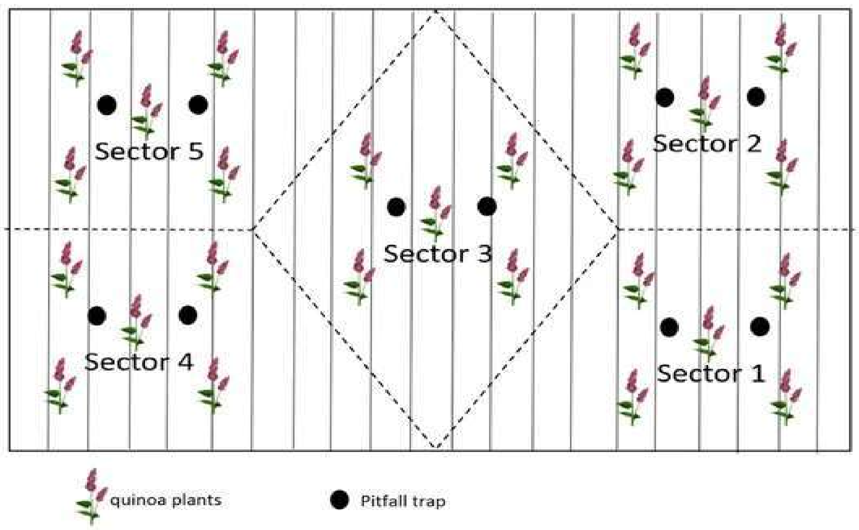

Figure 22. The sectorization and sampling scheme used for the quinoa insect pest survey.

Data collection

Comprehensive field data were collected using KoboCollect, a digital data collection platform. The survey recorded key site-level information, including a unique site ID (beginning with 1), the date and time of data collection, and precise location details such as dzongkhag, gewog, and chiwog. GPS coordinates were captured to accurately geolocate each quinoa field. Additional agroecosystem characteristics were documented, including the total area under quinoa cultivation, measured in decimals (where 1 acre equals 100 decimals). Data on the quinoa variety grown, the phenological stage of the crop at the time of observation, and the irrigation method employed were also recorded. Pest assessments were conducted on 25 quinoa plants per field, with five plants sampled from each of five predefined field sectors.

2.6.3 Results and Discussions

The survey across five quinoa growing dzongkhags, Wangdue Phodrang , Chhukha, Samtse, Dagana, and Tsirang, identified a complex of eight insect pests affecting quinoa crops, as shown in **annexure 2**. The most prevalent were leaf miners (*Liriomyza* spp.), black bean aphids (*Aphis fabae*), green peach aphids (*Myzus persicae*), and Lygus bugs (*Lygus* spp.). Leaf miners were present in over 70% of surveyed sites, especially during the vegetative and early reproductive stages, causing significant foliar damage through serpentine mining patterns. Similar findings by (Cruces, de la Peña, & De Clercq, 2021) also highlight leaf miners as a major pest of quinoa. Aphid infestations, particularly in Tsirang and Wangdue, were dominant during the flowering and grain filling stages, with potential implications for virus transmission. Lygus bugs were commonly observed during the reproductive phase, where their feeding may impact grain development.

Minor pests included shield bugs, loopers, caterpillars, and a single incidence of stem borer in Dagana. These pests occurred at low densities and did not cause significant economic damage, but signal the need for continued vigilance. Pest incidence varied by agroecological zone; subtropical dzongkhags like Dagana and Tsirang exhibited higher pest diversity and intensity compared to cooler temperate zones such as Phobji. Infestations were minimal in newly germinated fields, indicating a temporal pattern, with peak pest pressure occurring from vegetative to reproductive stages, likely driven by increased resource availability during those phases.

From the weeds survey in the Quinoa crop, *Galinsoga quadriradiata, Persicaria nepalensis, Bidens pilosa, Ageratum conyzoides, and Polygonum aviculare* were the dominant weeds in the quinoa crop, but compared to dryland crops such as maize and vegetables, quinoa fields exhibit lower weed diversity and infestation, as shown in **Annexure 3**. For the disease infestation, only downy mildew was recorded during the survey in the quinoa field at Darkar gewog, Wangdue Phodrang.

Overall, the findings underscore the emerging significance of insect pests, weeds, and disease infestation in quinoa cultivation under Bhutanese agroclimatic conditions. The dominance of polyphagous pests such as leaf miners and aphids suggests the potential for rapid population growth, emphasizing the need for Integrated Pest Management (IPM) interventions. Regular monitoring, field sanitation, and biological control, especially targeting leaf miners, should be prioritized.

2.6.4 Conclusion

This study provides the first field-level documentation of insect pests, weeds, and disease incidence in quinoa *(Chenopodium quinoa Willd.*) production in Bhutan. Surveys across five dzongkhags recorded eight insect pests, dominated by *Liriomyza spp., Aphis fabae, Myzus persicae, and Lygus spp.;* the principal weed species were *Galinsoga quadriradiata, Persicaria nepalensis, Bidens pilosa, Ageratum conyzoides, and Polygonum aviculare*; and a single occurrence of downy mildew was observed at Darkar gewog, Wangdue Phodrang. Pest and weed pressure were higher in subtropical mid-hill zones and peaked from vegetative to reproductive phenophases, with leaf miners present in over 70 percent of sites and aphids prevalent during flowering and grain fill.

These findings indicate a multi-trophic threat to quinoa productivity via direct tissue loss, impaired grain development, and potential virus transmission. Development of an integrated pest management program tailored to Bhutanese agroclimatic conditions is recommended, prioritizing standardized monitoring, cultural practices, and field sanitation to disrupt pest and pathogen cycles, targeted weed management, and conservation or augmentation of biological control agents. The results provide a baseline for future surveillance and sustainable pest management interventions.

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3. Weed Science Program

3.1 Efficacy evaluation of synthetic weedicides for the management of Wetland Weeds in Transplanted Paddy cultivation

3.1.1 Introduction

Rice is the country's most important staple cereal, with the government aiming for at least 30% self-sufficiency in rice production during the 13th Five-Year Plan (13FYP). To reduce reliance on imports, farmers must overcome challenges such as pests, diseases, labor shortages, irrigation issues, nutrient deficiencies, and wildlife damage. However, weed-related yield losses are often underestimated, as their detrimental effects typically appear late in the crop's growth, making timely control difficult.

The Department of Agriculture (DoA) has tried various weed management tools, but mechanical methods proved impractical due to the terrain and fragmented fields. Consequently, since the early 1980s, the DoA introduced safer herbicides, with the National Plant Protection Centre (NPPC) distributing Butachlor from 1989 to control weeds in transplanted rice. Without effective organic alternatives, manual weeding remains the main control method. The NPPC's Weed Science Program continues to evaluate newer, environmentally safer herbicides. This study evaluates the effectiveness of two such synthetic herbicides in managing paddy weeds.

Butachlor 5G, a selective pre-emergence herbicide, has been the primary chemical used for weed control in rice fields for over thirty years. Despite a decline in the area planted with paddy, farmer demand for Butachlor has continued to rise, suggesting either the development of weed resistance or labor shortages in rural areas. Although a new herbicide, ethoxysulfuron (15% WDG), was introduced in 2019 following extensive research, it has not replaced Butachlor because it only targets broadleaf weeds in transplanted rice, leaving grassy weeds uncontrolled. This highlights the ongoing need for an alternative herbicide to effectively manage grassy weeds in paddy cultivation.

To address this, the Weed Science Program has been testing two new herbicides for their ability to control grasses and other weeds in rice fields and greenhouse conditions over one season. The current study marks the second season of this trial, which is being conducted across different sites. The objectives are to evaluate the efficacy of two new weedicides as post-emergence weedicides against complex weed flora in transplanted rice and compare the effectiveness of current weed management practices (i. Butachlor+sunrice, ii. Sunrice) with the new weedicides under study.

3.1.2 Materials and methods

Study site and period

The trial was conducted at three different sites, one of which was the on-station greenhouse at NPPC. Although the trial was established there on June 5, 2024, during the previous financial year, the treatment application and data collection for this site took place in the current financial year. Therefore, this report focuses solely on the treatment application details and data collection results from the on-station greenhouse.

In addition to the greenhouse trial, field trials were established at two locations: a farmer's field in Wakhuna, Kabisa, on June 14, 2024, at the same site as the previous season's trial, and the on-station field at ARDC-Bajo on June 22, 2024. The timing of these field trials was aligned with the paddy transplantation season and adjusted according to the specific conditions of each site.

Like the previous trial, this season's trial was conducted using a Randomized Complete Block Design (RCBD) with four treatments, each replicated three times.

Treatment specification

i. Treatment 1: Ethoxysulfuron 15% WDG (50 gm/acre)
ii. Treatment 2: Fenoxapro-p-Ethyl 9.3% EC (250 ml/acre)
iii. Treatment 3: Triafamone 20% + Ethoxysulfuron 10% WG (90 gm/acre)
iv. Treatment 4 (Control): Butachlor 5% G + Ethoxysulfuron 15% WDG (50 gm/acre)

Experimental setup

At the farmer's field, the farmer carried out the field preparation and transplantation, with plots varying in size; each plot was measured individually to adjust the herbicide application rate according to its specific area. While the experimental site at ARDC-Bajo was established by regional plant protection officers with support from the field crops team, establishing uniform plot sizes of 5 m X 2 m.

Application of treatments (weedicides)

Butachlor, a pre-emergent herbicide, was applied five days after paddy transplantation in the field and five days after planting turions in the greenhouse, targeting weeds before their emergence. In contrast, the post-emergent herbicides, Sunrice, Ricestar, and CouncilActiv were applied approximately 15 days after transplantation or planting, once the weeds had reached the 2 to 4 leaf stage.

Considering the varying plot sizes at different sites, the application dosage for each treatment was carefully calculated based on the recommended rates indicated on the product labels, adjusted to the size of each individual plot. To ensure accurate dosing, a digital weighing balance was used for granular formulations, while a measuring cylinder was employed for liquid formulations. These tools helped guarantee precise measurement of the herbicide quantities applied to each plot.

A water level of 10 cm above the soil surface was maintained during herbicide application and for 10 days thereafter. This practice is essential because pre-emergent herbicides require consistent moisture to activate and effectively penetrate the soil to control weeds. Maintaining a uniform water level ensures even distribution and absorption of the herbicide throughout the entire plot, minimizing the risk of uneven application and preventing localized herbicide concentrations that could potentially damage the crop.

Data Collection

Data collection was conducted both before and after weedicide application. In the field trials, the square method was employed using a 1 x 1 meter quadrant placed randomly at a position which was demarcated to ensure that subsequent data were gathered from the same location. In the greenhouse, data were collected from the entire chamber or plot. Subsequent collection of data was consistently taken within the same marked areas throughout the experiment. All collected data were recorded and systematically organized using Microsoft Excel for further analysis.

Pre-spray data collection

Pre-spray data collection involved identifying the different weed species present within the sampled and demarcated quadrants. The number of individual weeds of each species was counted and recorded for every quadrant. This data was collected one day prior to the application of weedicides to establish a baseline for comparison with post-application observations.

Post-spray data collection

Post-spray data collection began 14 days after the herbicide application and was subsequently carried out every 14 days (biweekly) until 56 days after sowing (DAS). During each interval, the number of individual weeds for each species previously identified during the pre-spray data collection was recorded from the same demarcated quadrant to ensure consistency. In addition, any new weed species that appeared within the sampled quadrant and were not observed during the pre-spray assessment were also identified and their counts recorded.

During the final round of data collection, the aerial parts of all weed species present within the demarcated areas of each plot were harvested. The fresh biomass was then oven-dried at 120°C for 72 hours to obtain the dry weight. The dry weight of each weed species was recorded and used to calculate the Weed Control Efficiency (WCE), which indicates the effectiveness of a weed control treatment in reducing weed biomass.

The WCE was calculated using the formula proposed by Mani et al. (1973) and is expressed as a percentage:

$$WCE = \frac{(DWC-D\quad)}{DWC} \times 100$$

Where, DWC=Dry weight of weeds in control treatment
DWT=Dry weight of weeds in treatment plot

Data Analysis

Data entry, processing, and the calculation of Weed Control Efficiency (WCE) were performed using Microsoft Excel. Statistical analysis was carried out using R software, focusing on two key parameters: weed count and WCE, calculated based on the dry weight of weed species. For detailed analysis, one dominant weed species was selected from each site—*Potamogeton distinctus* from both the greenhouse and the Wakhuna, Kabisa site, and *Paspalum* from the ARDC-Bajo site. The analysis was based on the average weed counts and dry weights obtained from all replications for each treatment.

Variations in weed counts and Weed Control Efficiency (WCE) across different treatments were analyzed to assess the effectiveness of each treatment. Treatments 1, 2, and 3 were compared against Treatment 4, which served as the control. The dry weight of the weed species was used to calculate WCE, allowing for a clear comparison of biomass reduction among the treatments and determining the relative efficiency of each weedicide in suppressing weed growth.

3.1.3 Results and Discussions

The study revealed that *Potamogeton distinctus* was the most dominant weed species present in farmers' fields in Kabisa. In addition, *Monochoria vaginalis*, *Paspalum distichum*, and *Cynodon dactylon* were found to be more abundant than other species such as *Alternanthera sessilis* and *Acmella uliginosa*. Weed count and Weed Control Efficiency analyses were conducted specifically on two major species: *Potamogeton distinctus* in the greenhouse and farmers' field and *Paspalum distichum* in ARDC Bajo.

Weed count and Weed Control Efficiency in the Greenhouse experiment

a) Weed count (*Potamogeton distinctus)* comparison across the treatments

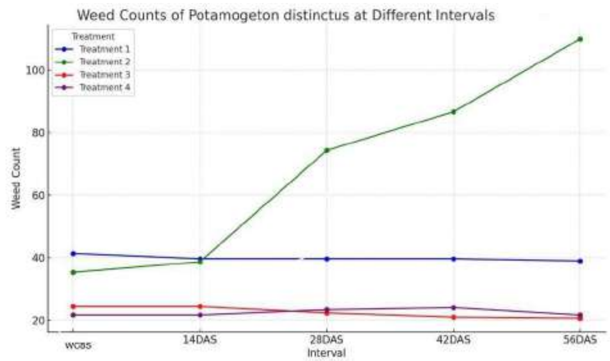

Figure 1. *Potamogeton distinctus* counts at different intervals

The study demonstrated that Treatment 2 was ineffective in controlling broadleaved weeds such as *Potamogeton distinctus*, as evidenced by a sharp increase in weed count over time shown in the graph. In contrast, Treatments 1 and 3 displayed moderate effectiveness. Treatment 4 was the most effective, successfully suppressing the initial weed population and preventing substantial regrowth throughout the study period.

b) Weed Control Efficiency

Summary of average WCE based on the average dry weight for the Greenhouse trial

Treatment	Average WCE (%)
T1	-99.63
T2	-2704.76
T3	-50.8

The average values indicate that Treatments 1 and 3 were less effective than Treatment 4, as they resulted in higher weed dry weights compared to the control. Treatment 2 had the poorest performance, showing the highest dry weight and thus the least effectiveness in controlling *Potamogeton distinctus*. The negative values suggest that these treatments resulted in greater weed biomass than the control (treatment 4). In this study, Treatment 4 served as the control and represents the standard weed management practice commonly used by farmers. It involves the application of Butachlor 5% G + Ethoxysulfuron 15% WDG at a rate of 50 grams per acre and proved to be the most effective method for controlling *Potamogeton distinctus* among all the treatments tested.

Weed Count and Weed Control Efficiency in farmers' fields in Kabisa

a) Weed count (*Potamogeton distinctus)* comparison across the treatments

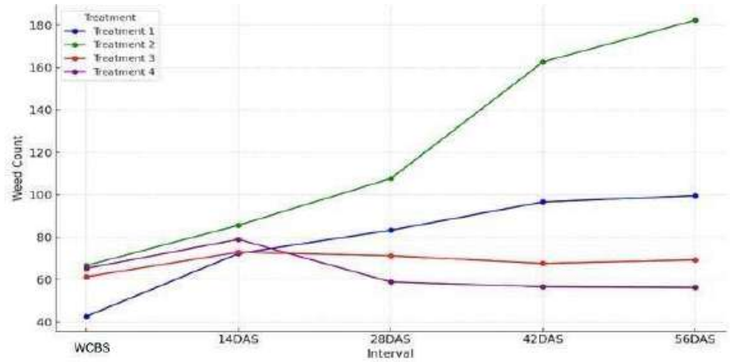

Figure 2. *Potamogeton distinctus* counts at different intervals

The study in farmers field in Kabisa also showed that Treatment 2 is the least effective against broadleaved weeds. Treatment 2 showed a continuous and sharp increase in weed population, indicating poor weed control. Treatment 4 initially had a similar weed count to the others but demonstrated a decline after 14 DAS and maintained the lowest weed count throughout the observation period, indicating it was the most effective in suppressing weed growth.

b) Weed Control Efficiency

Table 1. Summary of average WCE based on the average dry weight for the farmers field trial

Treatment	Average dry weight (g)	Average WCE (%)
T1	11.9	-202.29
T2	19.83	-404.58
T3	5.05	-28.49
T4	3.93	NA (0)

Treatments 1, 2, and 3 were less effective in managing *Potamogeton distinctus* compared to the control (Treatment 4). Among them, Treatment 2 showed the poorest performance, with the highest average dry weight (19.83 grams) and the most negative WCE (-404.58%), indicating minimal weed control. Treatment 3, with a lower average dry weight of 5.05 grams and a less negative WCE (-28.49%), demonstrated relatively better weed suppression than Treatments 1 and 2.

Weed count and Weed Control Efficiency in ARDC-Bajo experimental trial

a) Weed count (*Paspalum distichum*) comparison across the treatments

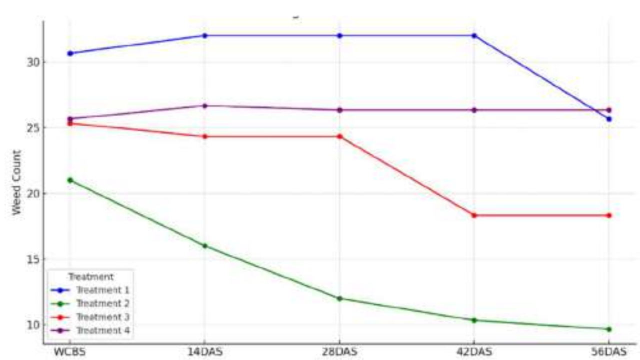

Figure 3. *Paspalum distichum* counts at different intervals

The study in ARDC Bajo showed that Treatment 2 is the most effective weed control specifically for *Paspalum distichum*, with a consistent decline in weed count. This indicates strong and sustained suppression of weeds over time. Treatment 3 also showed moderate effectiveness, while Treatment 1 maintained a consistently high weed count. Treatment 4 (control) showed minimal change throughout the trial, indicating limited suppression. Therefore, Treatment 2 provided the best performance in reducing weed infestation.

b) Weed Control Efficiency (WCE)

Table 2. Summary of average WCE based on the average dry weight for the ARDC-Bajo trial

Treatment	Average dry weight (g)	Average WCE (%)
T1	30.4	14.42
T2	12.9	56.13
T3	9.3	67.04
T4	31.6	NA (0)

Treatment 3 recorded the highest WCE, indicating it was the most effective in weed control. In contrast, Treatment 2 showed a moderate WCE, while Treatment 1 had the lowest WCE, making it the least effective among the treatments.

3.1.4 Conclusion

The study showed differences in the effectiveness of the four treatments over time compared to different weed types. Treatment 4 was effective in suppressing broadleaved weeds, maintaining relatively stable weed counts throughout the trial period. On the other hand, Treatment 2 showed a consistent reduction in overall weed count, particularly against grassy weeds, especially *Paspalum distichum*.

In the upcoming trial scheduled for the next season, one of the treatments will involve applying Butachlor 5% G as a standalone herbicide, without combining it with other weedicides. This approach is designed to allow a focused and clear assessment of Butachlor's individual effectiveness in controlling weeds. By isolating Butachlor from other weedicides, the study aims to determine its true potential for weed suppression on its own. Including this treatment will provide valuable insights into whether Butachlor alone can effectively manage weed populations, helping to refine and optimize weed management strategies for farmers. This information could also guide decisions on whether combining Butachlor with other herbicides is necessary or if it can be relied upon as a single-agent solution.

3.1.5 Reference

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3.2 Evaluating the Efficacy of a Synthetic Weedicide on Diverse Weed Populations

(broadleaved and grassy) in Maize

3.2.1 Introduction

Maize (*Zea mays* L.) is one of the most important cereals widely grown in the world and used as a primary staple food in many developing countries. It was among the five most produced cereal species in 2022 worldwide including wheat, rice, barley and sorghum (Food and Agriculture Organization [FAO], 2023). Similarly, Maize is Bhutan's second most important cereal crop after rice in terms of both area and production. It is highly adaptable to different ecological conditions and is cultivated across most parts of the country. The crop is particularly popular in the mid and low-altitude regions of the Eastern and Southern areas.

Maize production in Bhutan has been steadily declining each year, posing a potential risk to the country's food security. It reported a production of 25,118 MT from 17,140.71 harvested area (acre) in 2023. This was 863 MT less compared to the 2022 production, equivalent to a 3 percent decline, and 21,117 MT less than the 46,235 MT recorded in 2019. The harvest area for maize dropped by 1,288 acres compared to the 2022 harvest area (National Statics Bureau [NSB], 2024). This decline can be attributed to factors such as the reduction in cultivated land and the number of growers, crop losses caused by pests (including insects, diseases, weeds, and wild animals), declining soil fertility, and other challenges associated with domestic production (NSB, 2024).

Among these challenges, weed infestation stands out as a major constraint, primarily due to the high labor costs associated with weeding. Currently, manual weeding remains the only available method for weed control in maize cultivation, making it both labor-intensive and expensive. Weeds, both annual and perennial, significantly impact maize yield by competing for essential nutrients, water, and sunlight (Salceanu et al., 2024). Studies estimate that weeds contribute to approximately 37% of global maize production losses (Oerke & Dehne, 2004; Sharma & Rayamajhi, 2022). Furthermore, in heavily weed-infested fields, maize plants struggle to develop strong root systems, further exacerbating yield losses. Therefore, the primary challenge in improving maize productivity lies in implementing effective weed management and control strategies (Güncan & Karaca, 2014).

During a weed inventory survey conducted across various regions of Bhutan, particularly in the east, the Weed Science team found that many maize growers faced significant challenges in managing diverse weed species in their fields. Farmers expressed concerns about the limited availability of labor and the rising costs of hiring workers, making weed control increasingly difficult. Currently, maize farmers in Bhutan rely primarily on manual weeding, as no selective herbicide is specifically available for weed control in maize fields. The only exception is Metribuzin, a selective herbicide effective against dryland weeds, though it is predominantly used for weed management in potato fields rather than maize cultivation. This lack of effective herbicide options further complicates weed management for maize growers, highlighting the urgent need for alternative weed control solutions.

Considering the importance of maize production in Bhutan and the challenges posed by labor shortages and weed infestations, it is vital to explore and assess new-generation herbicides for effective weed control. As part of this effort, the program has identified a potential candidate, and it is essential to evaluate its efficacy under local environmental conditions to ensure its effectiveness before it is made available for farmers' adoption. The objectives of the study are to evaluate the efficacy of Tembotrione 34.5% SC as a post-emergence weedicide against complex weed flora in maize and compare the weed control efficiency of manual weeding with the new weedicide under study.

3.2.2 Materials and methods

Background on the weedicide:

A selective, post-emergence herbicide developed for the control of a broad spectrum of broadleaf and grassy weeds in corn.

Product name: Laudis

Active ingredient: Tembotrione

Formulation: liquid end-use product (34.5%), Suspension concentrate

Crops for use: Maize/Corn

Mode of action:

- ✓ Systemic
- ✓ It is absorbed through the leaves of the weeds and then translocated throughout the plant.
- ✓ Works by inhibiting the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) in plants. This inhibition blocks the biosynthesis of carotenoids, leading to the depletion of these pigments. Carotenoids are critical for capturing light energy and protecting chlorophyll from photooxidation. When this process is disrupted, the affected weeds experience chlorosis (yellowing of leaves) followed by necrosis (death of plant tissue), ultimately resulting in their elimination from the crop field.

Rate: 115 ml of a.i per acre

Dilution: 115 ml of a.i in 200 L of water

Pre-harvest interval: 55 days (Waiting period b/w the last application and harvest)

Time of application: Applications should take place between crop emergence to the V8 (more than 8 visible leaves) developmental stage of corn. Best control of broadleaf weeds is achieved when weeds are less than 6” in height and actively growing. The best control of grass weeds is achieved prior to tillering and when grasses are actively growing.
Weed growth ceases within hours after its application. Symptoms on susceptible weed species progress from yellowing and bleaching to necrosis resulting in eventual plant death generally within 7 to 14 days after application.

Experimental Sites and Period of Execution

The trial was established at four locations: the on-station greenhouse of NPPC, maize fields at ARDSC-Tsirang, ADRC-Wengkhar, and a farmer’s field in ChhalingGewong under Monggar. The experiment was initiated in March and will go on until August 2025. The activity will be

completed in the next fiscal year.
Trial set up at the on-station site of ARDC-Wengkhar: **March 13**
Trial set up in a farmer's field in Chhalinggewog: **March 14**
Trial set up in the on-station greenhouse of NPPC: **March 28**
Trial set up at the on-station site of ARDSC-Tsirang: **March 31**

Trial Design Details

- **Experimental Design:** Randomized Complete Block Design (RCBD)
- **Treatments:** Four treatments

  T1: Manual management

  T2: Lower dose of weedicide

  T3: Higher dose of weedicide

  T4: Control (no treatment)
- **Replications:** Three replications
- **Total Experimental Plots:** 12 plots (4 treatments × 3 replications)
- **Plot Size:** 3 m × 2 m (6 m^2 per plot) in field and 2.10 m × 0.95 m (1.995 m^2 per plot) in greenhouse

Field preparation and seed sowing (ADRC-Wengkhar and ARDSC Tsirang)

The selected fields for the trial were initially ploughed using a power tiller one day prior to establishment, with assistance provided by the respective Centres. On the day of establishment, soil loosening was carried out manually using spades to facilitate bed preparation and seed sowing. Following soil loosening, the entire field area was carefully measured and divided into 12 uniformly sized plots. Each plot was prepared as a bed measuring 3 m × 2 m, with a 60 cm inter-bed spacing maintained to ensure ease of movement during field operations. After bed preparation, all plots were uniformly fertilized using farmyard manure (FYM) sourced from the Centres. Each plot received 20 kg of FYM, which was evenly distributed and thoroughly incorporated into the soil prior to seed sowing.

The maize variety Yangtsepa was used for the trial, sourced from the Field Crop Sector of the Centre. Each plot was designed with 20 planting points, arranged with a 40 cm spacing between points along the 2 m width, and 60 cm between rows along the 3 m length of the bed. Planting holes were created using wooden dribblers at the designated positions, and three seeds were sown per hole. Following the completion of seed sowing across all plots, the entire field was adequately irrigated to ensure proper seed germination. As the final step in plot establishment, tag poles were installed in each plot. These tags clearly indicated the specific treatment and replication details corresponding to each plot.

Figure 4. Field preparation at ARDC-Wengkhar

Figure 5. Field preparation at ARDSC-Tsirang

Figure 6. Application of FYM

Figure 7. Sowing seed (left) and installation of tag poles (right)

Field preparation at a farmer's field in Khampasadong village in Chulabi Chiwog under Chhalinggewog, Monggar Dzongkhag

The trial site was selected in collaboration with the Plant Protection focal officer from ARDC-Wengkhar, the Agriculture Extension Officer of the gewog, and the field owner. A specific area within the field was identified and measured to ensure it could uniformly accommodate all 12 experimental plots. Soil preparation commenced with manual ploughing and loosening using spades. During this process, large stones and plant debris were removed to create a clean and workable seedbed.

Similar to the setup at the on-station field, 12 beds were prepared, each measuring 3 m × 2 m, with a 60 cm gap between beds to facilitate movement. The beds were organized into two rows of six, and for experimental design purposes, four beds (two from each row in sequence) were grouped to form one replication. The placement of replications and treatments was randomized to maintain experimental integrity. Unlike the on-station setup, no farmyard manure (FYM) was applied at this site, as FYM was not available on the farmer's property. The maize variety Yangtsepa, obtained from the Field Crop sector of the Centre, was used for the trial. Seed sowing was conducted following the same procedure as that employed at the research centre.

Figure 8. Field measurement (left) and preparation/ploughing (right)

Figure 9. Bed preparation

Plot preparation (on-station greenhouse of NPPC)

The greenhouse experiment was conducted using 12 chambers, each measuring 2.10 m × 0.95 m (1.995 m²), which served as the experimental plots. To facilitate uniform weed germination, the soil in each chamber was loosened and tilled prior to treatment application. No seed sowing was carried out in the greenhouse. Instead, the experiment was designed to observe the effect of the weedicide on the naturally emerging weed population within each chamber.

Application of treatments (weedicides)

Following the pre-spray data collection, treatments were prepared and applied as per the protocol.

The plots designated for Treatment 1 in all three replications were weeded manually by hand. Treatments 2 and 3 were treated with the herbicide Laudis. For T2, a solution of 0.3 ml weedicide mixed with 300 ml of water and 0.74 ml of surfactant was prepared for each plot. For T3, a solution of 0.6 ml weedicide with 300 ml of water and 4.44 ml of surfactant was used per plot.

For efficiency, the entire solution for T2 was prepared at once and then divided equally among the three replications. The same approach was followed for T3. A 100 ml measuring cylinder was used to measure the weedicide and surfactant, while a 1000 ml measuring beaker and jug were used for water and solution measurements.

Initially, a 100 ml hand sprayer was used for application, but due to time constraints, a 500 ml mineral water bottle modified with small needle-punched holes in the cap was used to spray the solution evenly. The solution was uniformly applied to all designated plots. Treatment 4 plots were left untreated to serve as the control.


Figure 10. Solution preparation for the treatment


Figure 11. Herbicide treatment at: ARDC Wengkhar trial (L); Farmers' field (R)

Data Collection

Data collection was carried out both before and after the application of the weedicide. In the field trials, the square method was used, involving a 1 x 1 meter quadrat placed randomly within a designated area to ensure that all subsequent data collections were made from the same location. In the greenhouse, data were collected from the entire chamber or plot. Throughout the experiment, all follow-up data collection will be consistently conducted within these marked areas. To date, only one round of post-treatment data has been collected, with further data collection planned for the next financial year. All gathered data have been systematically recorded and organized using Microsoft Excel for future analysis.

Pre-spray data collection

Pre-spray data collection involved identifying the various weed species present within the sampled and marked quadrants. The number of individual weeds for each species was counted and documented in every quadrant. This information was gathered prior to the weedicide application to establish a baseline for comparison with post-treatment observations.

Pre-spray data collection at ARDC-Wengkhar and farmers' field in ChhalingGewog Pre-spray weed data was collected with the assistance of a few ARDC officials, including the interim Plant Protection (PP) Officer. Prior to data collection, 1-square-meter quadrants were randomly established within each treatment plot using jute rope and four small wooden stakes positioned at the corners.

Weed data from each marked quadrant was collected and recorded in an online Excel sheet. As per protocol, all individual weed species present within the demarcated area were identified, and the number of individuals of each species was counted before weedicide application. The same data collection format was used for the farmer's field in ChhalingGewog.

During the pre-spray data collection, a total of 19 weed species were recorded across both sites, with most species being common to both locations. At the ARDC-Wengkhar trial site, the dominant weed observed in all treatments was *Galinsoga quadriradiata*, while in the farmers' field, *Bidens pilosa* was most prevalent, followed by *Galinsoga quadriradiata*.

Table 3. List of weed species recorded in both the trial sites

SI.No.	Name of Weeds	Presence of weed in the Trial site (ARDC & Farmer field)
1	*Galinsoga quadriradiata*	Both
2	*Galinsoga parviflora*	Both
3	*Bidens Pilosa*	Both
4	*Persicaria runcinata*	Both
5	*Persicaria nepalensis*	Both
6	*Chenopodium album*	Both
7	*Cynodon dactylon*	Both
8	*Digitaria*	Both
9	*Oxalis latifolia*	ARDC
10	*Amaranthus viridis*	Both
11	*Pennisetum clandestinum*	ARDC

12	*Cyperus roduntus*	Both
13	*Pteridum aqualinium*	Both
14	*Capsella bursa pastoris*	ARDC
15	*Commelina benghalensis*	Both
16	*Rumex nepalensis*	ARDC
17	*Trifolium repens*	ARDC
18	*Sonchus arvensis*	ARDC
19	*Ageratum conyzoides*	Farmers field

 

Figure 12. Demarking the sampling area (left) and the Quadrant for data collection

 

Figure 13. Pre-spray data collection

Post-spray data collection

Post-spray data collection began 14 days after the herbicide application and will continue at 14-day (biweekly) intervals until 56 days after sowing (DAS). At each interval, the number of individual weeds for each species previously recorded during the pre-spray assessment will be counted within the same marked quadrant to maintain consistency. Additionally, any new weed species that emerge within the sampled quadrant and were not observed during the pre-spray assessment will be identified, and their numbers will be documented.

Overall, during the initial post-spray data collection, a reduction in weed count was observed across all previously recorded weed species in all treatment plots, except for the control.

Data Analysis

Data was managed in Microsoft Excel. The analysis focused on the two most dominant weed species at each site, *Galinsoga quadriradiata* and *Galinsoga Parviflora.* Selected for their high frequency and abundance as indicators of treatment efficacy. At the ARDC-Wengkhar site, *Chenopodium album* was additionally analysed for the same purpose. Mean weed count per treatment, averaged across all replications, served as the basis for the efficacy assessment.

3.2.3 Results and Discussions

The study showed that *Galinsoga quadriradiata*, *Galinsoga parviflora* and *Bidens pilosa* were the most dominant weed species present in all trial sites. Among them, *Galinsoga quadriradiata* and *Galinsoga parviflora* showed a strong response to the weedicide treatment. The weedicide application effectively suppressed their growth, reducing their population density compared to unmanaged plots. Beyond these species, the study also recorded the presence of *Persicaria runcinata, Persicaria nepalensis, Chenopodium album*, *Oxalis latifolia,* and *Digitaria spp***.** in significant numbers. The weedicide exhibited effectiveness against these species, resulting in suppression across all the treated plots except *Bidens pilosa*, maintaining its dominance across all the trial fields and the results indicated that weedicide was ineffective in controlling this species.

Efficacy based on weed count in ARDSC Tsirang

a) *Galinsoga quadriradiata*

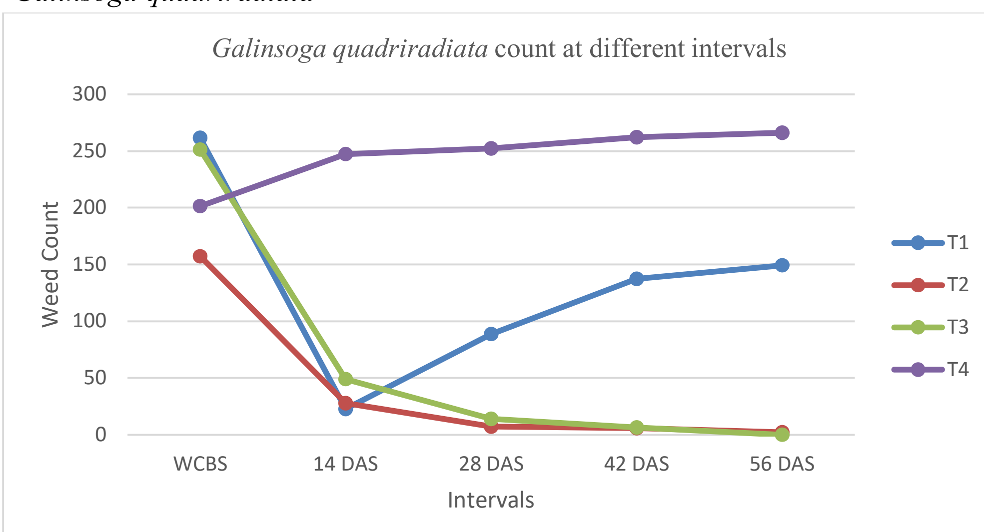

Figure 14. *Galinsoga quadriradiata* count at different intervals

The study exhibited the counts of *Galinsoga quadriradiata* at different intervals, *i.e,* (WCBS, 14 DAS, 28 DAS, 42 DAS, and 56 DAS) under four various treatments (T1, T2, T3, and T4)

- **T4 (Control**): This treatment recorded the highest weed counts. Starting at 200 numbers during WCBS (weeds count before spray), the population steadily increased. This trend indicates that in the absence of weedicide, *Galinsoga quadriradiata* proliferated and dominated the field.

- **Treatment 1 (Hand weeding):** Initially, Treatment 1 showed a high weed population density, similar to that of the control, but after 14 DAS, there was a substantial reduction. However, from 28 DAS onwards, the weed population gradually increased, indicating that treatment T1 having a temporary suppression against the weed population.
- **Treatment 2 (Lower dosage)**: This treatment exhibited effectiveness in controlling *Galinsoga quadriradiata* growth. The weed count was reduced significantly by 28 DAS, results indicating a strong and lasting efficacy of treatment T2 in controlling this particular species.
- **Treatment 3 (Higher dosage)**: Comparable to T2, the treatment T3 also achieved a significant decline in weed population density. The weed suppression remained consistent up to 56 DAS, indicating that T3 was also highly effective.

Treatments T2 and T3 proved highly effective, completely eliminating *Galinsoga quadriradiata* by 28–42 days after sowing (DAS). Treatment T1 showed partial efficacy, providing only temporary weed suppression followed by regrowth. In contrast, the untreated control (T4) exhibited unchecked weed growth, highlighting the rapid proliferation of *G. quadriradiata* without management.

b) Galinsoga parviflora

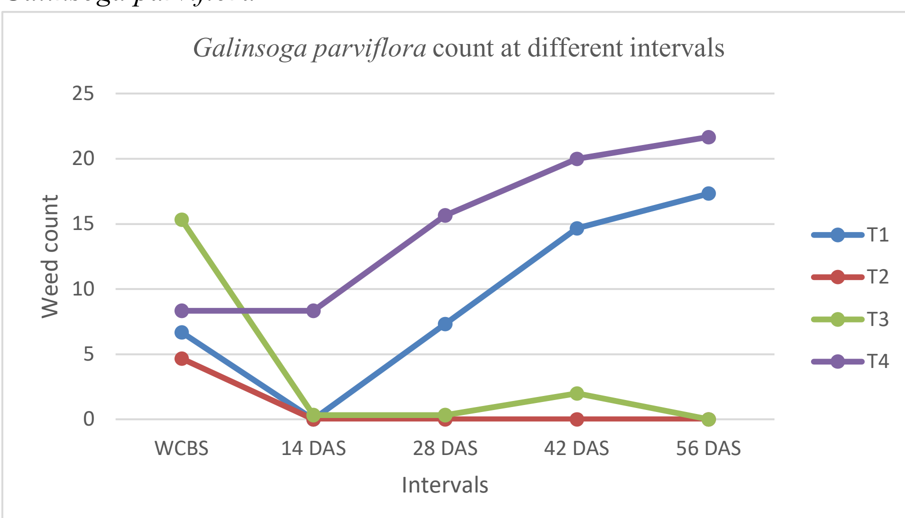

Figure 15. *Galinsoga parviflora* count at different intervals

The data demonstrate the efficacy of four treatments (T1, T2, T3, and T4) in reducing the population density of *Galinsoga parviflora* at multiple intervals (WCBS, 14 DAS, 28 DAS, 42 DAS, and 56 DAS) over a 56-day period. In the untreated control (T4), *G. parviflora* density remained consistently high. Treatment T1 showed high weed density comparable to the control, with only moderate suppression at 14 DAS that proved transient, as counts increased again after 28 DAS. In contrast, T2 and T3 provided highly effective in controlling *Galinsoga parviflora*, exhibiting sustained suppression over the duration of the study.

Efficacy based on weed count in ARDC Wengkhar

a) *Galinsoga quadriradiata*

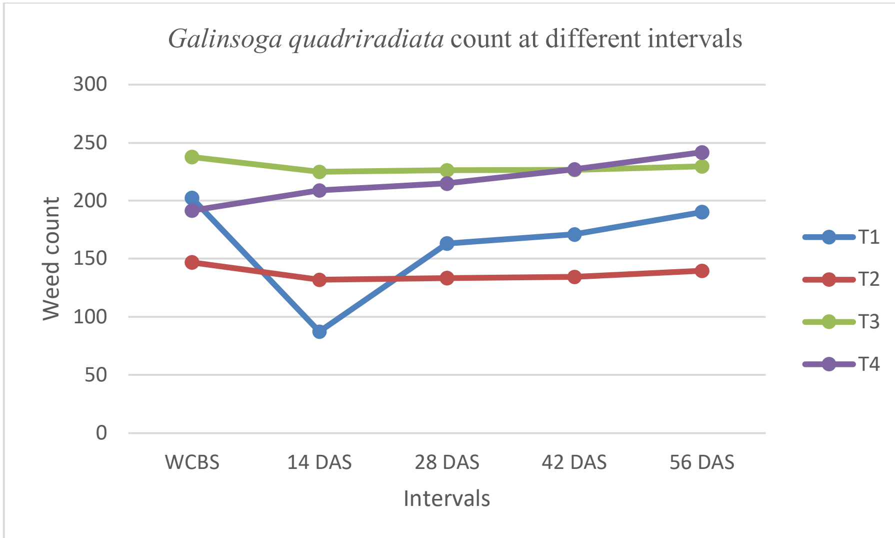

Figure 16. *Galinsoga quadriradiata* count at different intervals

The figure represents the weed count of *Galinsoga quadriradiata* recorded at different intervals under four treatments (T1, T2, T3 and T4). T1 indicated strong initial effectiveness but gradually the weeds recurred. T2 and T3 maintained relatively stable and sustained suppression of *Galinsoga quadriradiata*. However, T4 (control) reflected the rapid growth of the weed in the absence of treatment.

b) *Chenopodium album*

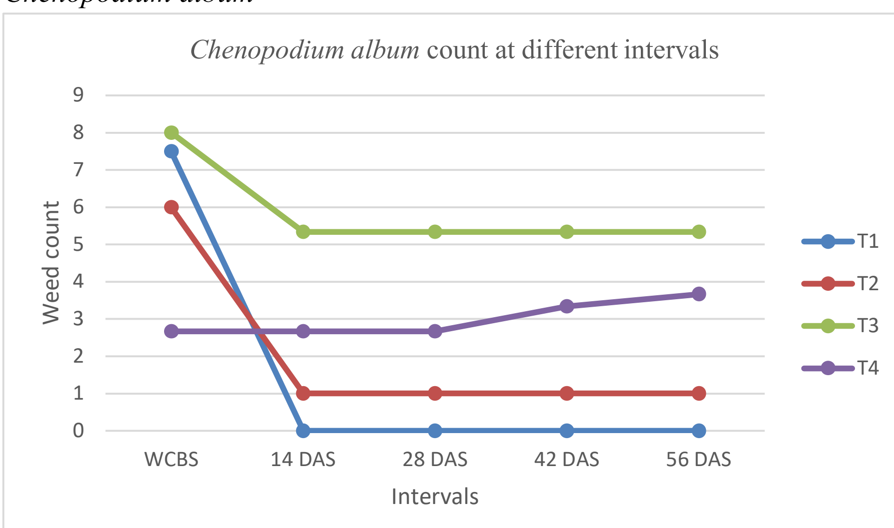

Figure 17. *Chenopodium album* count at different intervals

This figure illustrates the weed counts of *Chenopodium album* under four treatments (T1, T2, T3, and T4) at various intervals. T1 (hand weeding) was highly effective, reducing counts to zero by 14 DAS and maintaining complete control through 56 DAS. T2 also achieved strong suppression, sharply lowering the population and sustaining low counts throughout. In contrast, T3 was less effective than T2, with relatively higher and stable weed densities across intervals. The untreated control (T4) showed natural weed proliferation, with counts increasing over time.

Efficacy based on weed count in the Farmers' field, Chhalinggewog

a) *Galinsoga quadriradiata*

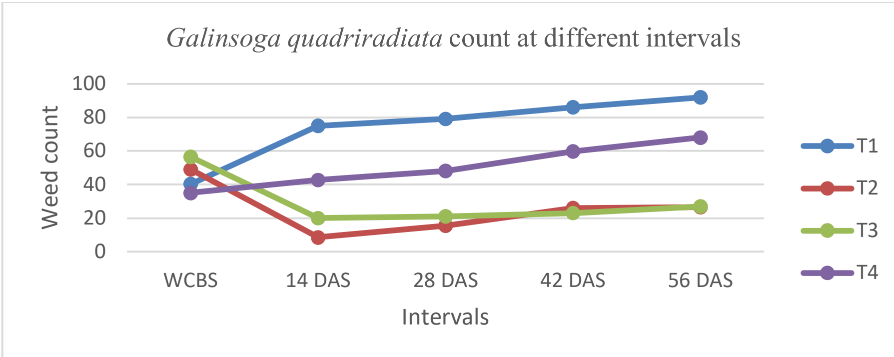

Figure 18. *Galinsoga quadriradiata* count at different intervals

The figure represents the weed count of *Galinsoga quadriradiata* recorded at different intervals under four treatments (T1, T2, T3 and T4). T2 and T3 were the most effective treatments, maintaining low weed densities throughout the study period. T1 (hand weeding) reduced weeds temporarily but allowed rapid regrowth, resulting in higher counts at later intervals. T4 (control) shows steady increases in weed population over time.

b) *Galinsoga parviflora*

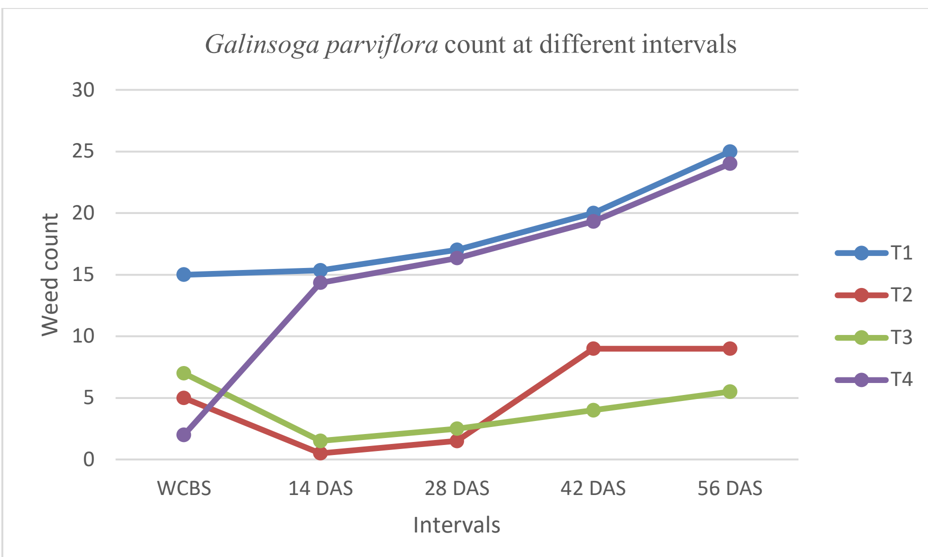

Figure 19. *Galinsoga parviflora* count at different intervals

The figure above exhibits the weed count of *Galinsoga parviflora* recorded at different intervals under four treatments. Manual weeding (T1) was initially effective in removing the weed, but regrowth was observed after some time. The application of a lower dose of synthetic weedicide (T2) suppressed *Galinsoga parviflora* more effectively than manual weeding, although some regrowth still occurred later. In contrast, the higher dose of weedicide (T3) provided the most effective and prolonged control, significantly reducing the presence of *Galinsoga parviflora* and minimal regrowth was observed throughout the study period. Meanwhile, in the control plot (T4), where no weeding or treatments were applied, the entire plot was observed to be overgrown with *Galinsoga parviflora* throughout the study period.

3.2.4 Conclusion

The study showed that the application of synthetic weedicides in T2 and T3 was highly effective in suppressing weed growth, such as *Galinsoga parviflora*, *Galinsoga quadriradiata, Persicaria runcinata, Persicaria nepalensis, Chenopodium album*, *Oxalis latifolia,* and *Digitaria spp*. Manual weeding in T1 provided partial control, though weed regrowth was observed after some time. In contrast, T4, maintained as the untreated control, recorded heavy infestation with unchecked weed growth. The results indicate that the weedicide treatment in T2 and T3 were better to manual weeding and the untreated control.

3.2.5 Reference

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3.3 Farmers' Awareness and Perceptions of Glyphosate Use in Agricultural Production and Its Impacts

3.3.1 Introduction

In the last 2-3 decades, the use of herbicides, particularly glyphosate, has gained prominence to enhance agricultural productivity. Glyphosate is a widely used herbicide known for its effectiveness in weed control, cost-efficiency, and ease of application. Glyphosate, a broad-spectrum herbicide discovered in the 1970s and introduced in the market under the trade name

Roundup® is known for controlling both broadleaf and grass weeds. It has become one of the most widely used herbicides in the world due to the wide scale adoption of transgenic, glyphosate-resistant (GR) crops after their introduction in 1996. In Bhutan, it was introduced around 1990s for general weed control. Since mid-2000s it has been widely used in potato fields prior to harvest to readily remove weeds and haulms to facilitate convenient tuber gathering. In recent years, its use has also extended to clearing grass around electric fences, controlling weeds on the terrace bunds, and cleaning up bushes along the roadsides.

However, concerns have emerged regarding the potential ecological and health impacts of glyphosate, and it is a restricted product in the EU countries pertaining to its adverse effects on human health, soil, and the environment. As Bhutan strives to maintain its commitment to sustainable and organic farming practices, it is crucial to evaluate the consequences of glyphosate use on farming communities. Moreover, the actual benefits and negative impacts of glyphosate have not been thoroughly assessed in Bhutan despite its widespread use. Thus, the study aims to conduct a comprehensive farmer interview survey to understand farmers' awareness and perceptions of glyphosate use in agriculture. Furthermore, to study the impacts of glyphosate and the purpose of its usage by gathering firsthand insights from farmers that use Glyphosate.

The study through questionnaire-based survey, interviewing farmers was conducted from August to December 2024, in Six Gewogs (Gangteng, Phobji, Bjenag, Ruebisa, Chhapchha, and Kar-tshog) under Wangdue Phodrang , Chhukha and Haa Dzongkhags. The objective of the study is to obtain a holistic understanding of glyphosate use in the surveyed area, including farmers' knowledge, practices, concerns, and the observed impact on crops, soil, health, and environment.

3.3.2 Materials and Methods

Study Site

The questionnaire-based survey was conducted in the key potato-growing regions, specifically Phobji, Gangteng, Bjenag and Ruebisa Gewogs (Wangdue dzongkhag); Chhapchha Gewog (Chhukha dzongkhag) and Kar-tshog Gewog (Haa dzongkhag).

Gangteng and Phobji Gewogs, with all 10 Chiwogs included, are among the top potato-producing gewogs with high glyphosate use as per the NPPC pesticide distribution data. In Chhapchha and Kar-tshog Gewogs, surveys were conducted in multiple villages known for cultivating diverse crops like potatoes and carrots, with potatoes being the major crop.

The survey was conducted in the key potato-growing areas of Bjenag and Ruebisa Gewogs, specifically within the Khotokha valley, which includes several villages under both gewogs. The farmers in Khotokha valley primarily focus on potato production.

Target population**:** The survey interview included all households within the selected survey site for sample size calculation.

Sample size and sampling method

The sample size was calculated using Yamane's formula: $\boldsymbol{(n = N/(1+N(e)^2)}$**,** where *n* represents the sample size, N is the population size, and e is the margin of error. A simple random sampling method was used to ensure an unbiased representation of the population.

The actual number of farmers surveyed was lower than the calculated sample size due to several factors. Some households were unoccupied at the time of the visit. Others were unavailable because family members were engaged in work outside the village. Additionally, certain respondents were elderly individuals who faced difficulties in comprehending or responding to the survey questions, limiting their participation.

Survey Details and Key Highlights

Table 4. Survey details

Place of survey (Gewog & Dzongkhag)	Date	No. of female respondents	No. of male respondents	Total respondents
Gangteng, Wangdue Phodrang	13th -15th Aug 2024	111	76	187
Phobji, Wangdue Phodrang	16th -18th Aug 2024	68	78	146
Kar-tshog, Haa	28th Aug-2nd Oct 2024	26	94	120
Chhapchha, Chhukha	2nd -5th Oct 2024	22	32	54
Bjenag, Wangdue Phodrang	8th -12th Dec 2024	16	13	29
Ruebisa, Wangdue Phodrang	13th - 15th Dec 2024	20	18	38
Total farmer participants				**574**

Data collection

The survey was conducted using a structured questionnaire to collect data on various key areas. These include socio-demographic characteristics, farmers' knowledge and history of glyphosate use, patterns of glyphosate application, and its perceived effects on crops, soil, the environment, and human health. Additionally, the questionnaire covered the development of glyphosate-resistant weeds. The data collection involved face-to-face interviews with farmers and was conducted as a one-time event.

The questionnaire was integrated into the open-source digital application **Epicollect5**, enabling interviewers to use their mobile phones for data collection. This approach has simplified the process and reduced the need for paper resources. The survey gathered both qualitative and quantitative data to evaluate farmers' awareness and perceptions of Glyphosate use and its impacts.

Figure 20. Interviewers gathering information from farmers at Gangteng and Phobji

Figure 21. Interviewers interacting with farmers to gather information at Chhapchha and Bjenag

Figure 22. Face-to-Face interview with the farmers at Khotokha

3.3.3 Results and Discussions

Findings from survey conducted at Gangteng and Phobji

Demographics

The survey team conducted interviews with 333 farmers, including 146 from Gangteng Gewog and 187 from Phobji Gewog. Out of these 333 respondents, 179 were male and 154 were female. Most respondents (n=201) were uneducated, while 19 had attended non-formal education. A total of 110 respondents had formal education ranging from pre-primary to class 12, and only 3 had pursued higher education up to a degree level. Out of the 333 farmers surveyed, the majority (n=128) have a household size of three. Among the farmers surveyed, 226 have over 20 years of farming experience, 98 have between 6 and 20 years, and only 9 have less than 5 years of experience.

Crops cultivated

Potato is the primary crop cultivated in both Gewogs, with turnip, radish, and buckwheat being commonly grown as subsequent crops. These secondary crops are typically used as cattle feed during the winter season.

Farmers' knowledge on Glyphosate and the History of Glyphosate

When questioned about the purpose of Glyphosate, nearly all the farmers (n=331) indicated they were aware of its use, with only two respondents unaware of its purpose. All respondents who knew its purpose stated that it is used for weed control. Out of the 333 respondents, 318 reported obtaining Glyphosate from the Gewog Centre through their Agriculture Extension Officer. Eleven others sourced it from alternative channels, such as fertilizer suppliers (ASSR[2]) or directly from India. Meanwhile, two respondents obtained it from either the Gewog Centre or NPPC, another one from both the Gewog Centre and a local seller, and one solely from a local seller. Sixty-five farmers stated that they are aware of when Glyphosate was first introduced in their region.

Glyphosate usage

Of the 333 respondents, 332 reported using Glyphosate, while one stated they do not use it due to having a small cultivation area where weeds can be managed manually without relying on Glyphosate. Those who use it highlighted its effectiveness in controlling weeds in potato fields, reducing the need for manual labor and saving time. Additionally, it aids in harvesting by desiccating weeds and haulms, making tuber collection more efficient and convenient.

This year, 103 farmers used glyphosate: 64 relied on previous stock, 5 purchased it from local sellers, 2 bought it from the gewog office while also using previous stock, and 32 obtained it directly from the gewog office. Usage duration varied among 332 respondents, with 256 having applied it for over 10 years, 50 for 7–10 years, 22 for 4–6 years, and 4 for 1–3 years.

Glyphosate application

A total of 232 respondents applied Glyphosate once, typically just before the potato harvest, while 98 applied it twice - once pre-emergence and again before the harvest. On average, farmers used 200-300 ml of Glyphosate per 20-25 litres of water (equivalent to 10 ml per litre). The application was typically foliar, using Power Knapsack sprayers. Most farmers reported wearing some protective gear, including gumboots, long pants, long-sleeved shirts, and some used face

[2] Agriculture Sales and Service Representative

masks and plastic coverings to protect themselves from Glyphosate exposure.

Crop harvest interval

Farmers adhered to a crop harvest interval of 2-3 weeks (minimum 15 days) to prevent crop damage and ensure the healthy growth of subsequent crops. Some respondents noted that failing to maintain this interval led to potato rotting and poor growth of the following crops, as they usually broadcast the subsequent crop immediately after the potato harvest to prevent weed growth.

Soil and Health Observations

Majority of the respondents (n=229) observed that their soil had become softer over time, which facilitated easier potato harvesting and ploughing. While most farmers were aware of the health hazards associated with Glyphosate, due to repeated advisories from agriculture extension, but they lacked knowledge about the environmental risks.

Weed challenges

The most common and problematic weeds identified by the farmers include *Spergula arvensis, Persicaria runcinata, Digitaria sanguinalis, Rumex acetosella, Poa annua, Galium aparine, Galinsoga quadriradiata,* and an unknown grass resembling fodder grass. Respondents reported that Glyphosate could effectively kill these weeds within 15-20 days of application. However, the selective herbicide metribuzin was found to be less effective, particularly against *Rumex acetosella, Galinsoga quadriradiata, Poa annua, and Digitaria sanguinalis,* even when applied at higher dosages (15-20 grams per liter of water).

Other Observation

Farmers in both Phobji and Gangteng Gewogs are experiencing significant issues with white grub infestations in their potato crops and fodder grass. They reported that the severity of infestations has increased this year compared to previous years, although the problem has been present for the past 2-3 years. Additionally, farmers in Koombu, Gangteng gewog are facing escalating issues with potato rot, which has worsened annually. They have already informed the Gewog Agriculture Office and Gewog Administration about this concern. The survey team assured the farmers that the issue would be reported to the Gewog authorities and then the Gewog Administration will submit an official report to the National Plant Protection Centre (NPPC) for further laboratory assessment. The team also advised farmers to avoid leaving infected tubers in the field and to burn any plant debris during the winter to prevent the spread of these issues.

Figure 23. Potato field heavily infested with *Spergula arvensis* (L) and Potato infested with *Rumex acetosella* (R)


Figure 24. Potato infested with *Digitaria sanguinalis* (L) and *Persicaria runcinate* (R)


Figure 25. Hand weeding by farmers

Findings from survey conducted at Chhapchha and Bji

Demographics

The survey team conducted interviews with 174 farmers, including 54 from Chhapchha Gewog and 120 from Kar-tshog Gewog. Out of these 174 respondents, 48 were male and 126 were female. Most respondents (n=102) were uneducated, while 3 had attended non-formal education. A total of 68 respondents had formal education ranging from pre-primary to class 12, and only one had pursued higher education up to a degree level. The average household size dedicated to farm work was three people per household.

Crops cultivated

Potato and Carrot are the primary crops cultivated in both Gewogs, with chili, cabbage, buckwheat, and wheat being commonly grown as subsequent crops.

Glyphosate usage

Of the 174 respondents, 42 reported using glyphosate, while 132 had never used it. Among users, 27 obtained it from the gewog agriculture extension office, 6 purchased it from local sellers, and 1 bought it from across the border. Usage duration varied, with 30 having applied it for 1–3 years, 4 respondents used it for 4–6 years, and 2 respondents used it for 7–10 years.

Glyphosate application

A total of 33 respondents applied Glyphosate once, while only 3 applied it twice during the cropping season. The majority of farmers in both Gewogs use Glyphosate in field peripheries to clear bushes and prevent their encroachment into cropping areas. The application was typically foliar, using Power Knapsack sprayers, and a few respondents used buckets.

Most farmers reported wearing some protective gear, including gumboots, long pants, long-sleeved shirts, face masks, and plastic coverings to protect themselves from Glyphosate exposure.

Soil and Health Observations

Respondents who used Glyphosate observed some soil softening, which they suspect is due to poor rooting after killing shrubs and weeds with the herbicide.

Weed challenges

The most common and problematic weeds identified by the farmers include *Rumex nepalensis, Persicaria runcinata, Galium aparine, Galinsoga quadriradiata*, and bamboo-like shrubs growing adjacent to the fields. Respondents reported that Glyphosate could effectively kill these weeds within 15-20 days of application. Farmers from both Gewogs use Metribuzin to control weeds in potato fields, applying it once, 2-3 months after potato sowing.

Other Observation

Farmers in Chhapchha Gewog have been using illegal herbicides like glyphosate and paraquat, obtained from local sellers and cross-border imports. Prior to the awareness program, they were unaware of these chemicals' harmful effects. They now recognize the critical need to avoid such practices to protect community safety.

Farmers from both Chhapchha and Kar-tshog Gewogs do not use Glyphosate on their crops, as it would kill the plants. Instead, they use Glyphosate to clear bushes when preparing fallow land and to manage peripheral bushes like small bamboos.

Findings from the survey conducted at Bjenag and Ruebisa (Khotokha)

Demographics

The survey team conducted interviews with 67 farmers, including 29 from Bjenag Gewog and 38 from Ruebisa Gewog. Out of these 67 respondents, 31 were male and 36 were female. The majority of respondents (n=21) were aged between 31 and 40 years. This was followed by 14 respondents aged between 20 and 30 years, while 13 respondents each fell within the age ranges of 41–50 years and 51–60 years. Only 6 respondents were over 60 years old.

Among the respondents, 40 were uneducated. Thirteen had received formal education up to the primary level (PP-6), 12 had studied between class 7 and class 10, and only 2 had attained a degree-level qualification. The majority of respondents (n=19) reported a primary household size of two, followed by 16 respondents with a household size of four. Additionally, one respondent reported a household size of nine, and another reported a size of twelve. Six respondents indicated having no other family members, 4 had a household size of six, 7 had a size of five, and 13 had a size of three.

Among the farmers surveyed, 40 have over 20 years of farming experience, 6 have between 16 and 20 years, 6 have between 11 and 15 years, 6 between 6 and 10 years and only 9 have less than 5 years of experience.

Crops cultivated

Potato is the primary crop cultivated in both Gewogs, while secondary crops include mustard, Cole crops, chili, turnip, radish, buckwheat, and wheat. Turnip and radish are commonly used as cattle feed during the winter months, whereas mustard, Cole crops, and chili are grown both for self-consumption and for commercial purposes.

Farmers' knowledge on Glyphosate and the History of Glyphosate

When questioned about the purpose of Glyphosate, all 67 farmers indicated they were aware of its use, stating that it is used for Weed control. However, most admitted to not knowing the recommended dosage. Among the few who claimed to know, their answers varied. Out of the 67 respondents, 48 reported obtaining Glyphosate from the Gewog Centre through their Agriculture Extension Officer. Seven mentioned sourcing it from both the Gewog Centre and local sellers, while 11 relied solely on local sellers. One individual sourced it through alternative channels, such as fertilizer suppliers (agents).

Regarding the history of Glyphosate's introduction in their gewogs, 62 farmers stated they were unaware of when it was first introduced, while 5 respondents were familiar with its history. Among the 5, 3 were uncertain about the exact year of introduction, 1 reported it was introduced in 2016, and another said it was in 2017. According to these respondents, a commission agent who supplies fertilizer introduced Glyphosate to their area, while one mentioned it was introduced by the agriculture sector. Two respondents mentioned that Glyphosate was introduced as a method of weed control in potatoes, while one said it was brought in for general weed control, not for a specific crop. Another respondent noted that it was introduced to control weeds in bamboo plants. One farmer indicated that it was introduced for both weed control and soil fertility improvement.

Glyphosate usage

All 67 respondents reported using Glyphosate due to its effectiveness in controlling weeds in potato fields, which helps reduce manual labor and saves time. Additionally, it facilitates harvesting by desiccating weeds, making tuber collection more efficient and convenient. This year, 13 farmers from Bjenag Gewog and 10 farmers from Ruebisa used Glyphosate, with 14 using it from previous stock, 6 purchasing it from local sellers, 2 purchased from Gewog and 1 obtained it from the neighbour. The duration of Glyphosate uses among farmers varied, with 31 having used it for more than 10 years, 14 for 7-10 years, 18 for 4-6 years, and 4 for 1-3 years.

Glyphosate application

A total of 62 respondents applied Glyphosate once, typically just before the potato harvest, while 5 applied it twice, i.e. once during the pre-emergence and again before the harvest. The majority of farmers from Bjenag (n=25) & Ruebisa (n=29) Gewogs reported using Glyphosate in agriculture fields exclusively during potato cultivation. However, 7 farmers used it both in agriculture fields and along electric fence line to clear the fence peripheral and 6 farmers from Ruebisa Gewog used it in agriculture fields as well as around their settlement areas. Farmers use it exclusively in potato fields and avoid applying it to other crops, as they report that its use on any other crop completely destroys them.

Farmers apply Glyphosate close to harvest time, but only after the haulms (the above-ground parts of the potato plants) have naturally dried up. The application of Glyphosate is done specifically to kill or dry out the weeds. They explained that applying Glyphosate before the haulms dry out can damage the potato tubers, causing them to rot.

The application was typically foliar, using Power Knapsack sprayers. They reported applying it at sunrise and avoiding its use during or immediately after rainfall. The application was mostly done by men. Most farmers reported wearing some protective gear, including gumboots, long pants, long-sleeved shirts, and some used face masks and plastic coverings to protect themselves from Glyphosate exposure. However, 4 farmers admitted to not using protective equipment, citing forgetfulness or lack of awareness as reasons.

Alternative weed control methods

A total of 63 farmers reported using alternative methods in place of Glyphosate around the time of harvest. Among them, 34 used Metribuzin, 11 relied on manual weeding, 12 combined manual weeding with Metribuzin, 2 used weeders, 3 combined manual weeding with weeders, and only 1 practiced mulching.

Crop harvest interval

No respondents harvest potatoes right after applying Glyphosate. The majority (n=40) wait for 2-3 weeks before starting the harvest, 10 wait for one week, and 17 wait for up to a month before harvesting. They explained that they wait until all the weeds have dried up, clearing the field for harvesting. The potatoes are mainly exported to India and a few farmers keep some for self-consumption and selling the rest to local dealers.

Regarding the cultivation of subsequent crop, 53 farmers plant the next crop (turnip, mustard, radish, buckwheat) immediately after harvest to prevent weed growth, while 14 farmers wait for a period ranging from 1-2 days to 1 month before planting.

Impact of Glyphosate through observations (Crop, Environmental and Human Health)

When asked about their knowledge of Glyphosate's impact, 43 farmers stated they were unaware of its effects, while 24 farmers reported being aware of its impacts on human health, the environment, and crops.

Impact on crops

Among the respondents, 5 farmers reported observing changes in the primary crop. Two observed positive changes such as increased tuber size and improved growth, while 3 noticed tubers rotting when excessive quantity of Glyphosate was applied. Similar to the primary crop, 14 farmers reported observing changes in the subsequent crop. They noted damage such as yellowing of leaves, gradual drying of plants from top to bottom, and, in some cases, no growth, particularly in mustard when planted immediately after the potato harvest. However, such issues were not observed in crops like turnip and radish.

Impact on Environment

Although 60 respondents did not observe any environmental changes, 7 farmers mentioned noticing changes such as trees near the Glyphosate-applied fields drying up and a decline in soil health. A few farmers also suspect that the death of insects and worms may be caused by Glyphosate. Some respondents observed soil softening, which they believe is due to poor rooting caused by the elimination of shrubs and weeds with the herbicide, while others noticed soil hardening after using Glyphosate.

Impact on Human Health

Among the respondents, 44 farmers reported not noticing any effects of Glyphosate on human health, while 23 farmers noted its impacts during use. Those who observed effects reported symptoms such as headaches, drowsiness, and nausea from inhaling its smell, eye and skin irritation upon contact, and tongue tingling if ingested.

Weed challenges

The most common and problematic weeds identified by the farmers include *Spergula arvensis, Persicaria runcinata, Rumex nepalensis, Galium aparine, Galinsoga quadriradiata, Equisetum spp., Thlaspi arvense*, *Bidens pilosa*, *Cynodon dactylon* and *Pennisetum clandestinum*. Respondents reported that Glyphosate could effectively kill weeds within a week of application and that it takes over a month for the weeds to regrow after its application.

Some farmers in Bjenag reported that Glyphosate is ineffective against certain weeds, such as *Equisetum* spp., *Rumex nepalensis*, and *Pennisetum clandestinum*. Similarly, a few farmers in Ruebisa noted that it fails to control weeds like *Equisetum* spp. and *Cynodon dactylon*.

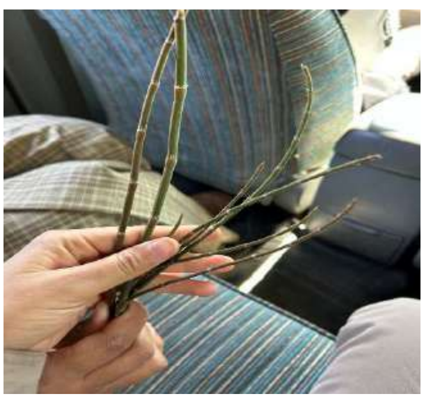



Figure 26. Equisetum spp. (left) and Thlaspi arvense (right)

Additional Observations

- Farmers from both gewogs reported that Glyphosate is more effective than Metribuzin. Some noted that Metribuzin can harm crops like beans and cabbage if planted immediately after its application and the potato harvest.

- A few farmers expressed concerns about the negative impacts of Glyphosate and are willing to stop using it. However, some farmers wish to continue using it despite being aware of its harmful effects.

- In Ruebisa, some farmers know glyphosate supply has stopped, but most remain unaware of the reasons or even the discontinuation itself. In Bjenag, by contrast, most farmers are aware of the supply cessation and understand its causes

- Several farmers emphasized the importance of raising awareness about Glyphosate's impacts, as many lack sufficient knowledge on the subject. Additionally, many expressed serious concerns about illegal imports of the pesticide. They emphasized that if Glyphosate is completely banned, the government must implement strict regulatory measures to prevent illegal imports. They pointed out that even if they refrain from using it, illegal products brought in and used by others could still have widespread harmful effects on their health, crops, and the environment.

3.3.4 Conclusion

The survey revealed that glyphosate is widely used for effective weed control and labor reduction, particularly in potato cultivation, though its use varies across regions. While farmers are generally aware of its purpose, knowledge of its health and environmental impacts remains limited. Some have observed benefits like soil softening and easier harvesting, while others reported crop damage, health symptoms, and environmental concerns linked to misuse. These findings highlight the need for strengthened awareness, improved regulation to address illegal imports, and promotion of safer, sustainable weed management alternatives.

3.4 Awareness campaign on safe & efficient use of pesticides

3.4.1 Introduction

Pesticides play a crucial role in modern agriculture by helping to control pests, weeds, and diseases, thereby enhancing crop yields and ensuring food security. However, their misuse poses significant risks to human health, the environment, and agricultural sustainability. Recognizing these challenges, the National Plant Protection Centre (NPPC), the leading agency for pesticide management in the country, collaborated with the Bhutan Food and Drug Authority (BFDA) and the Royal Centre for Disease Control (RCDC) under the Ministry of Health to conduct awareness campaigns on the safe and efficient use of pesticides.

These campaigns were conducted from August to October 2024, in Gangteng and Phobji Gewogs (Wangdue Phodrang dzongkhag) Chhapchha Gewog (Chhukha dzongkhag) and Kar-tshog Gewog (Haa dzongkhag). The program aimed to educate farmers on the judicious use of pesticides, including proper handling, storage, and application techniques. The campaigns also sought to raise awareness of the potential health hazards linked to pesticide exposure, ranging from acute toxicity to long-term chronic effects, and the environmental impacts such as soil degradation and water contamination.

Another key focus of the campaigns was regulatory compliance, with an emphasis on preventing the illegal import and distribution of unauthorized pesticides, which often have unregulated toxicity levels and can endanger both human and environmental health. Ultimately, the campaign highlighted the need to balance farming productivity with safety, protecting the environment, and following existing rules for pesticide use. The objectives of the campaign were to educate farmers on safe pesticide handling to minimize health and environmental risks and to ensure farmers understand regulations on pesticide procurement and prevent illegal imports.

Campaign Details and Key Highlights

Table 5. Campaign details

Place of Awareness campaign (Gewog & Dzongkhag)	Date	No. of female participants	No. of male participants	Total Farmer participants
Gangteng, Wangdue Phodrang	12/08/2024	185	115	300
Phobji, Wangdue Phodrang	19/08/2024	133	162	295
Chhapchha, Chhukha	25/09/2024	35	30	65
Kar-tshog, Haa	27/09/2024	104	25	129
Total farmer participants				**789**

3.4.2 Awareness campaign at Gangteng Gewog

Program Introduction

The pesticide awareness campaign in Gangteng Gewog was attended by 300 farmers and local officials (Gewog Thrizin, Mangmi, Tshokpa of five Chiwogs and Gewog Agriculture extension). The program began with opening remarks from the Gewog Agriculture Officer and the Thrizin, who stressed on the relevance of pesticide safety to the community and individual users. The Principal Plant Protection Officer, Mr. Jigme Wangchuk from NPPC then provided an overview of the NPPC, detailing its mandate, roles, and responsibilities. He emphasized the importance of the awareness program, focusing on the potential impacts of pesticide use on health and the environment. Additionally, he introduced the farmers to the newly implemented online system for pesticide indenting, known as the Plant Protection Product Information and Management System (3PIMS), explaining its significance in streamlining and monitoring pesticide use.

Presentation (Main program)

The presentation on the safe and efficient use of pesticides, covering topics such as the different types of pesticides, toxicity labelling, and safe handling practices, including proper application, storage, and disposal, was delivered by Mr. Nidup Dorji from the NPPC. The session also provided guidance on first aid for pesticide exposure and outlined key dos and don'ts. Following this, Dr. Gyem Tshering from the Royal Centre for Disease Control (RCDC) presented the health risks associated with pesticide exposure, sharing findings from a 2021 study on its long-term effects. Mrs. Kezang Choden, representative from Bhutan Food and Drug Authority (BFDA) emphasized the regulatory framework, including penalties under the Pesticides Act of Bhutan 2000, which enforces strict measures against illegal pesticide imports to protect public health and biosecurity.

The participants were shown two impactful documentaries showcasing the devastating health and environmental consequences of improper pesticide use, with a focus on Endosulfan misuse in India and the dangers of Glyphosate exposure. These reinforced the importance of safe handling practices and protective measures.

Question & Answer Session (Key concerns and feedback)

During the Q&A session, two key concerns were raised by the audience: alternatives to Glyphosate and the illegal importation of unregulated herbicides. The Principal Plant Protection Officer explained that the phase-out of Glyphosate was necessary due to its harmful effects on human health and soil quality. However, no safer and equally effective alternatives are currently available, as other herbicides with similar efficacy have already been banned for their toxicity. On the issue of illegal imports, a BFDA representative clarified that violations are prosecuted under the Pesticides Act of Bhutan 2000, with offenders facing a minimum one-year prison sentence. BFDA assured the audience of enhanced monitoring at border and integrated check posts to address this challenge.

The session concluded with closing remarks from the Gewog Agriculture Extension Officer and the Mangmi, who expressed gratitude to all participants and highlighted the importance of safe and responsible pesticide use. They acknowledged the farmers' efforts in attending the campaign during their busy potato harvest season and assured them of continued collaboration between the Gewog administration and NPPC to mitigate pesticide-related risks and support sustainable farming practices.

Figure 27. Group photo of participants at Gangteng Gewog

Figure 28. Welcome address by Gangteng Gewog Thrizin (left) and Mr. Jigme Wangchuk providing an overview of NPPC (right)

Figure 29. Presentation from NPPC (L) and Presentation from BFDA (R)

3.4.3 Awareness campaign in Phobji Gewog

Program introduction

On 19th August 2024, an awareness program on the safe use of pesticides was held at Phobji Gewog. The campaign was attended by 295 farmers from the five Chiwogs of Phobji Gewog, along with Gewog officials (Gewog Thrizin, Mangmi, 5 Tshokpa, Gewog Adm and Officials from National Seed Sub-Centre). The event began with an introduction by the program coordinator, Mr. Nidup Dorji, followed by a welcome speech from the Gewog Thrizin. The Thrizin emphasized the importance of the program in safeguarding farmers' health and preserving the soil and environment, expressing gratitude to the National Plant Protection Centre (NPPC) and the organizing team for their timely initiative.

Figure 30. Awareness programs group photo at Phobji Gewog

The Program Director of NPPC, Mrs. Yeshey Dema, then delivered the opening remarks, providing an overview of the NPPC's mandate, roles, and various programs. She highlighted the critical impact of pesticide use on human and soil health and the environment. The program director advised that any plant protection issues should be reported through the gewog agriculture extension to ensure proper communication and resolution rather than reporting to the media or to members of parliament.

Main presentation

Following the Program Director's opening remarks, the NPPC presented on the safe and efficient use of pesticides. This was followed by a representative from public health presenting the impact of pesticides on human health and preventive measures. The final presentation, by Mr. Sherab Zangpo, a representative from the BFDA, covered the importance of biosecurity, the risks of illegal pesticide importation and distribution, and an overview of relevant rules, regulations, and acts. The content and outline of the presentation is same as that of awareness program held in Gangteng. The same impactful documentary videos shown to the participants of Gangteng were also shown to the participants of Phobji, reinforcing the dangers of improper pesticide handling and highlighting the serious health risks involved.

Question & Answer Session (Key Concerns and feedback)

During the Q&A session, participants raised three main concerns. The first was the replacement of Glyphosate, as potato growers feared difficulties in weed management affecting their yields. The program coordinator, Mr. Nidup Dorji, explained that Glyphosate is typically used 2-3 weeks before harvest to kill weeds and ease harvesting, while Metribuzin is provided for weed control during critical growth periods. The NPPC is actively exploring safer alternatives. The second and third concerns revolved around the illegal import and distribution of herbicides. Participants highlighted border monitoring loopholes and called for stricter oversight. The Program Director assured that integrated check posts would tighten controls and encouraged anonymous reporting of illegal distributors. A request for timely pesticide delivery was also made, which the NPPC pledged to address.

The Program Director emphasized that all pesticides are inherently toxic, regardless of their classification, and advised strict use of safety precautions and personal protective equipment (PPE). She reiterated the importance of minimizing pesticide use. Concluding the session, the Gewog Mangmi expressed gratitude to NPPC, BFDA, and RCDC for the program and stressed its importance for protecting health, soil, and the environment. He suggested revisiting the cash collection process during pesticide indenting and proposed that NPPC manage PPE procurement to ensure uniformity and technical standards, enabling farmers to access affordable, standardized protective gear.

Figure 31. Awareness campaign on Safe & efficient use of pesticides at Phobji Gewog

Figure 32. Participants attending the campaign

3.4.4 Pesticide awareness in Chhapchha Gewog

Program introduction

In Chhapchha Gewog, 65 farmers, along with local officials (Gewog Thrizin and Mangmi, Tsokpa of 6 Chiwogs, Gewog Agriculture extension) attended the awareness program. The program commenced with an introduction by the Gewog Agriculture Officer, followed by a welcome address from the Gewog Thrizin, who shared the significance of the meeting. The Thrizin expressed gratitude to the National Plant Protection Centre (NPPC) and the organizing team for the awareness program, highlighting how it would greatly benefit farmers by safeguarding their health and preserving the soil and environment. The Thrizin also emphasized the importance of organic farming. He shared his concerns about pesticide residues in vegetables marketed to schools, stressing that most of these vegetables are consumed by our own children. Therefore, careful consideration of pesticide application is crucial, as it indirectly impacts the health of our children and consumers.

Following the welcome address, the Principal Plant Protection Officer of NPPC delivered the opening remarks. He provided an overview of the NPPC, detailing its mandate, roles, and responsibilities. He emphasized the importance of the awareness program, highlighting the potential impacts of pesticide use on health and the environment. Additionally, he introduced the newly implemented online system for pesticide indenting, known as the Plant Protection Product Information and Management System (3PIMS), and explained its significance in streamlining and monitoring pesticide use.

Figure 33. Awareness programs group photo at Chhapchha Gewog

Main presentation

Following the opening remarks, the NPPC delivered an extensive presentation on the safe handling and efficient use of pesticides. The presentation covered several critical topics, including the background and various types of pesticides, the importance of toxicity labelling, and the color-coding system for pesticide packaging. It also addressed the importance of pesticides in agriculture, highlighting both the benefits and associated risks and challenges. Farmers received practical guidance on safe handling practices, application methods, proper storage, and the safe disposal of pesticide containers. The presentation concluded with essential information on first aid and emergency procedures related to pesticide exposure, along with a list of dos and don’ts for safe pesticide use.

Following this, Dr. Gyem Tshering a representative from the RCDC under the Department of Public Health presented on the negative impacts of pesticides on human health. This presentation outlined various routes of exposure to pesticides, detailed first aid procedures for pesticide toxicity, and emphasized preventive measures to minimize health risks. Additionally, the representative shared findings from a study conducted in 2021, which shows the effects of pesticide exposure on human health, providing crucial insights into the long-term impacts.

The program continued with a presentation by Mr. Tsewang Dorji, a representative from the

BFDA, who covered pesticide use regulations, biosecurity importance, and penalties for illegal pesticide importation. He highlighted that, under the Pesticides Act of Bhutan 2000, individuals illegally importing and distributing pesticides face severe penalties, including a minimum one-year prison sentence. This strict enforcement aims to protect public health and ensure compliance with national biosecurity standards. Same impactful videos were shown like the previous program in Gangteng and Phobji.

Question & Answer Session (Key concerns and feedback)

The participants provided positive feedback, noting that the awareness program was highly informative and taught them numerous essential practices for handling pesticides. They also requested that the NPPC facilitate the yearly procurement of personal protective equipment (PPE) alongside pesticides.

The awareness campaign concluded with closing remarks from the Assistant Dzongkhag Agriculture Officer (ADAO), who expressed gratitude to all officials, representatives from relevant agencies, and farmers for their participation. Emphasizing the importance of using pesticides safely and responsibly to avoid potential hazards, the ADAO thanked the farmers for attending despite their busy harvest season. He summarized whole program for easy understanding for the farmers and repeatedly urged the farmers to use pesticides safely and judiciously.

Figure 34. Mr. Jigme Wangchuk from NPPC delivering opening remarks (left) and Welcome address by Chhapchha Gewog Thrizin (right)

Figure 35. Presentation from NPPC and Presentation from BFDA

Figure 36. Presentation By DoPH on Health Impacts and Summary of the Program by ADAO

3.4.5 Pesticide awareness in Kar-tshog Gewog

On September 27, 2024, an awareness program on the safe use of pesticides was held at Kar-tshog Gewog, attended by 129 farmers from the five Chiwogs of Kar-tshog Gewog, alongside Gewog officials. Additionally, agricultural extension officers from four other Gewogs attended to disseminate the information in their respective Gewogs.

Figure 37. Awareness program at Kar-tshog Gewog

The event began with an introduction by the temporary Gewog agriculture extension of Kar-tshog, followed by a welcome speech from the Gewog Mangmi. The Mangmi introduced the program's importance in safeguarding farmers' health and preserving the soil and environment. He expressed gratitude to the National Plant Protection Centre (NPPC) and the organizing team, urging participants to refrain from bilateral discussions during presentations and to ask questions after the presentation. The Mangmi also shared the overall status of pesticide usage in the Gewogs, particularly in vegetable and potato farming, highlighting the necessity of such training programs.

The Principal Plant Protection Officer (PPPO) of NPPC, Mr. Jigme Wangchuk, then delivered the opening remarks like in the previous awareness program conducted in other Gewogs. He provided an overview of NPPC's mandate, roles, and various programs and their functions in the Centre. Mr. Jigme Wangchuk also shared the status and trends of pesticide usage in the country and concerns related to these trends. He detailed the procedures for pesticide indenting and distribution, explaining the rationale behind these systems. Additionally, he emphasized alternative methods of pest management, urging farmers to use pesticides only as a last resort, due to the negative impacts of pesticide use on human health, soil health and the environment.

Main presentation

Following the Principal's opening remarks, Mr. Nidup Dorji, presented on the safe and efficient use of pesticides, highlighting the negative impacts on human health, the environment, and soils. The methods and timing of pesticide application were key points shared with scientific justifications.

The final presentation, by Mr. Gembo Dorji the representative from the BFDA, covered the importance of biosecurity, the risks of illegal pesticide importation and distribution, and an overview of relevant rules, regulations, and acts. Due to the absence of a health representative in Kar-tshog Gewog, the NPPC covered the health impacts of pesticides, including a summary report on pesticide residues in the human body. As in other Gewogs, two impactful documentary videos were shown to the participants, reinforcing the dangers of improper pesticide handling and highlighting the serious health risks posed by restrictive pesticide use and improper application.

Question & Answer Session (Key concerns & Feedback)

The farmer participants raised concerns about the hazardous nature of certain pesticides, urging the government and NPPC to restrict and monitor the illegal import of such pesticides. They highlighted that some individuals are importing and selling illegal pesticides, which pose significant hazards and dangers to the community.

One participant requested that the NPPC produce short videos on the dosage and application rates for each pesticide, enabling uneducated farmers to follow the recommended guidelines. Given that many farmers are illiterate and unfamiliar with the specific dosage requirements, they also requested that the EA's and ASSR's provide the recommended rates and explain the application methods during the distribution of pesticides.

The concluding remarks were delivered by the Gewog Agriculture Extension, who expressed gratitude to the NPPC, and representative from the BFDA for organizing and raising awareness on the safe and judicious use of pesticides in Kar-tshog Gewog. She explained that the program would greatly benefit pesticide handlers by helping them protect their health, the soil, and the environment. She also highlighted the importance of continuous education and training in ensuring safe agricultural farming. She encouraged all participants to share the knowledge gained with their communities and to remain vigilant against the misuse of pesticides. Finally, she stressed the significance of collective effort in preserving the environment and ensuring the well-being of future generations.


Figure 38. Pesticide awareness campaign in Kar-tshog Gewog, Haa

3.4.6 Conclusion

The pesticide awareness campaigns in Gangteng, Phobji, Chhapchha, and Kar-tshog Gewogs successfully educated 789 farmers and local government officials on safe pesticide use, regulatory compliance, and sustainable practices. They also addressed concerns about Glyphosate alternatives, illegal imports, and access to protective gear. Through presentations, impactful documentaries, and discussions, the programs emphasized minimizing pesticide use, safeguarding health, and protecting the environment. These initiatives were made possible with funding support from Bhutan for Life, enabling collaboration between NPPC, BFDA, RCDC, and local stakeholders in educating the farmers on safe and efficient use of pesticides and the negative impacts of pesticides on human health, soil, and the environment.

3.5 Developing Weed Herbarium

3.5.1 Introduction

The development of a weed herbarium is an essential initiative aimed at advancing the identification, research, and educational efforts surrounding weed species. By collecting, drying, pressing, and mounting weed specimens, the herbarium becomes an invaluable resource for studying weed biology, management strategies, and distribution patterns. An herbarium serves as a crucial reference for the future by preserving plant specimens and their associated data. By maintaining a comprehensive archive, herbariums ensure that knowledge about weeds remains accessible and impactful for years to come.

Over the year weed science program has successfully collected and developed over 100 weed specimens and digitized both old and newly developed herbarium specimens, ensuring their archival for future reference.

3.5.2 Benefits of the Weed Herbarium

- **Identification:** Offers an authoritative reference for the accurate identification of weed species.
- **Research:** Promotes deeper insights into weed biology, ecology, and management practices.
- **Education:** Serves as a vital educational tool for students, researchers, and the wider community.
- **Data Archiving:** Facilitates systematic documentation and analysis of weed distribution and prevalence.
- **Monitoring and Trend Analysis:** Supports long-term observation of weed population dynamics and environmental changes.

3.5.3 Collection and Preparation

Sample Collection

- **Locations:** Weed samples were collected across diverse regions of Bhutan, representing various agro-ecological zones, habitats, and soil types. Targeted areas were chosen based on weed prevalence and species diversity.

- **Timing:** Seasonal and growth-stage considerations were carefully observed to ensure specimens captured representative ecological and phenological traits.
- **Plant Material:** Efforts were made to collect entire plants, including roots, stems, leaves, and flowers, depending on seasonal availability. Each specimen was handled meticulously to avoid damage.

Specimen Preparation

- **Drying and Pressing:** Specimens were placed between absorbent sheets and pressed using plant presses equipped with corrugated cardboard, with heavy objects applied to achieve optimal flatness and preservation.
- **Special Handling for Succulent Weeds:** Absorbent sheets were regularly replaced to prevent mold or decay in moisture-rich plants, ensuring the quality of the specimens.

Figure 39. Collecting herbarium samples

Figure 40. Preparing Herbarium Specimens

Figure 41. Drying Samples with Newspaper

3.5.4 Mounting and Labelling

Mounting

- Dried specimens were affixed to acid-free herbarium sheets using non-acidic adhesives like paper glue, guaranteeing long-term durability and quality preservation.

Figure 42. Recording sample information

Figure 43. Laying out the specimens

Labeling

- Detailed labels were prepared for each sheet, including:
  - Correct scientific and common names.
  - Botanical family classification.
  - Collection date and location.

- Collector's name for attribution.

Figure 44. Pressing Specimens

Systematic Organization

- Mounted sheets were arranged systematically according to recognized taxonomic classifications, such as genus-wise and botanical families, ensuring logical and user-friendly accessibility.

Figure 45. Specimen mounting

Figure 46. Specimen label preparation.

Figure 47. Mounting & Labelling

3.5.5 Herbarium Storage & Digitization

Storage

- The herbarium was systematically organized and stored in a controlled environment, free from pests and excess humidity. Key specimens were displayed prominently to showcase Bhutan's major weed species.
- The records of each specimen were maintained.

Digitization

- A total of 331 herbarium specimens, including both old and newly developed collections, were scanned using the Microtek ObjectScan 1600.
  - Old Herbarium: 216 specimens collected by Chris Parker and his team during 1991–1993 were scanned and archived.
  - New Herbarium: 115 newly developed specimens were scanned and incorporated into the digital archive.

- Digitization efforts aimed to enhance accessibility, streamline research, and ensure the long-term preservation of Bhutan's weed biodiversity.

Figure 48. Herbarium Specimen scanning for digital archiving

Figure 49. Completed Herbarium

4. Pest Surveillance Program

4.1 Barberry survey

4.1.1 Introduction

Wheat rust ranks among the most devastating diseases of wheat crops, inflicting substantial global production losses annually. This fungal pathogen relies on both sexual and asexual reproduction to complete its life cycle. The asexual phase occurs on its primary host (wheat), while the sexual phase takes place on alternate hosts such as barberry and Mahonia. Unlike many other pathogens, wheat rust requires these distinct host plants to thrive. Alternate hosts are pivotal in rust epidemiology, as they enable sexual recombination on their tissues, generating millions of progeny variants and spawning new, potentially more virulent races each year

The Himalayan region is the centre of origin and diversity for *Berberis* spp., alternate hosts of the yellow rust pathogen (*Puccinia striiformis*) and stem rust pathogen (*Puccinia graminis*) affecting cereal crops. Population studies of yellow rust in this area reveal high genetic diversity, signalling sexually recombining populations. Surveillance suggests regular migration of key wheat yellow rust races from the Himalayas to East Africa. Recent findings of exotic stem rust races in Nepal and Bhutan, such as TTKTT (Ug99 group) and TKFTF + TKKTF (clade IV-F), heighten the risk of recombination on these alternate hosts. Such events could produce even more virulent strains that spread beyond the region, threatening global cereal production. These dynamics emphasize the urgent need for deeper understandings into alternate hosts' roles in pathogen variation and disease epidemiology across Bhutan and the broader Himalayas.

4.1.2 Methods and Materials

Barberry bushes were located and documented at each site during surveys conducted in Paro and Haa Valleys. These sites were chosen based on the previous wheat season's rust reports, which detected a novel race/pathotype unique to these areas and absent elsewhere in the country. Identified bushes were tagged for easy relocation on future visits. Monthly observations and data collection continued until dormancy, with aeciospore samples gathered from any infected bushes. These samples were lab-analysed to assess barberry's potential in infecting wheat and other cereals. If infections appear on wheat from these aeciospores, affected plant samples will be collected and shipped to GRRC, Denmark, for pathotype analysis.

4.1.3 Field observations:

The identification of barberry bushes in Haa and Paro valleys started in the first week of February 2025. Different species of barberry plants were identified and tagged, and the tagged bushes visited once a month for data recording and to collect aeciospores, which will then be taken to the laboratory and do testing on the wheat plants. The detailed field observation is reflected in the table below:

Table 1. Survey details

S.No	Date of visit	Site	Barbery plant stage	Aeciospore observed/not	Sample collected (Yes/No)	Remarks
1	3/2/2025	Paro valley	Vegetative	Old aeciospores recorded from broad/oval-leaved barberry (Figure 3).	Yes	Two distinct types of barberry plants were recorded (broad-leaved and narrow-leaved) (Figures 1& 2).
2	4/2/2025	Haa valley	Dormant to vegetative	No	No	Three distinct types of barberries were observed: narrow-leaved with long spines, narrow-leaved with shorter spines, and red-stemmed barberry (Figures 4, 5 & 6).
3	14/3/2025	Paro valley	Vegetative	No	No	-
4	15/3/2025	Haa valley	Vegetative	No	No	-

5	14/4/2025	Paro valley	Flowering	Aeciospores observed on the broad-leaved barberry; it must have first appeared one to two weeks ago, as the aeciospores that we observed from those plants have already become old.	Yes	Taken to the laboratory, dried properly, and stored for future use
6	15/4/2025	Haa valley	Most plants are at flowering stages, and very few are at vegetative and dormant stages, depending on the species	No	No	-
7	11/5/2025	Paro valley	Fruiting	Yes, observed pycnidia on the barberry plants (on both broad and narrow-leaved), which are grown in proximity to the wheat fields infected with the yellow rust. In addition, we also recorded a few aecia spores from the broad-leaved barberry plants.	Yes	-
8	10/5/2025	Haa valley	Vegetative to fruiting stages	Yes, now the barberry plants located near those wheat fields where the initial infection of rust was recorded, started to produce pycnidia on their young leaves	Yes	-

9	27/5/2025	Haa valley	Flowering to fruiting stages	Aeciospore was observed on all the barberry bushes in all the locations (Figure 7).	Yes	Samples were brought to the office and were inoculated on different lines of wheat to test the presence of wheat rust.
10	28/52025	Paro valley	Late flowering to fruiting stages	Aeciospore observed on all the barberry bushes in all the locations	Yes	Samples were brought to the office and were inoculated on different lines of wheat to test the presence of wheat rust.
11	21/6/2025	Paro valley	Fruiting/fruit maturing stage	Very few aeciospores were observed	Yes	-
12	22/6/2025	Haa valley	Fruiting	Very few aeciospores were observed	Yes	-

Figure 1. Broad-leaved barberry plant in Susuna, Nagya, Paro

Figure 2. Narrow-leaved barberry plants

Figure 3: Aeciospores collected from broad-leaved barberry plants, Susuna Paro

Figure 4: Narrow-leaved barberry with long spine   Figure 5: Narrow-leaved barberry with short spine

Figure 6: Red colored stem barberry plants in Haa

Figure 7: Aeciospores from narrow-leaved Barberies, Haa valley

Multiple barberry species were documented at the same sites, as they grow abundantly nationwide. Flowering and fruiting times vary by species, influencing aeciospore observation based on both plant type and location.

In general, aeciospores were recorded on oval/broad-leaved barberry in Paro Valley, though they were aged. Fresh aeciospores were found on few barberry bushes in both Paro and Haa valleys. This first appearance of aeciospores on the alternate host coincided with the initial infection of yellow rust on wheat grown in the nearest wheat field. From this season's monitoring, infections on tagged barberry plants peaked from the second week of May through mid-June 2025, after which only sparse aeciospores remained on most bushes.

4.1.4 Laboratory work on barberry

4.1.4.1 Materials and Methods

Materials

- 50mL Self-standing centrifuge tube (base-cut)
- Differential seed- Six varieties of wheat consisting of four locally released varieties from ARDC-Bajo (Bajoka 3, Bajosokha, Gumasokha, and Bumthangka Drukchu) and one local variety each from Haa and Paro dzongkhags.
- Slow-release granular fertilizer (such as Osmocote) or regular granular fertilizer.
- Maleic hydrazide (MH)
- Plastic container or bucket (at least 40mm or 1¼') high with lid
- Plastic bags (gallon-size or larger)
- Spray bottle with distilled water
- Sand or potting soil or coco peat
- Petri Dishes
- Germination paper
- Plastic trays
- Tweezer
- Marker
- Labels

Methods

Growing clean plants for inoculation

Step A: Seed pre-germination (Figure 8): to ensure even germination and seedling sizes for inoculation, pre-germination of the seed of each line in petri dishes was carried out. For that, we have carried out the following steps:

1. Added germination paper to the bottom half of the Petri dish
2. Added distilled water until the paper is saturated, but there is no free water.
3. Labeled the Petri dish with the name of the line and date.
4. Added up some seeds per Petri dish
5. Closed the petri dish and left it on the table for 24 hours.

6. After 24 hours, the germinated seeds were transplanted into prepared 50 ml centrifuge tubes.

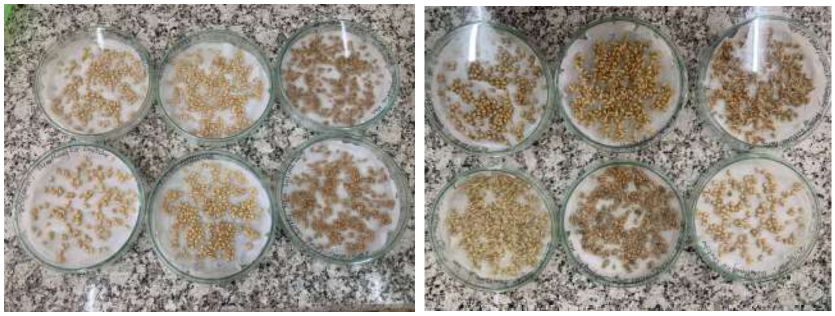


Figure 8: Pre-germination of seeds

Step B: Planting on Centrifuge tubes as follows:

1. Mixed cocopeat with slow-release granular fertilizer 30:1 (Figure 9)
2. Filled the tube with moist potting soil and left 2 inches of headspace
3. Labeled the differential line name
4. Planted 5 seeds of the differential lines in each tube
5. Filled the tubes with potting soil, leaving ½ inch of head space for watering with maleic hydrazide solution (Figure 10).

Figure 9. Coco peat and fertilizer mixture   Figure 10. Planting pregerminated seeds in a centrifuge tube

Step C: Growing clean plants (Figure 11)

1. Placed the labeled seeded tube on the tube holder
2. Placed the seeded tubes on a tray with water, in a place with sufficient light for cereal seedlings to grow.
3. Prepared a maleic hydrazide (MH) solution in water (full-strength 0.3g/L, half-strength 0.15g/L). Note: *Use half-strength when toxicity is observed, especially on barley and oats. Normally, wheat is fairly tolerant unless under very hot conditions. Recommended to use full strength.*
4. When the seedling tip is visible (about 3-4 days after growing), the wheat seedlings were treated with MH by pouring over 5ml of the MH solution per tube over the top. *Note: Using a 5- or 10-ml syringe makes the process easier (Figure 11).*

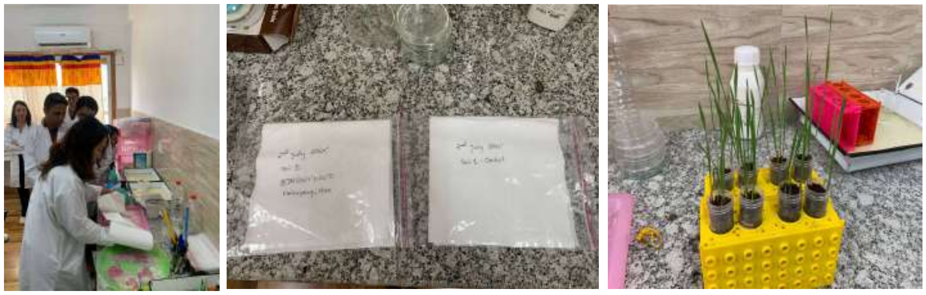
5. Continued growing the plants until ready to use (about 7-10 days post-watering @ 22±3°C) until the plants are 4-6 inches tall.
6. Then the plants were carried to the inoculation room in a container with a lid on, protecting them from contamination

Figure 11: Growing clean plants in a tube and treatment of plants with maleic hydrazide

Step D: Inoculation (Figure 12)

1. Each of the aecia-infected barberry leaves was washed with clean water to wash off contaminants, such as urediniospores from the field, that might be present on the barberry leaf samples by alternatively immersing leaves in a beaker full of water and moving leaves around. The washed barberry leaves were placed into a petri dish and sealed to prevent contamination.
2. Pre-label the plastic bag or plastic tub with the sample code and date. Line the plastic bag or tub with a paper towel. Moistened a paper towel to saturation by spraying with distilled water. If using a plastic bag, place it onto a tray.
3. Gently rubbed each leaf of the differentials and control plants between two fingers to remove wax.
4. Laid the seeded tubes flat into a plastic container or a gallon-sized plastic bag lined with paper towels. For multiple tubes, arrange so that they are facing each other with the seedling leaves overlapping.
5. Mist the plants by spraying distilled water lightly into the container (or bag).
6. Arranged each infected barberry leaf on the top of the differential plants with aecial pustules facing the plants and touching the leaves.
7. Sprayed the leaf and plants lightly with the spray bottle using distilled water.
8. Sealed the inoculation box (or bag) but not anaerobically
9. Placed the setup at a location away from direct sunlight and heat sources.

I. Aeciospores II. Washing off contaminants



III. Preparing inoculation setup (Preparing plastic bags & pre-labeling)

IV. Rubbing leaf to remove wax

V. Inoculation of plants with aeciospores

Figure 12: Inoculation procedures

Note: If using tubes or small pots, a wire mesh can be placed over the seedling leaves and the infected barberry leaves placed onto the wire mesh over the seedling leaves, aecia side down (Figure 13).

Figure 13: Placing aeciospores on plants for inoculation

Step E: Infection period in the dew chamber

1. Incubated the inoculated first set of plants (set 1) in the Plastic bag/tub (dew chamber) overnight (Figure 14). *Note: Ideally, if you can maintain 15-20 degrees Celsius during the dew period, you can get both stripe and stem rust; the lower (up to 12 degrees Celsius), the better for getting stripe rust. If the temperature is higher than 27 °C, it is difficult to get an infection, even for stem rust. No chance of getting stripe rust. Putting a dew chamber outside is a good option for stripe rust (if cooler than indoors).*
2. Moved the same barberry leaves onto a new set of clean plants (set 2) the next day. Follow the same inoculation method as set 1.
3. Continued to incubate the plants of set 1 for another 24 hours in a dew chamber (a total of 48-hour infection period).

4. Handled the plants of set 2 similarly to set 1.
5. To be able to collect uredinospores from the infected differential set for further race analysis, put each set into an isolation chamber, preventing contamination. Remove the seedling set from the plastic bag/tub. Place them on a tray with water (or in a container with water). Cover with an isolation chamber. NB Only 1 set of plants should be in the isolation chamber (but it is possible to combine 2 controls into a single isolation chamber).

Figure 14: Successful sporulation of aeciospores onto test seedlings

Step F: Incubation for rust development (Figure 15)

1. Plants should be grown with an ambient temperature of @22±3°C and exposed to 12-16 hours of light.
2. Watered plants as needed (daily if necessary) without getting leaves wet. Using a tray or saucer for the bottom water is best.
3. Rust infection should be visible 6-7 days post-inoculation
4. Infection types could be noted 12-14 days post-inoculation
5. Rust spores could be collected into gelatin capsules and sufficiently desiccated
6. Rust spores from different crop species/varieties should be kept separately

Figure 15: Inoculated plants in the incubation chamber

Collecting spores:

1. Scrape into capsules (see collection into gelatin capsules part https://www.youtube.com/watch?v=MGYQffJZ6T0&t=117s), use a mouth collector, or use a vacuum collector. In the latter two cases, you will need a cyclone collector and a special connector.
2. After collecting spores, please make sure to desiccate spores for 4-5 days in a desiccator with dry (blue) silica gel/crystals. before storing them in a freezer -20 °C or -80 °C. Gelatin capsules can be placed in an open, labelled Eppendorf tube in the desiccator.
3. You can also save infected wheat leaves in paper envelopes to dry for a week or so and then refrigerate.
4. Dry spores in the closed Eppendorf tube can be sent to international labs under permit in the same way as infected leaf samples.

Include a control set.

Include a control (misted test seedlings but not inoculated with barberry sample) with each seedling set tested (include a control for set 1 and set 2). This will inform you of any potential contamination from an ambient environment during growth or operations (Figure 16).

Figure 16: Control set in the experiment

Barberry Sub-Sample for confirmation

1. Kept a few infected barberry leaves as a subsample for confirmation. The leaves were properly dried in a paper envelope and stored in the fridge. If some important virulence is found in the experiment, it will help to have the subsample for confirmation by running DNA.

General Notes:

1. The aecia on barberry appeared to be reddish aecia of stripe rust tend to be this color. Stem rust is more yellowish.

4.1.4.2 Results

Three sets of experiments have been carried out using aeciospores from both narrow and broad-leaved barberry plants from both the Haa and Paro valleys. Though flecking on the wheat lines used for testing in the second experiment was recorded (Figure 17). Further development of the uredospore is not observed.

Figure 17: Flecking observed on the differential lines during the experiment

4.1.4.3 Conclusion

The reason the uredospore was unable to develop further could be that, although the aeciospore landed on the wheat crop, it did not develop into rust disease because the uredospore belongs to a different rust type that affects other grasses or cereals, not wheat. Another possible reason is that the wheat lines used may be resistant varieties. Therefore, in the next experiment, we will use the most susceptible varieties imported from the CIMMYT office.

4.2 Wheat Rust Sentinel Plot Establishment

4.2.1 Introduction

The National Plant Protection Centre, in collaboration with the CIMMYT office (DEWAS Subgrant) has established wheat rust sentinel plots in four different locations (Figure 18), one in the lowest elevation at ARDC-Samtenling located at 392 Masl in southern region, two in mid elevation at ARDC-Bajo located at 1184 Masl in west central region and at ARDC Wengkhar in the eastern region located at an elevation of 1588 Masl and the fourth one in the highest elevation in Susuna in Nagya gewog under Paro dzongkhag located at 2363 Masl in the western region. The sentinel plots were established to trap rust spores, which will be collected and sent to the Global Rust Reference Centre in Denmark for rust race analysis, and to check the resistance of different wheat varieties against rust diseases under different environmental conditions.

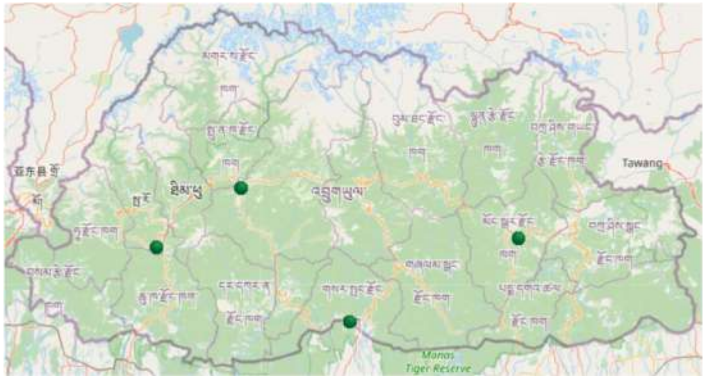


Figure 18: Sentinel plots established at four locations

4.2.2 Materials and Methods

In total, there are 28 lines of seeds sown in each sentinel plot. In addition to the 24 different lines of wheat seeds imported from the CIMMYT office, Mexico, four locally released varieties are included (Table 2). The sentinel plot establishment at different locations was done as reflected in Table 2. Seeds were sown by preparing two plots of 70cm x 600cm. Each lines (20grams) were divided into two sets (10grams each). One set of lines is sown in one plot and the second in the second plot by keeping approximately 20cm line-to-line distance. The data from the sentinel plots were collected using ODK applications, and the samples of rust were collected if we came across any rust diseases during the time of data collection and were shipped to GRRC, Denmark, for rust race analysis.

Table 2: List of wheat lines included in the sentinel plots

Si. No	**Lines**
1	YR5/6*AOC **(CX86.6.1.20-OMXI**)
2	SUPER KAUZ **(CM67458-4Y-1M-3Y-OB)**
3	YR10/6*AOC **(CX93.53.3.1-OMXI)**
4	YR15/6*AOC (**CX89.1.1.27-OMXI**)
5	YR26/3*AOC (**CX96.17.1-OMXI**)
6	OPATA/PASTOR (**C05607-A020**)
7	AOC-YR3*/PASTOR (**CGSS00Y00207T-099M-1Y**)
8	YR33 **(###513182-OMXI**)
9	Yr35 98M71 **(###734610-OMXI**)
10	Yr37 **(###734609-OMXI**)
11	Yr51 **(###734607-OMXI**)
12	YRSP/6*AOC (**CX94.14.1.15-OMXI**)
13	W2691Sr13 **(###23300)**
14	SWSR22T.B. (**II.70.565.11-?)**
15	LC-SR25-ARS **(###250760**)
16	EAGLE-SR26, SR9G (**EAGLE-SR26, SR9G**)
17	COORONG TRITICALE-SR27 (**COORONG TRITICALE-SR27**)

18	SR31/6*LMPG (**SR31**)
19	W2691 SR36TT1 (**W2691 SR36TT1**)
20	TETRA CANTHATCH/AE. SQ-SR33, SR5 (**RL5405**)
21	W3763=SR35 (W3763-SR35)
22	SR50/4/3*KACHU*2/3ND643/2*PRL/2*PASTOR (**CMSS16BO1884T-099MABY-099M-099Y-47M-OWGY)**
23	KASUKO (**CMSS11BOO123S-099M-099NJ-1RGY-0B-4M-ORGY**)
24	CNSSRTMP (**SRTMP)**
25	Bumthangka Drukchu
26	Gumasokha
27	Bajosokha
28	Bajoka 3

Table 3: Sentinel plot establishment at various locations

Si.No	**Location**	**Elevation (Masl)**	**Date of seed sowing in each location**
1	Susuna, Nagya, Paro	2363	25th October 2025
2	ARDC-Bajo, Wangdue Phodrang	1184	24th Noveber 2025
3	ARDC-Wengkhar, Monggar	1588	6th November 2025
4	ARDC-Samtenling, Sarpang	392	14th November 202

4.2.3 Field observations

a. ***Sentinel plots in ARDC-Samtenling, Sarpang***

This site is located at the Agriculture Research Development Centre, Samtenling, under the Sarpang dzongkhag, which is located at an elevation of 392 Masl in the southern part of the country (Figure 19). The data collection from this plot was done monthly, starting from the tillering stages until it matured. Similar to the previous year, a very low incidence of leaf rust was recorded on CIMMYT line numbers 15 and 20. While the plot is free of any yellow rust or stem rust diseases.

Figure 19. Sentinel plot established in ARDC-Samtenling

b. ***Sentinel plots in ARDC-Bajo, Wangdue Phodrang***

This site is located at the Agriculture Research Development Centre, Bajo, under Wangdue Phodrang dzongkhag, which is located at an elevation of 1186Masl (Figure 20). Three times data collection was carried out from this site, when the crops were at the tillering to dough stages. The first data recording was done on 15th March 2025, when the crops were at the tillering stage. Only the yellow rust was recorded from the majority of the lines during that time. As the plant grew older, both the incidence and the severity of yellow rust became higher, infecting even the spikes. Similarly, even the leaf rust started to appear, and its incidence and severity became higher as the plant got older. In general, the incidence of yellow rust was very high, while the leaf rust incidence was recorded to be very low, with varying severity and the reaction types for both diseases (Figure 21). Stem rust was not recorded from this plot. The samples of both leaf rust and the yellow rust were collected and sent to GRRC, Denmark, for pathotype analysis.

20. Sentinel plot established in ARDC-Bajo

Figure 21. Diseases observed

c. Sentinel plots in ARDC-Wengkhar, Monggar

This site is located at the Agriculture Research Development Centre, Wengkhar, under Monggar dzongkhag, which is located at an elevation of 1588 Masl in the eastern part of the country (Figure 22). The data collection from this plot was done on a monthly basis starting from February 2025, when the crops were at tillering stages, until it is at flowering stages. Very low incidence of yellow rust was recorded from this plot when the plants were at flowering stages (symptoms observed on CIMMYT line numbers 7, 9, and 16), with low severity showing varying host plant reaction towards its pathogen. While the trace of leaf rust was recorded on locally released varieties such as Gumasokha and Bajosokha, which were also at flowering stages, showing moderately resistant to moderately susceptible reaction type. On the other hand, though the plot was found to be quite free of rust diseases, almost all the lines in the plot were severely infected with the powdery mildew starting from tillering until maturity stages (Figure 23). Samples of both leaf rust and the yellow rust were collected and sent to GRRC for analysis.

Figure 22. Sentinel plot in ARDC-Wengkhar, Monggar

Figure 23. Diseases observed in this sentinel plot

d. Sentinel plots in Susuna, Paro

This site is located in the western part of the country. It is established at Susuna in Nagya Gewog under Paro dzongkhag, which is located at the highest elevation among the four plots established in the country. It is located at an elevation of 2363 Masl. Data recording from this plot was done on a monthly basis starting from when the plants were at tillering until it is matured. Disease started to appear in this plot when the crops were at boot to flowering stages; a trace of yellow rust was recorded from a few CIMMYT lines during that time. Similar to the plot established in ARDC-Bajo, in these plots as well, we have recorded a very high incidence of yellow rust with varying severity and the host plant reaction types depending on the lines in the plots (Figure 25). In some lines, the severity was very high so that the rust had even infected its spikes as well (Figure 25). The last data collection was carried out in the third week of June 2025; all the lines in the plots were matured and dried except the CIMMYT line number 1, very little green tissue was observed, and recorded a low incidence of leaf rust on that line (Figure 25). Other than yellow rust and leaf rust, we have not observed any disease in this plot.

In addition to this, the symptoms of yellow rust on *Dactylis glomersta*, a common forage grass, and the stem rust on Lolium species grass grown next to the sentinel plot were observed (Figure 26). The samples of both types of rust from the sentinel plots and the grass were collected and shipped to GRRC, Denmark, for pathotype analysis.

Figure 24. Sentinel plot in Susuna, Paro

Figure 25. Diseases recorded from the plots in Susuna, Paro

Figure 26. Yellow rust on *Dactylis glomersta* and the stem rust on Lolium species grass

4.2.4 Conclusion

Overall, both leaf rust and the yellow rust were recorded from all the sites except for the one that was established at the lowest elevation in the southern part of the country, where only a very low incidence of leaf rust was observed. The incidence and the severity of both yellow rust and leaf rust were recorded to be higher in all the sites as compared to leaf rust. Stem rust was not found in any of the plots. Apart from rust diseases, a very high incidence and the severity of powdery mildew were observed in the plots established in ARDC-Wengkhar. While, low incidence of powdery mildew was also recorded from the plots in ARDC-Bajo. The samples of both yellow rust and the leaf rust were collected and shipped to the Global Rust Reference Center, Denmark, for the pathotype analysis.

4.3 Wheat disease surveillance
4.3.1 Introduction

Wheat rust survey is a regular activity that is conducted every year by the NPPC in collaboration with the ARDCs and the Extension agents, with the funding support from the CIMMYT office. The activity is also a part of the global wheat rust monitoring system, wherein rust outbreaks and possible development of new rust races are monitored. The wheat rust surveillance for this wheat growing season started from the second week of February 2025 till the end of June 2025.

Objectives:

1. To monitor rust outbreaks in the major wheat-growing areas
2. To know the rust type and the races of rust present in the surveyed area
3. To support the collection of data on the early warning information dissemination reach

4.3.2 Materials and Methods

The rust monitoring survey was conducted in Major wheat-growing areas of Punakha, Wangdue, Trongsa, Bumthang, Trashigang, Monggar, Lhuntse, Tashiyangtshe, Zhemgang, Thimphu, Haa, and Paro dzongkhags. The survey was performed using the ODK application by scanning the QR code of the project customized by the CIMMYT office for carrying out the wheat rust surveillance for the specific country. In each gewog, major wheat fields were randomly selected. Within a field, a diagonal pattern is taken to observe plants at every 10-15 steps. Information on location, variety, crop stage, rust type, and its incidence; rust severity and reaction type were recorded. The rust live samples, including the rust found on grass and the barberry plants grown next to wheat fields, were also collected from the field for analysis of rust races present in the country. Similarly, the samples of diseases which we cannot confirm their identity in the field were also collected and brought to the office for confirmation through observation of their spores under a microscope, which have been cultured using wet tissue culture.

4.3.3 Field observations

a. Zhemgang Dzongkhag

The rust survey in this region was conducted on 16th and 17th November 2024 in Bardo and Shingkhar gewog under Zhemgang dzongkhagt. People in this area cultivated only two varieties of barley, which are locally known as Mukulung (awnless barley) (Figure 27) and Sangchan (awned barley) (Figure 28). The crops were at late heading to dough stages depending on the places where the barleys are cultivated. Rust is not recorded from any of the sites visited except for one site in Nyimzhong, Shingkhar gewog; a trace of leaf rust was observed from one field (Figure 27). Similarly, a very low incidence of loose smuts was also recorded from this area (Figure 28).

Figure 27: Leaf rust on barley

Figure 28: Loose smuts on barley

b. Samtse Dzongkhag

The wheat disease surveillance in Samtse dzongkhag was conducted in mid-February 2025 in Tading, Pemaling, Tendruk, Namgyalchhoeling, and Sangngagchhoeling gewogs. Farmers cultivated only bread wheat of various varieties, and in some pockets, they grew oats for fodder purposes. The crops were at tillering to dough stages, depending on the varieties and the location where the crop was cultivated. Wheat growers in this area cultivated mostly the local varieties except in Tading gewog, where almost all of them cultivated the Gumasokha variety. They grew wheat in both dryland as well as in wetland. People in this dzongkhag started to sow wheat in the field as early as September, and it goes up to December as per their convenience. In this wheat growing season, in all the locations, the wheat crop was very healthy, and we have not observed any wheat rust or any other diseases. The very low incidence of aphids was recorded from some sites. Interestingly, we have not recorded any barberry plants in this dzongkhag.

c. Trongsa Dzongkhag

The wheat disease surveillance in the lower part of Trongsa dzonghkag was carried out in last week of February 2025, covering two gewogs (Draagteng and Langthil). In general, the farmers in this area grew only the bread wheat of various local varieties. The crops were at tillering to maturing stages, depending on the varieties and the places where it is cultivated. Farmers start to sow seeds in the field from September to November, depending on their work pressure on the farm. Low incidence of both yellow rust and leaf rust was detected from the wheat fields in Draagteng gewog (Figures 29 & 30). In addition to this, a very low incidence of crown rust was also recorded from one of the sites in Draagteng gewog, where the field is cultivated with oats. Similarly, the low incidence of yellow rust was recorded from Langthil gewog. No leaf rust was observed in this area during this season. However, the low incidence of Fusarium head blight was recorded from two sites, and this is our first detection of Fusarium head blight in this region (Figure 31). Alternate hosts such as mahonia and barberries were observed in this area (Figure 32). The samples of both rusts and the Fusarium head blights were collected. The collected rust samples were shipped to Denmark, while Fusarium head blight samples were observed under a microscope in the laboratory through wet tissue culture methods to confirm the disease (Figure 33), and it was confirmed that it really is the FHB (Figure 34).

Similarly, a one-day survey was carried out in the upper part of Trongsa dzongkhag in Nubi gewog in the first week of April 2025. The wheat growers in this area grew only the bread wheat of different local varieties. The crops were at booting stages in the majority of the sites visited.

Low incidence of leaf rust was recorded from almost all the sites, while yellow rust was found only in three of the sites surveyed. Barberry plants were also seen abundantly in all the locations. Live samples of both leaf rust and the yellow rust were collected and sent to GRRC, Denmark.

Figure 29. Leaf rust

Figure 30. Yellow rust

Figure 31. Fusarium head blight

Figure 32. Mahonia

Figure 33. Wet tissue culture

Figure 34. FHB spores under the microscope

d. Bumthang Dzongkhag

The two days of wheat disease surveillance were carried out in Bumthang dzongkhag in Tang and Chhumig gewog in the third week of May 2025. The crops were at tillering to flowering stages, depending on the varieties and the location where they are cultivated. Wheat growers in this dzongkhag cultivated only bread wheat of different local varieties. Both the incidence and the severity of leaf rust and the yellow rust were observed to be very low in all the sites visited. The severity of leaf rust was just a trace, while the severity of yellow rust was found to be much higher than compared of leaf rust. The samples of both types of rust were collected and sent to GRRC for analysis. The very low incidence of loose smuts was also recorded from some of the fields.

e. Punakha Dzongkhag

Wheat rust (Yellow rust) in this area was first reported in the second week of February 2025 on durum wheat, which was at tillering to boot stages by the gewog extension agent. Following that report, three days of wheat disease surveillance were conducted in this dzongkhag from 9th to 11th March 2025, covering all the major wheat-growing gewogs. The farmers in this area grew both bread wheat as well as durum wheat. The wheat crops, especially the bread wheat, were at tillering to dough stages depending on the varieties and the locations where they were cultivated, while all the durum wheat was at flowering stages. Both leaf rust and the yellow rust were recorded from this dzongkhag (Figures 35 & 36). Leaf rust was observed only from one of the sites in Toep gewog with moderate incidence and severity of 5% showing a moderately resistant reaction towards its pathogen, while yellow rust was recorded from almost all the sites that we have visited. The incidence of yellow rust was found to be quite high in every field, with 5% to 90% severity, with the majority of the sites showing moderately resistant to moderately susceptible reaction. Durum wheat growing farmers have sprayed their fields with fungicide (Propiconazole/Mancozeb) in the last week of February when the crops were at heading stages to control yellow rust. Alternate hosts such as barberry plants were also observed abundantly in this area.

A one-day follow-up survey was conducted in this dzongkhag in the second week of April 2025. The crops were at the milk to dough stage. This was our second visit to all the sites. Compared to the first visit, the severity of yellow rust was found to be decreased in the majority of the sites, as the farmers have already taken action to spray their fields with fungicide (Propiconazole/Mancozeb). The symptoms of yellow rust were found mostly on the spikes and awns. While the incidence of Fusarium head blight was observed to be increased in the fields cultivated, especially with durum wheat. We also have observed quite a high incidence of stem borers in almost all fields (Figure 37). Similarly, a few incidences of loose smuts were also recorded. The live samples of rust from both wheat as well as from the grass that are grown near wheat fields were collected and sent to GRRC, Denmark.



Figure 35. Yellow rust severity and the reaction of the host plant towards its pathogen

Figure 36. Leaf rust

Figure 37. Stem borer infestations

f. Wangdue Phodrang dzongkhag

The wheat disease surveillance in this dzongkhag was executed from 12th to 15th March 2025, covering all the major wheat-growing gewogs. Like the Punakha dzongkhag, people in this area also cultivated both types of wheat (bread wheat and durum wheat). The crops were at the tillering to flowering stages during the time of the survey. Both yellow rust and leaf rust were recorded from this region (Figures 38 & 39). Leaf rust was recorded only in the Baylangdra area under Kazhi gewog, while yellow rust was recorded from the majority of the sites visited. Compared to Punakha, both incidence and the severity of yellow rust were found to be quite low in the Wangdue region. Alternate hosts such as barberry were observed in all the locations, while mahonia was recorded only in Baylangdra. Surveys of barberry plants were also undertaken. Symptoms similar to Fusarium head blight were recorded from one of the sites in Gase Tshogongm gewog, where the field is cultivated with durum wheat (Figure 40). Samples were collected and brought to the office for laboratory confirmation. The laboratory result on confirmation of FHB was obtained, and it was confirmed that the field was infected with FHB (Figure 41)**.** Similar to Punakha dzongkhag, here as well, this is our second visit in the second week of April 2025. The crops were at the flowering to dough stages. The incidence and the severity of the yellow rust were reduced in the fields where the durum wheat is cultivated, as they have sprayed fungicides to control the disease. While the incidence and the severity of rust have increased in the fields cultivated with bread wheat as they have not taken any management action. We have also recorded the stem borers and the loose smuts from the majority of the sites visited (Figure 40). The live samples of rusts were collected and sent to GRRC, Denmark, for race analysis.


Figure 38. Yellow rust


Figure 39. Leaf rust


Figure 40. Loose smuts


Figure 41. Fusarium Head Blight


Figure 42. FHB spores

g. Lhuentse Dzongkhag

Wheat disease surveillance in this dzongkhag was conducted in the last week of March 2025. People in this area cultivated only durum wheat. The wheat was at the booting to flowering stages, depending on the location and the varieties cultivated. This year, we have not recorded any rust. A trace of Fusarium head blight was recorded from some of the sites visited (Figure 43). Interestingly, only mahonia is seen abundantly grown everywhere in this area, while no barberry plants were recorded in any of the sites visited (Figure 44).

Figure 43: FHB Figure 44: Mahonia

h. Monggar Dzongkhag

People in this dzongkhag have not grown any wheat this year. Therefore, wheat fields in the ARDC-Wengkhar research station were monitored from March end to the first week of April 2025. They have grown only the durum wheat in the plot. The crops were at the flowering stage. Low incidence of yellow rust was recorded, showing a severity of 5% with moderately resistant to moderately susceptible host plant reaction towards its disease pathogen (Figure 45). The samples of it were collected and sent to GRRC for analysis. Similarly, a very low incidence of Fusarium head blight was also observed (Figure 46).

Figure 45: Yellow rust Figure 46: FHB

i. Trashi Yangtse Dzongkhag

The people in this dzongkhag have cultivated both durum and bread wheat. The wheat disease surveillance in this area was carried out in the first week of April 2025. The crops were at the dough to maturing stages during the time of the survey. The low incidence of leaf rust was recorded on both kinds of wheat (Figure 47). However, the Fusarium head blight was observed only on the durum wheat (Figure 48). Live samples of leaf rust were collected and sent to GRRC, Denmark.

Figure 47: Leaf rust

Figure 48: Fusarium head blight

j. Tashigang Dzongkhag

The wheat disease surveillance in this dzongkhag was conducted in the first week of April 2025. The wheat growers in this dzongkhag cultivated both durum as well as bread wheat of various varieties. The crops were at boot to maturing stages, depending on the location and the varieties cultivated. Low incidence of both yellow rust and leaf rust was found in this area (Figure 51). The trace of Fusarium head blight was also recorded from almost all the fields cultivated with durum wheat (Figure 49). While very low incidence of loose smuts was recorded from very few sites cultivated with local wheat varieties (Figure 50). Neither barberry nor the mahonia plants were observed at any of the sites visited.

Figure 49: FHB

Figure 50: Loose smuts

Figure 51: Leaf rust

k. Haa Dzongkhag

Wheat disease surveillance in this valley was done in the second week of April 2025. Farmers in this area grew only the bread wheat of different local varieties. The crops were at the tillering stage. No disease was recorded from all the sites except for one of the sites located in Samar gewog. Very low incidence of yellow rust exhibiting moderately resistant to moderately susceptible reaction types by the host plant towards its pathogen (Figure 52). The samples were collected and sent to GRRC, Denmark, for its pathotype analysis.

The follow-up survey in this region was done on a monthly basis after the first visit. In total, we have visited four times until now. From our monthly observation, initially both the incidence and the severity of yellow rust were found to be low in every field visited, and their incidence and severity became higher each time we visited the field, infecting even the spikes (Figure 26b). While we have not observed any leaf rust in any of the sites visited until the second visit on 27th May 2025. Similar to yellow rust, both its incidence and severity increased and became much higher, and have overtaken the yellow rust in some of the places as the temperature became warmer (Figure 53). The stem rust was also recorded from one of the sites from Samar gewog on the stump of the already harvested crops. Samples of all three types of rust from the wheat, as well as from the grass grown at the border of the wheat fields, were collected and sent to GRRC for pathotype analysis. Apart from rust, a very low incidence of powdery mildew was also recorded (Figure 55). The barberry plants were recorded to be grown abundantly near every wheat field (Figure 54)

Figure 52: Yellow rust on wheat at the seedling stage and on spikes

Figure 53: Leaf rust

Figure 54: barberry plants grown near a wheat field

Figure 55: Powdery mildew

l. Paro Dzongkhag

The survey in this area was conducted in the second week of April 2025. People cultivated only bread wheat of various varieties. Crops were at their tillering to boot stages, depending on the locations and the varieties they had grown. The trace of yellow rust was recorded from a few fields. Live samples of rusts from both wheat as well as from grass grown in and near wheat fields were collected and brought to the office, which will be sent to GRRC, Denmark.

Similar to the Haa dzongkhag, follow-up in this region was done every month after the first visit. In total, we have visited three times until now. From our monthly observation, initially, both the incidence and the severity of yellow rust were found to be low in every field visited, and their incidence and severity became higher each time we visited the field. While we have not observed any leaf rust in any of the sites visited until the second visit on 27th May 2025. Similar to yellow rust, both its incidence and severity increased and became much higher, and have overtaken the yellow rust in most of the sites as the temperature became warmer. Samples of both leaf rust and yellow rust from wheat, as well as from the grass grown at the border of the wheat fields, were collected and sent to GRRC for pathotype analysis.

4.3.4 Conclusion

To summarize, all three types of rust were detected in the country in this wheat-growing season. The incidence and the severity of yellow rust were found to be highest among all, followed by leaf rust, whereas the stem rust was recorded only in one site (Figures 56, 57 & 58). Both incidence and the severity of all types of rust were observed to be increasing every year. Similarly, the Fusarium head blight disease was also detected in almost all regions on both bread wheat and durum wheat; its incidence was found to be rising each year (Figure 59). Apart from those four major wheat diseases, very low incidence of leaf spots and loose smuts was also recorded, especially on the local wheat varieties. Quite a high incidence of stemborers was also recorded from some of the s, like Punakha and Wangdue Phodrang, especially at the later stages of the crop growth.

Figure 56. Yellow rust incidence

Figure 57: Leaf rust incidence

Figure 58: Stem rust incidence

Figure 59. Fusarium head blight incidence

4.4 Disease Sample Collection and Shipment
Background

The samples of all three types of rust from the main host, accessory host, and the alternate hosts (Figure 60) were collected from various locations across the country during the time of wheat disease surveillance in the farmers' fields, as well as in the research plots and the sentinel plots annually. Collecting samples and getting them pathotyped is essential for the pathologist to determine which races of disease are present in the region, and helps in monitoring the pathogen population over the period and the area. Knowing all those helps the pathologist to provide informed advice to the wheat breeding program and develop effective and efficient disease management packages. Similarly, it also provides information on whether the alternate host is functional or not in the rust disease epidemiology.

Figure 60. Various host plants of the rust pathogen

Materials and methods

Material: Paper envelopes, scissors, pencil, 70% ethanol, cotton, cool box, unique ID codes, and cryovials.

Methods:

Sample number: There are no absolute rules, but too many samples are usually better than too few. **The aim should be to get a representative sample of the wheat-growing areas and cultivars. Any susceptible reaction on a previously resistant cultivar/line should be sampled as a priority (e.g., from trap/sentinel plots), and collecting samples from accessory hosts (grasses, other cereals, etc) and volunteer plants is also essential. Younger (upper) leaves are usually better than older leaves (i.e., more viable and greener), if possible, with clearly separated lesions are the best (Figures 61 & 62).

Figure 61: Young and lush leaf with clearly separated lesions   Figure 62: Older leaves

***All three types of rust sample collection of both live sample and dead sample collecting protocols can be found in this link *http://rusttracker.cimmyt.org/wp-content/uploads/2011/11/2013-Updated-BGRI-protocols-v2-web.pdf*

After the samples were collected following the designed protocol, it was then shipped to the Global Rust Reference Center, Denmark, Cereal Disease Laboratory, ARHS, USA, for the virulence pathotyping and genotyping.

Shipment procedure:

Required documents: Obtain a Material Transfer Agreement (MTA) from the National Biodiversity Centre; a Clearance certificate from BFDA, and the import permit from the recipient organization.

For this wheat growing season, we have collected more than 160 samples of yellow rust and leaf rust from various host plants (wheat, barley, grasses, and the barberries of different species). The collected samples were air-dried properly (Figure 63) and then sent to GRRC (Figure 64).

Figure 63. Sample drying at room temperature

Figure 64. Sample packing and shipment

4.5 Strengthening the capacity of Agriculture Extension Agents on wheat diseases

Historically, wheat was a minor crop in Bhutan, grown on a small scale compared to staples like rice and maize. Wheat diseases posed no significant concern until recent years, leaving agriculture officials, including extension staff, with limited knowledge of identification and management. To address this, the National Plant Protection Centre (NPPC), funded by WISER

AP Phase-II under the CIMMYT project, trained officials from key wheat-growing dzongkhags: Punakha and Wangdue Phodrang (West Central), Thimphu, Chhukha, Paro, and Haa (Western), and Bumthang, Trongsa, and Zhemgang (Central).

The training was conducted at four locations: 1) Punakha for Punakha and Wangdue extension agents, 2) Paro for Thimphu and Chhukha, 3) Haa for Haa and Paro extension agents, and 4) Bumthang for the extension agents of Bumthang, Zhemgang, and Trongsa dzongkhags. Of the total 77 participants, 38 were male and 39 female (Figure 65). The two-day trainings consisted of theoretical sessions on the first day were both pre- and post-tests were carried out in order to assess the knowledge of the participants in all the locations. In all the sites, the theoretical sessions on the identification of the major and the emerging wheat diseases were delivered, followed by their management practices. They were also trained on how to collect data on wheat diseases using ODK, sample collection procedures of all types of diseases of both live and dead samples, including the sample collection protocols of both live and dead samples of its alternate host, such as barberry. The awareness of wheat blast diseases prevalent in the neighboring country was created and trained to look for its symptoms and its management should they come across such a disease in the future. They were also updated on the status of all wheat diseases in the country recorded from the last and this season's survey findings. Similarly, the information on all the activities that the National Plant Protection Centre, in collaboration with the CIMMYT project, including information on the funding support, activity status, and our achievements, was shared with them.

The second day session involved hands-on training in the wheat fields located near the training venue (Figure 66). In all the locations, extension agents were divided into groups and tasked to carry out wheat disease data recording using all the knowledge that they had gained from the first day training session. Group presentation of their findings from the field visit and what they had learnt from these two days' training were also included (Figure 67).

Observation:

From the recaps and the group presentation made at the end of the training, the results were quite impressive. They all did a very good job in summarizing what they have learned on day one and also did a nice presentation, demonstrating that they can collect data using ODK, able to identify all the key wheat diseases, their reaction types, scoring on disease incidence and its severity, sample collection protocols and what needs to be included on the sample envelopes while sending rusts samples. They also understood how those diseases should be managed, and everyone expressed that this training is very useful and informative for them, especially the practical session. From the observation of the pre- and post-test data, it also showed that they have learned a lot from this training program.

Figure 65. Group images of the participants at different locations

Figure 66. Practical sessions in the field at different locations

Figure 67. Participants working in groups and doing presentations on their findings

4.6 Awareness Campaigns on key wheat diseases and their management practices to the farmers of key wheat-growing areas

Wheat diseases such as rust diseases, especially the yellow rust and other diseases like fusarium head blight is an increasingly becoming a concern among wheat growers. From our observations through annual general wheat disease surveillance in the country, there has been an increased incidence and the severity of wheat diseases in recent years, causing a reduction in wheat productivity. Since both farmers and the agriculture extension agents have very limited knowledge of those diseases and their management practices. The National Plant Protection Centre, in collaboration with funding support from WISER AP Phase II subgrant under the CIMMYT project, decided to carry out the one-day wheat disease awareness workshop for the farmers of major wheat-growing areas. The awareness workshops were created at four locations in Gatse Tso Gom under Wangdue Phodrang, Barp gewog under Punakha, and Tang and Chhumig gewogs under Bumthang dzongkhag (Figure 68). There were 151 participants in total, where 104 were female participants and 47 were male participants. In all the locations, the talk on the importance of rust diseases and how the impact of rust disease management practices in the country contributes to the global wheat disease epidemiology. We also explained how our country plays a significant role in wheat rust disease epidemiology due to the presence of various species of barberry plants in the country, grown everywhere starting from the mountains to the banks of the rivers. They were familiarized with the symptoms of diseases such as rust (yellow rust, brown rust, and stem rust) and the fusarium head blight, and how those diseases affect their crop productivity. The participants were also taught how the disease should be managed using IMP technology and were advised to use fungicides as a last resort to control diseases. In addition to that, awareness of wheat blast disease was also created, as it is already prevalent in Bangladesh. They were advised to be vigilant and informed to report immediately to their respective extension agent if they come across symptoms similar to those of blast. We also demonstrated how deadly this disease can be and taught them how this disease should be managed if they encounter it in any of their field in the future. The awareness workshop ended with the question-and-answer sessions and had some discussions at the end.

Observations:

In all the locations, farmers were found to be interested in the short-duration wheat variety. Some also raised concerns about yellow rust infection increasing each year and requested disease-resistant varieties. Some raised issues about loose smuts in their fields, especially those cultivated with local varieties.



Figure 68. Images of participants (farmers) at different locations

4.7 Capacity building of Plant Protection officials on barberry work

The National Plant Protection Centre (NPPC), Semtokha, in collaboration with the CIMMYT project, organized five days of training on barberry surveys and the portable method for isolating cereal rusts from the alternate hosts. The training was attended by 25 participants in total, including the three experts, Dr. Dave Hodson from the CIMMYT regional office, Nepal, Dr. Maricelis from Cornell University, USA, and Dr. Yue Jin from USDA, ARS, Cereal Disease Laboratory (Figure 69). The majority of the participants were from the NPPC, while a few of them were from the Agriculture Research and Development Centers of four different regions.

The training held in a mixed-mode session, and it consisted of both theory as well as practical sessions in the field and the laboratory. Three-day field surveys were conducted along the Thimphu, Paro, and Haa Valleys, during which the participants were trained on how to carry out barberry surveys in the field using ODK applications, identification of aeciospores and their collection procedures, and how they should be handled after collection. The participants surveyed barberry bushes and the wheat fields using ODK and collected multiple samples of aeciospores and the wheat rust samples (Figure 70). The experts also taught how the samples of yellow rust and stem rust should be collected in the RNAlatter, which will be used for the MARPLE Diagnostics, and how they should be labeled and stored after reaching the laboratory. Two days of barberry work in laboratory training were very intensive. Dr. Dave lectured on the overview of wheat disease surveillance and early warning, including the MARPLE Diagnostics, while Dr. Maricelis gave stressed on the importance of barberry and barberry testing protocols. On the other hand, Dr Yue Jin gave a virtual talk on the importance of barberry in wheat disease epidemiology, followed by discussions after every session. The practical session included treating plants with melic hydrazide (MH), inoculation of spores on the wheat plants in the dew chamber, followed by transfer of inoculated plants from the dew chamber to the incubation chamber (Figures 71 & 72).

Figure 69: Group image of training participants

Figure 70. Barbarry survey in the field

Figure 71. Selecting the best aeciospores for inoculation

Figure 72. Hands-on training in the laboratory

4.8 Establishment of MARPLE Diagnostics Tools and Capacity building of Plant Protection and Research officials on MARPLE Diagnostics tools

The National Plant Protection Centre (NPPC), Semtokha, in collaboration with the CIMMYT project, established a MARPLE Diagnostics Laboratory in the centre and organized a four day training for plant protection and the research officials on MARPLE Diagnostics, starting from sample collection from the field to MinION sequencing, followed by basecalling and bioinformatics pipeline in the laboratory. There were 25 participants, including three experts, Dr. Dave from the CIMMYT Regional office, Nepal; Dr. Diane and Dr. Roland from the John Innes Centre (JIC), UK (Figure 73).

The training consisted of both theory as well as practical sessions in the laboratory (Figure 74).

The experts gave a lecture on each step of the protocols to run MARPLE, followed by a practical session, by dividing participants into two groups. The participants were briefed on the introduction to the MARPLE. "MARPLE" is a **M**obile **A**nd **R**eal-time **PL**ant dis**E**ase diagnostics, a technique for quickly identifying individual strains of the wheat yellow rust and wheat stem rust pathogens. Strains that are new to an area have the potential to be much more damaging than strains that have been present in the past, so identifying them as fast is key to protecting wheat crops. The experts explained each stages of the diagnostics followed by hands on sessions such as Sample collection from the field in RNAlatter solution that preserves nucleic acid integrity (Figure 75), DNA extraction of those samples using magnetic beads, DNA amplification and assessing DNA quality using gel and dye, preparation of library after knowing the quality of the DNA to select all the best DNA in the library, MinION sequencing flowed by basecalling and MARPLE bioinformatics pipeline, which will create phylogenetic trees and helps to explore the genetic relationship between current samples and the already known isolates of yellow rust or stem rust.

Figure 73. MARPLE Dianostics training participants

Figure 74. hands on training on Marple diagnostics

Practical sessions:

For this training, eight samples from four sites were used, two samples each from every site for running MARPLE Diagnostics:

i. Sample id BTN20251156 & BTN20251155 - Ge-Nyenkha, Thimphu
ii. Sample id BTN20251169A & BTN20251169B- Pangsundhima, Samar, Haa
iii. Sample id BTN20251142A & BTN20251142B- Susuna, Nagya, Paro
iv. Sample id BTN20251184A & BTN20251184B- Jangkhakha, Bji, Haa

After assessing the DNA quality, only six samples were selected for further processing. The MinION loading and gene sequencing were done as the training went on, and the results were as shown in the figure below after completing the MARPLE informatics pipeline (Figures 76 & 77).

Figure 75. Rust sample collection from the field in RNAlatter for MARPLE training

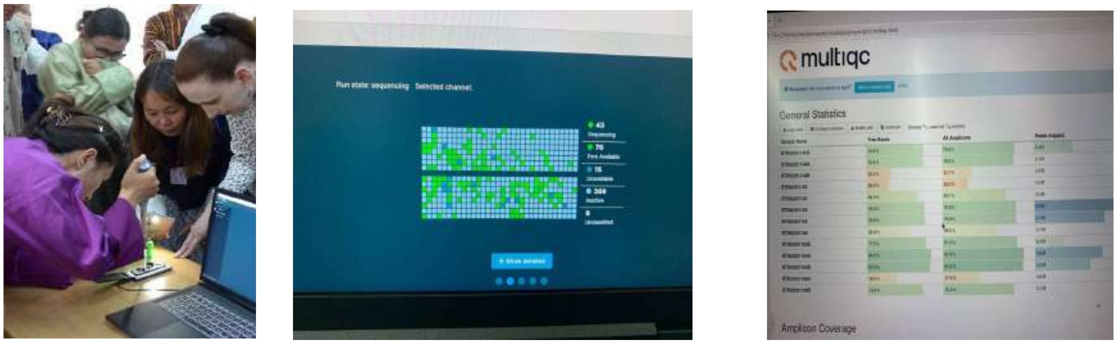


Figure 76. MinION loading, running gene sequencing, and Multiqc results

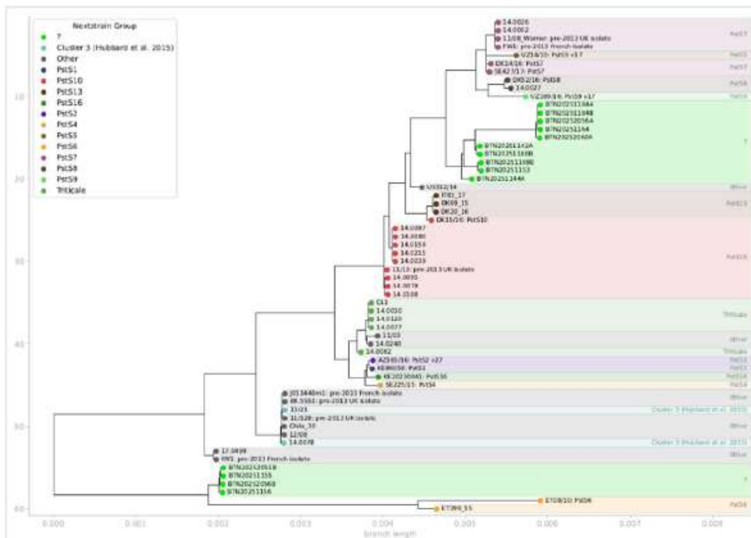


Figure 77. Sequenced DNA results

4.9 Post-training Laboratory work on the rest of the samples

Post training session, the staff of NPPC tried running the MARPLE diagnostics on the remaining samples (Figure 78). As per the training, eight samples were taken to run the MARPLE. Two samples from each place (Wangbama, Ge-Nyenkha, Thimphu with sample id BTN20251153 & BTN20251152; Norbugang, Samar, Haa with sample id BTN20251144A & BTN20251144B; Drugyel, Paro with sample id BTN202564 & BTN20251163; Doomchhog, Haa with sample id BTN20251188A & BTN20251188B). All the procedures were followed as instructed by the experts or mentioned in the MARPLE Diagnostics manual. After evaluating the DNA quality, two samples with id code BTN20251188A and BTN20251163 were left out as these samples had poor-quality DNA, and could not make it to MinION sequencing. After completing the sequencing and the bioinformatics pipeline, their results were as shown in the image below. Only four samples made it to the phylogenetics tree, one sample each from every place. While three could not be considered as the three bases of those, all three samples were below 40%. Therefore, the system automatically removed them from the pipeline.

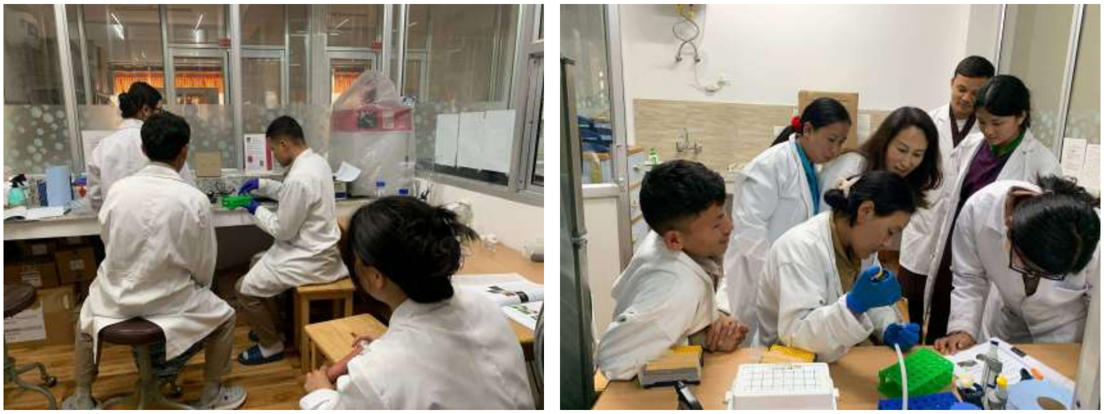


Figure 78. Post-training work on MARPLE Diagnostics by the Pest Surveillance & Pathology team

4.10 Production or publication of Extension materials/Leaflets

As a part of the CIMMYT project, the National Plant Protection Centre, Semtokha produced and printed 206 copies of a booklet "A Pictorial Guide for Field Identification of Wheat diseases" and 206 copies of the Rust Scoring Guide published by the CIMMYT office (Figures 79 & 80). These printed booklets were distributed to the plant protection officials of the centre, regional PP officers, agriculture research officers, wheat program officials, and the agriculture extension agents at the time of wheat disease awareness workshops and the training. These booklets produced will help agriculture officials to identify wheat diseases in the field during the time of wheat disease surveillance and help them to provide their management practices to the wheat-growing farmers if they record any diseases in the field during the time of surveys.

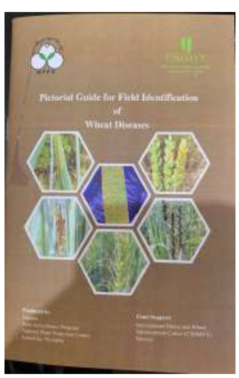


Figure 79. Pictorial booklet

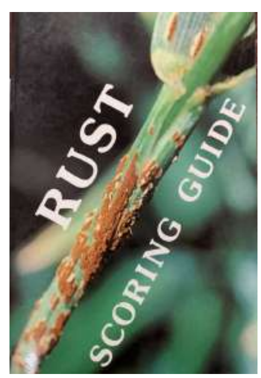


Figure 80. Rust scoring guide booklet

4.11 Wheat Disease Information Dissemination

With the increase in the number of wheat growers in the country and due to changes in the climate conditions, both the incidence and the severity of wheat diseases, especially the rust, were observed to be increasing each year. Similarly, there were also repeated reports of the problem of severe rust infection in the farmer's field. Therefore, the National Plant Protection Centre, in collaboration with the CIMMYT project, started to carry out wheat disease early warning during the whole wheat growing season to minimize crop loss due to rust infection.

The wheat rust disease forecasting was done weekly, starting from the start of the early wheat growing season till the wheat crops were harvested. The information was disseminated among all the agriculture officials throughout the country. Email and the NPPC web portal are the main channels through which the information was disseminated (Figure 81).

During the time of forecasting, the information on wheat rust spore deposition in the specific place and its infection efficiency was forecasted (Figures 82, 83 & 84). In addition to this, the information on the weather, such as precipitation and the temperature, was also disseminated (Figures 85 & 86). Having access to all that information will aid one in knowing whether there will be chances of rust infection in their respective regions, and it will help them to act in advance if there is the slightest chance of rust infection, which will ultimately help them to reduce crop losses.

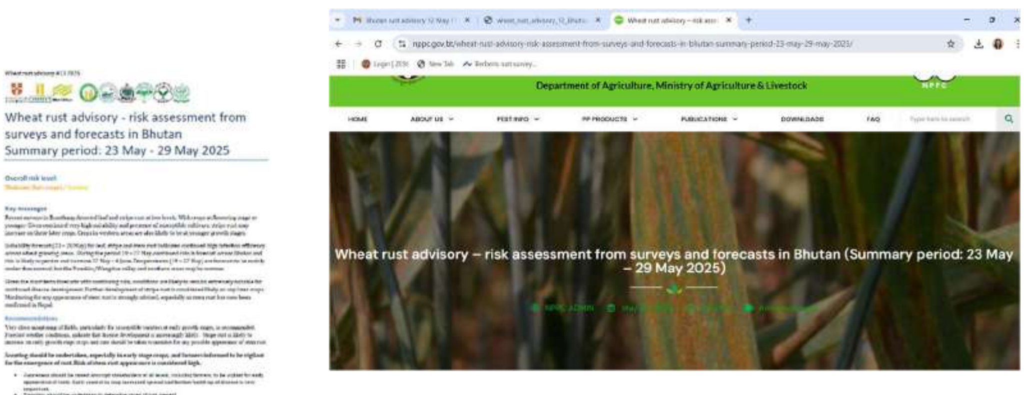
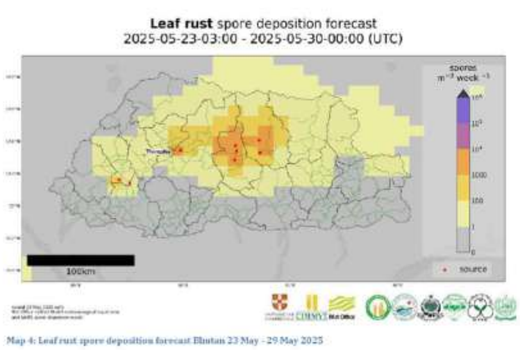


Figure 81: Information dissemination through email and NPPC web portal



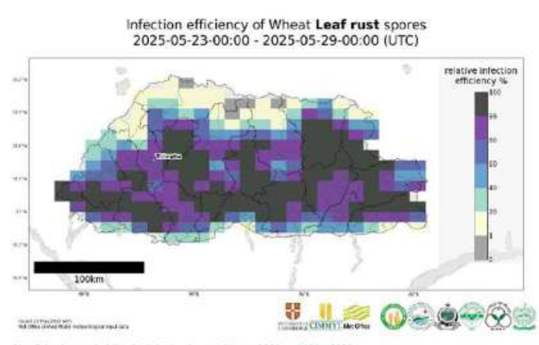


Figure 82: Leaf rust spore deposition and its infection efficiency forecast

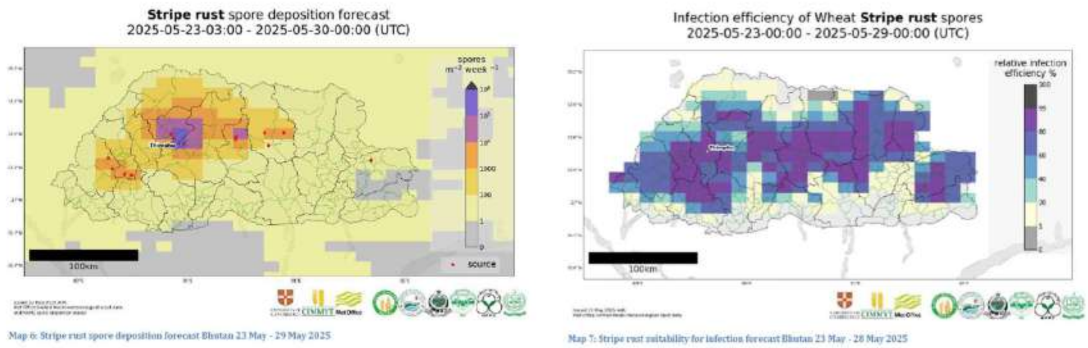


Figure 83: Yellow rust spore deposition and its infection efficiency forecast

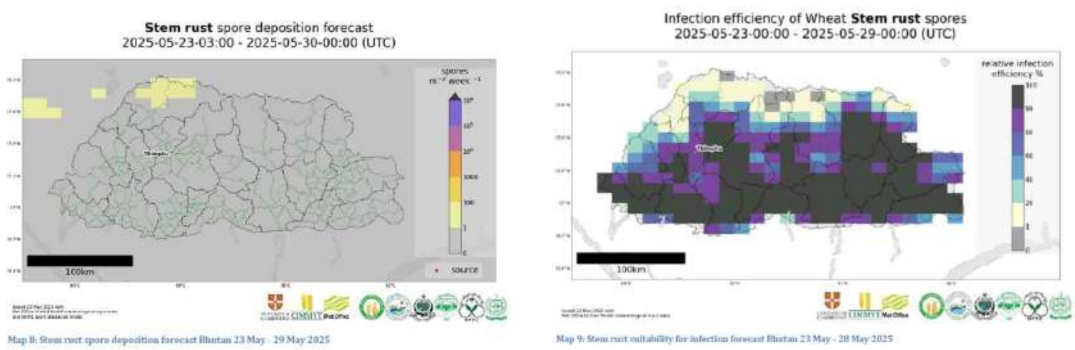


Figure 84: Stem rust spore deposition and its infection efficiency forecast

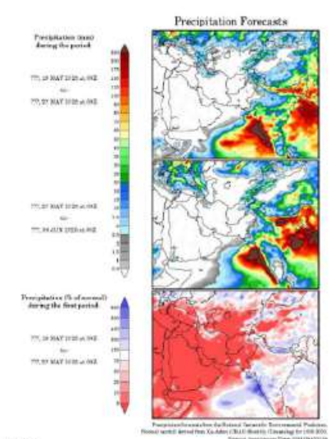


Figure 85: Precipitation forecast

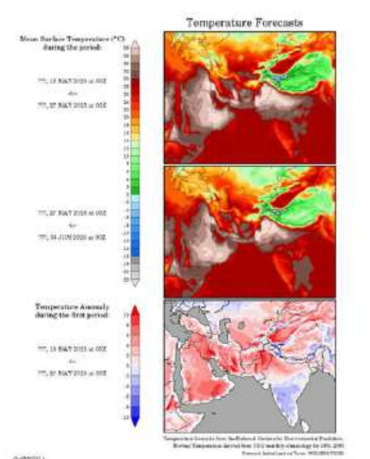


Figure 86: Temperature forecast

4.12 WISER AP Phase II Launch Meeting

The National Plant Protection Centre, in collaboration with the CIMMYT project, has organized a WISER AP Phase II meeting in the country. A three-day meeting was attended by around 15 members, including one member each from Bangladesh, Nepal and from the donor agency; four from the CIMMYT project, and the rest from Bhutan (Dzongkhag Agriculture offices, Extension office, Department of Agriculture, Agriculture Research Development Centre) and from the National Plant Protection Centre (Figure 87). For the first one ana d half day, the head of the project, Dr. Dave introduced the project and its objectives of the meeting; followed by an overview of the WISER AP Program by the program manager of the donor agency.

Dr. Dave highlighted the current achievements of the program in phase I and outlined the WISER AP Phase II. The session was followed by the updates from all three partner countries (Bhutan, Bangladesh, Nepal, and the team from the CIMMYT project) on phase I and the way forward focusing on the achievements, what worked well, the challenges, lessons learned, and the way forward. The team also made reflections on what had been done and collected feedback/inputs or guidance from the national stakeholders on the current status, what they would like to see achieved in phase II of the program (Figure 88). On the second day of the meeting, all the country partners worked on the work plan for phase II and did individual presentations on the outline of the proposed activities. On the third day, the team visited ARDC-Bajo (Figure 91) and visited some wheat growers to get their input on the activities to be carried out in phase II (Figures 89 & 90).

Figure 87: Group image of the participants

Figure 88: Meeting and discussions

Figure 89: Field visits and discussions

Figure 90: Meeting with farmers

Figure 91: At ARDC-Bajo

5. Plant Protection Product Program

The program has initiated the implementation of the online PPP-IMS system for the fiscal year 2024-2025 to manage the indent collection and distribution of Plant Protection Products. The program receives indents from various stakeholders, including farmers, public farms, and government agencies such as research centers and sub-centers using PPP-IMS. Following the indent, tender is floated to the eligible local suppliers for supply of the PP Products. The PPP are supplied to NPPC by the eligible suppliers. The gewog extension agents lift the products from NPPC and supply to the farmers as per their indented demand.

5.1 Protection (PP) Products Indents and Procurement

The Program has procured a total quantity of 39.50MT of insecticides, fungicides, and bio-pesticides, 820.24MT of herbicides, and 11,011 traps and lures worth approximately Nu.53.365 million as shown in ***Table 1***. The budget for these procurements has been utilized from the revolving fund secured under the RGOB source.

Table 1: Quantity of PP Products procured worth in Millions (Nu.)

S. N	Product types	Quantity (L/Kg)	Nu. in Million (M)
1	Insecticide	12267.4 L	6.23M
2	Fungicide	18784 Kg	8.16M
3	Herbicide	820238 Kg	11.70M
4	Butachlor	810000 Kg	23.56M
5	Bio-Pesticide	8458 L	2.42M
6	Traps/Lures	37181 numbers	1.26M

5.2 PP Product Distribution

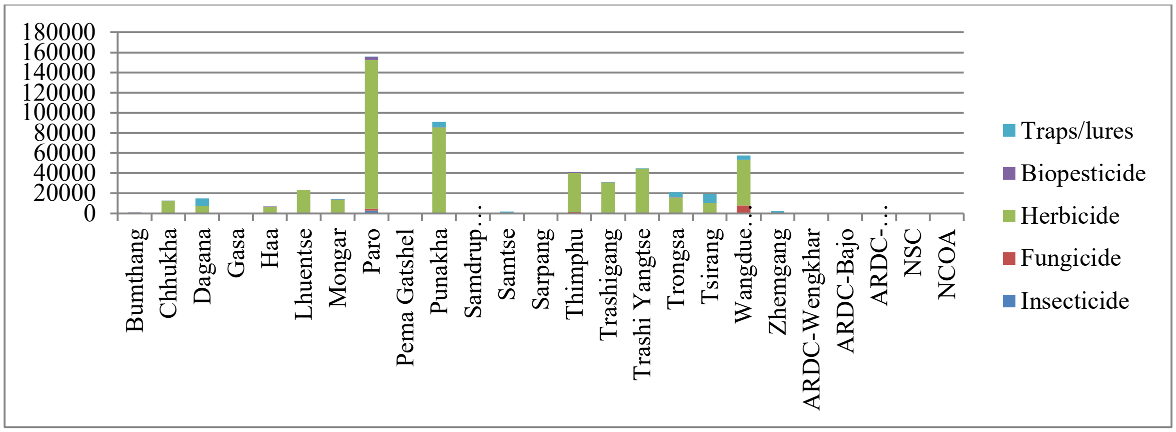


Figure 1. Overall, Dzongkhag-wise and central agencies' PP products distribution

Herbicides dominate pesticide usage. Paro, Punakha, and Wangdue Phodrang are the most active regions in terms of pesticide use, whereas Gasa and Sarpang report zero use of pesticides.

Insecticide distribution

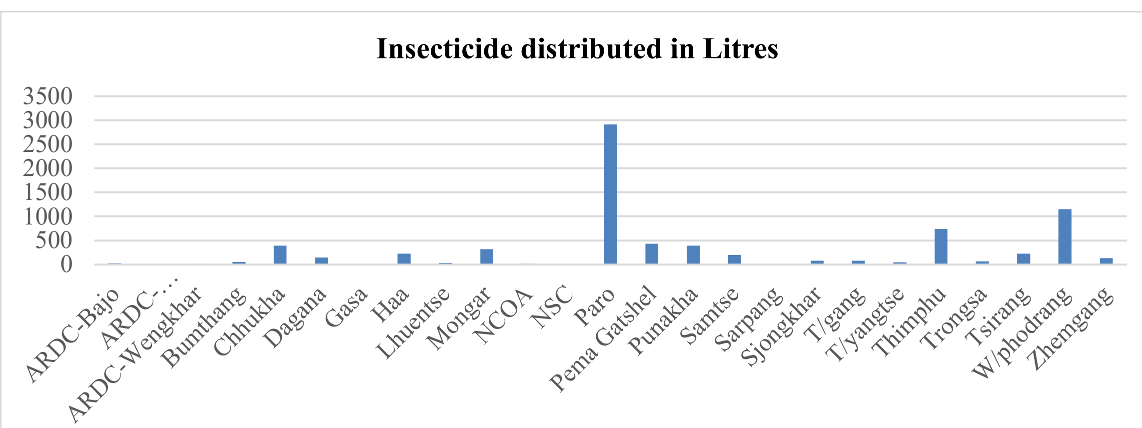


Figure 2. Insecticides distribution

A total of 7699.04 liters (85601 bottles/packets) of insecticide have been distributed across 20 Dzongkhags. Paro stands out with the highest distribution, close to 30,481 bottles**,** indicating a major use or demand for insecticides. Wangdue Phodrang and Thimphu follow next, each with around 12,145 and 7,914 bottles, respectively.

Fungicides distribution

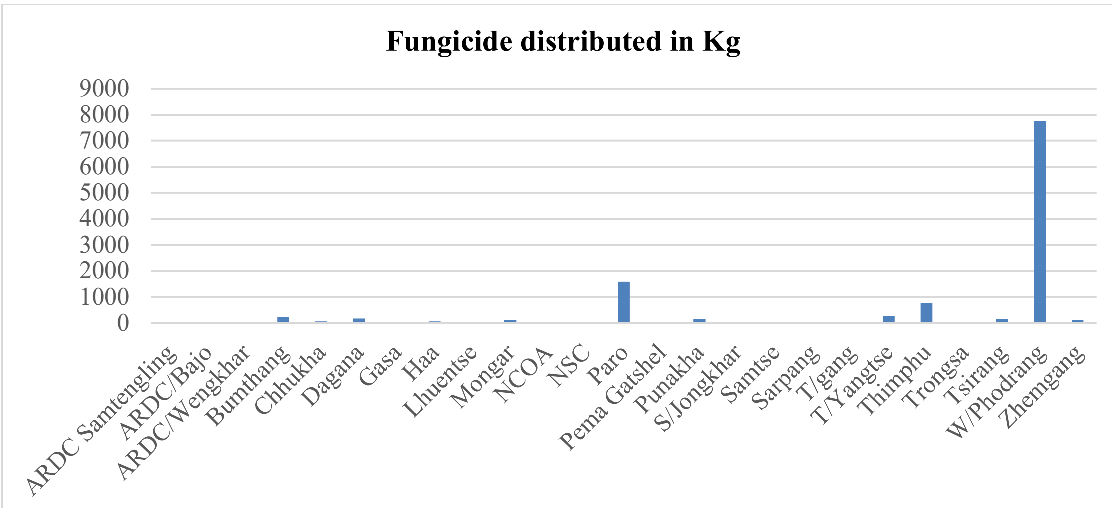


Figure 3. Fungicides distribution

A total of 11626.35 kg (48637 packets) of fungicides was distributed across 20 Dzongkhags. Wangdue Phodrang led with 30,959 packets, followed by Paro with 6,570 packets. Zhemgang recorded the lowest usage.

Herbicide distribution

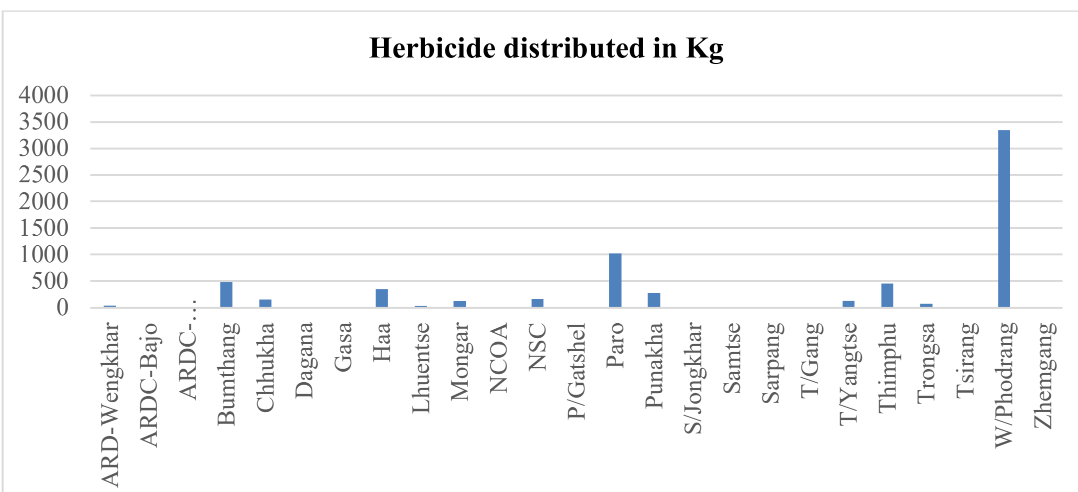


Figure 4. Herbicide distribution

A total of 6685.05 Kg (35016 packets/bottles) of herbicides was distributed across 20 Dzongkhags. The highest quantity was distributed to Wangdue Phodrang dzongkhag with a total quantity of 14723 packets, followed by Paro with a total quantity of 8423 packets, while Gasa, Samdrup Jongkhar, Samtse, Sarpang, and Zhemgang have not used any of the fungicides.

Butachlor *5G* Distribution

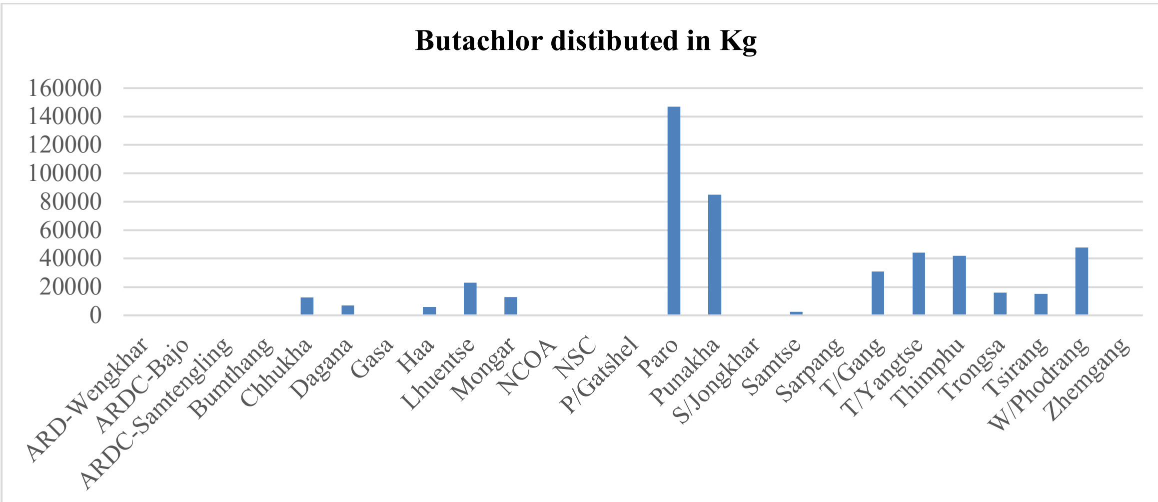


Figure 5. Butachlor 5G distribution

A total of 492050 Kg (49205 bags[3]) of Butachlor 5G was distributed across 20 Dzongkhags. Paro led the highest consumption of Butachlor 5G with 14,700 bags, followed by Punakha Dzongkhag with 8500 bags of Butachlor 5G.

Bio-pesticide distribution

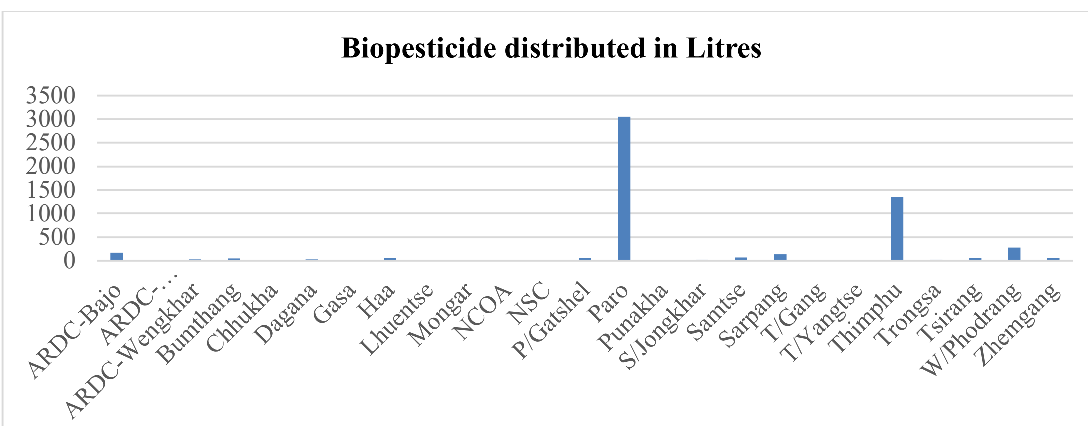


Figure 6. Bio-pesticide distribution

[3] 1 bag=10kg

A total of 5430.75 liters (9066 bottles) of bio-pesticide, including traps/lure, have been demanded. Paro has lifted the highest quantity of Bio-pesticide, followed by Samtse and Thimphu.

Traps/Lures distribution

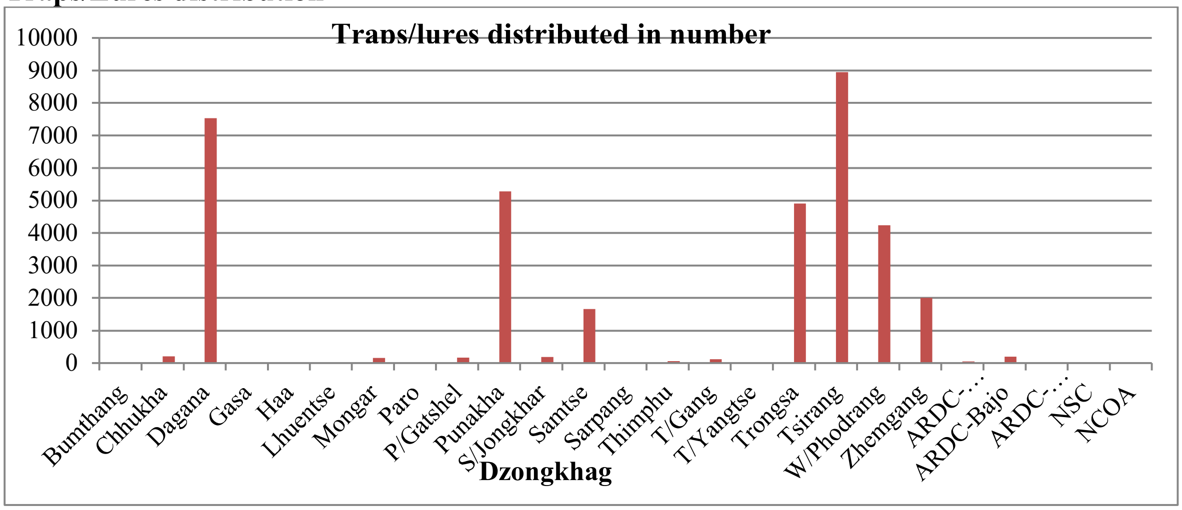


Figure 7. Traps/Lures distribution

A total of 35769 numbers of traps/lures were distributed for 20 Dzongkhags. The highest was distributed in Dagana with 7,527 numbers, followed by Punakha with 5280 numbers.

Conclusion

The procurement and distribution of PP products have increased compared to the previous year, which could be due to the occasional outbreaks of pests and diseases. Additionally, labor shortages may have encouraged farmers to opt for chemical applications to control pests and diseases, particularly weeds, as Integrated Pest Management (IPM) can be labor-intensive and time-consuming.

However, the Centre has initiated timely awareness programs on the environmental and public health impacts of the continual use of chemical pesticides. Such a program may encourage the farmers to opt for IPM, which is the key component of Good Agricultural Practices, the crucial standard for the international trade of agricultural products.

6. Bio-control Program

6.1 Isolation and Identification of Entomopathogenic Fungi from Fall Armyworm (*Spodoptera frugiperda*) in Bhutan

6.1.1 Introduction

The Fall Armyworm (*Spodoptera frugiperda* J.E. Smith) is a highly destructive polyphagous pest native to the Americas but now established across Africa, Asia, and Oceania (Goergen et al., 2016; Day et al., 2017). In Bhutan, the pest was first detected in 2019 and has since become a recurring threat to maize production, with outbreaks causing significant economic losses and food security concerns in major maize-growing regions (DoA, 2019). Its voracious feeding habit, high reproductive potential, and ability to adapt to diverse agro-ecological conditions make management challenging (Early et al., 2018).

While chemical insecticides have been the primary control strategy, their indiscriminate use can lead to pest resistance, non-target effects, and environmental contamination (Prasanna et al., 2018). Consequently, there is increasing interest in eco-friendly and sustainable pest management approaches, including biological control using entomopathogenic microorganisms. Entomopathogenic fungi (EPF), such as *Metarhizium anisopliae*, *Beauveria bassiana*, and *Nomuraea rileyi* (syn. *Metarhizium rileyi*), have been reported to infect *S. frugiperda* larvae under both laboratory and field conditions in different parts of the world (Garcia et al., 2011; Souza et al., 2019; Kumela et al., 2019).

These fungi invade the insect host via direct penetration of the cuticle, producing enzymes such as chitinases, lipases, and proteases, and ultimately killing the host through nutrient depletion, toxicosis, and tissue destruction (Vega et al., 2012). Besides their efficacy, EPFs are compatible with other IPM components and have minimal environmental impact, making them promising candidates for inclusion in sustainable FAW control programmes (Faria & Wraight, 2007).

In Bhutan, preliminary field observations have documented natural mortality of FAW larvae possibly associated with fungal pathogens (Dorji et al., 2023), but no systematic study has been undertaken to isolate and identify the causative EPF species. Isolation and identification of native EPFs is crucial for developing locally adapted biocontrol agents with high environmental fitness and pathogenicity against FAW under Bhutanese agro-climatic conditions.

6.1.2 Materials and Methods

Field Sample Collection

Naturally infected FAW larvae were collected from infested maize fields in Tading, Samtse dzongkhag (26°54'8"N, 89°16'16"E, 3,210 m asl). The larvae showed characteristic symptoms of fungal infection, including mummified bodies with visible powdery fungal growth (white or greenish). Samples were collected using sterile forceps and placed in sterile vials. They were transported to the laboratory in a cool box to preserve the viability of the fungal agents until further processing.

Figure 1: FAW larva showing visible symptoms of fungal infection (visible powdery fungal growth)

Surface Sterilization

Collected larvae were surface sterilized to remove external contaminants. Each larva was immersed in 70% ethanol for 30 seconds, followed by a 1-minute soak in 0.1% sodium hypochlorite. The larvae were then rinsed 3–4 times with sterile distilled water and dried using sterile filter papers in a laminar flow cabinet.

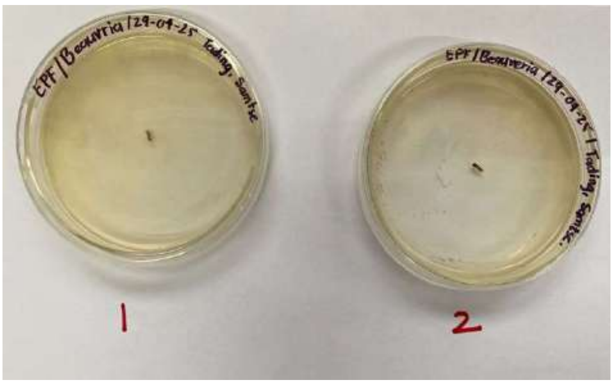
Preparation of PDA Medium

The culture medium was prepared by dissolving 39 g of Potato Dextrose Agar (PDA) powder in 1 L of distilled water. The solution was autoclaved at 121°C for 15 minutes. Once cooled to approximately 45–50°C, lactic acid (1 mL per 100 mL PDA) was added to inhibit bacterial contamination. The PDA was then poured into sterile Petri dishes inside a laminar airflow cabinet and allowed to solidify.

Figure 2. Laboratory staff pouring sterilized Potato Dextrose Agar (PDA) into Petri dishes under aseptic conditions for culturing entomopathogenic fungi.

Fungal Isolation and Plating

Under aseptic conditions, surface-sterilized larvae were cut into 2–3 segments using a sterile scalpel and forceps. These segments were placed onto the prepared PDA plates and gently pressed to ensure good contact. The plates were sealed with parafilm and incubated at 27°C to promote fungal growth.



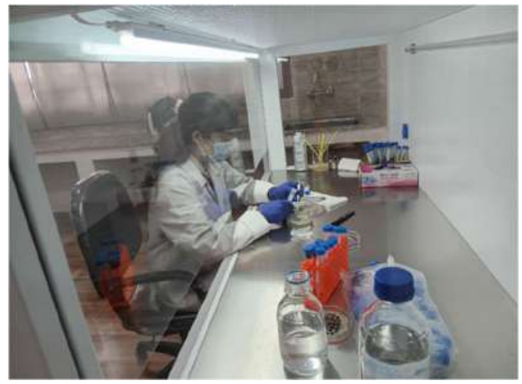


Figure 3. Laboratory staff culturing FAW larvae on Potato Dextrose Agar (PDA) media under sterile conditions (left); FAW larval segments placed on Potato Dextrose Agar (PDA) in a Petri dish for fungal isolation (right).

Fungal Growth and Subculturing

Initial fungal growth from larval cadavers was observed within 5–7 days. Hyphal outgrowths were subcultured onto fresh PDA plates using a sterile inoculating loop to obtain pure fungal colonies. These were incubated further to allow sufficient growth and to facilitate the isolation of distinct fungal morphotypes.

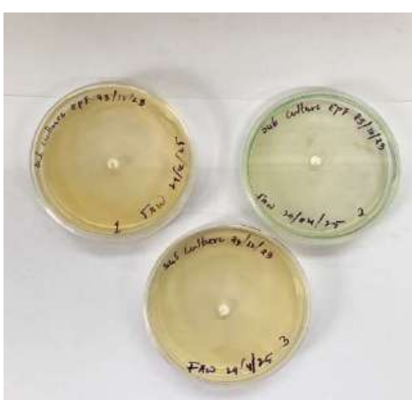


Figure 4. Lab staff sub-culturing fungal isolate (right); Fungal growth emerging from FAW larval segments on Potato Dextrose Agar (PDA) after incubation.

Microscopic Examination and Identification

A small portion of the fungal colony was mounted on a glass slide using lactophenol cotton blue stain. Microscopic observations were made under 40X and 100X magnification to identify morphological characteristics such as spore coloration, mycelial structure, and texture. Preliminary identification was based on morphological cues: *Beauveria* spp. showed white cottony growth, and *Metarhizium* spp. exhibited greenish conidia.

6.1.3 Results and Discussions

Two fungal isolates, *Metarhizium* spp. and *Beauveria* spp., were successfully recovered from infected FAW larvae and identified based on colony morphology and microscopic characteristics

Metarhizium spp.

Figure 5. Colony growth of *Metarhizium* spp. on Potato Dextrose Agar (PDA) medium

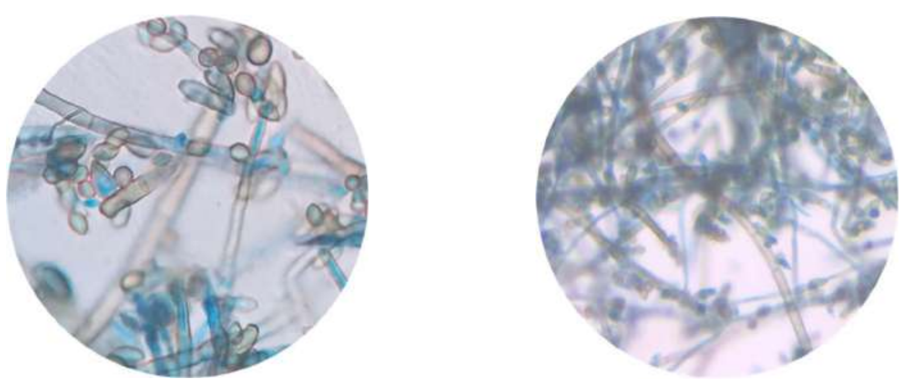


Figure 6. Microscopic features of *Metarhizium* spp. showing septate, hyaline hyphae (40x, left) and oval-shaped conidia (100x, right)

***Beauveria* spp.**

Figure 7. Colony growth of *Beauveria* spp. on Potato Dextrose Agar (PDA) medium

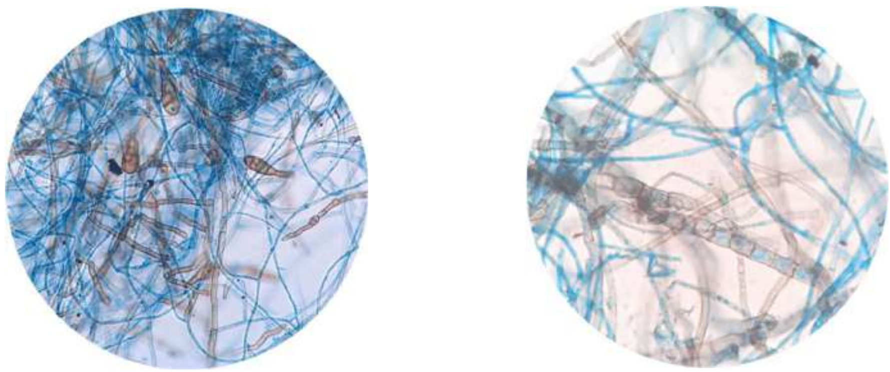


Figure 8. Microscopic features of *Beauveria* spp. showing septate, hyaline hyphae (10x, left), erect, branched conidiospores with round to conidia (100x, right)

Morphological examination confirmed the isolation of entomopathogenic fungi *Metarhizium* spp. and *Beauveria* spp. from naturally infected FAW larvae collected in Tading under Samtse dzongkhag. *Metarhizium* formed green powdery colonies with elongated conidia in chains, while *Beauveria* exhibited white cottony growth with oval conidia. These native EPF isolates offer promising potential for development as biological control agents in integrated pest management (IPM) programs targeting FAW in Bhutan

6.1.4 Conclusion

The successful isolation and identification of *Metarhizium* spp. and *Beauveria* spp. from naturally infected *Spodoptera frugiperda* larvae in Tading, Samtse, represents the first documented recovery of these native entomopathogenic fungi from FAW in Bhutan. The distinct morphological traits of green powdery colonies with elongated conidia in *Metarhizium* and white cottony colonies with oval conidia in *Beauveria* confirmed their identity. Given their known pathogenicity against lepidopteran pests worldwide, these native isolates hold strong potential as locally adapted biocontrol agents. Future research should focus on pathogenicity assays, mass production protocols, and field efficacy trials to integrate these EPFs into sustainable FAW management strategies under Bhutanese agro-ecological conditions.

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7. Vertebrate Pest Management Program

7.1 Portable Electric Fencing (PEF) Monitoring

7.1.1 Introduction

With financial support from the Bhutan for Life Project, monitoring was conducted between 19 April and 25 May 2025 at 33 sites across Bhutan to evaluate the effectiveness of Portable Electric Fencing (PEF) in mitigating wildlife incursions into farmlands. The activity covered Bumthang, Lhuentse, Trashi Yangtse, Samdrup Jongkhar, Zhemgang, Trongsa, Monggar, Trashigang, Sarpang, Dagana, Tsirang, Wangdue Phodrang, Haa, and Paro. The primary objectives were to assess the performance of installed PEF systems, provide maintenance where necessary, and document both successes and challenges experienced by farmers and local communities.

7.1.2 Installations Conducted During the Period

In Ngangla under Zhemgang dzongkhag, a PEF of approximately 1,211 metres was installed, protecting around 17.46 acres of land for seven households. Major crops cultivated include maize and buckwheat, with new orchards being established under the Million Fruit Tree initiative. In Bjoka, Zhemgang, approximately 800 metres of PEF now protect 4.6 acres of maize and citrus plantations, with porcupine damage identified as the main issue. Here, farmers have agreed to develop community bylaws to ensure standardized fence management.

7.1.3 Performance of PEF in each Dzongkhag

In Tsakaling under Patshaling Gewog of Tsirang dzongkhag, a 0.65 km PEF installation required no maintenance since commissioning, enabling uninterrupted crop protection. Similarly, in Khotokha under Wangdue Phodrang dzongkhag, a 0.6 km fence remained fully functional and in good condition throughout the year, requiring no repairs. These sites serve as models for effective installation and community stewardship.

In Sarpang, the Chhudzom Gewog site (1.2 km) protecting about 14 acres received minor wire and gate tightening, while the Singye Gewog site (1 km protecting 15 acres) remained effective despite ongoing primate pressure. In Samdrup Jongkhar, Martshala Gewog’s Raushing Chiwog (1.3 km) successfully deterred elephants for the first time, while Suzoong chiwog of Orong gewog (1 km) underwent minor wire and gate repairs. In Lauri Gewog’s Woongthig site (1 km), farmers reported marked declines in crop damage after the installation of the PEF.

Trongsa’s Tangsibje Chendenbji site proved effective against most vertebrates but continued to face primate challenges. In Nubi Gewog, the fence loop remains incomplete, necessitating additional materials such as ring insulators, MS flat iron poles, and poly wires. In Bumthang, Tang’s 1 km fence reduced the need for night guarding, while in Ura, a similar installation recorded zero crop loss since setup.

Monggar’s Drepoong site (1.3 km protecting 20 acres) underwent minor tightening and gate reinforcement, while Dramedtse’s 1 km fence reported no damage. Chali’s installation remained effective, Silambi’s 1.2 km fence maintained strong performance, and Saling’s 1.1 km protected 15 acres successfully. In Lhuentse, Khoma’s fence fault was traced to tree-cutting near the line and promptly rectified, Kurtoed's 0.4 km system remained effective, and Tsaenkhar's 1.2 km fence recorded no incursions.

In Trashi Yangtse, Tshergom (Boomdeling) maintained 2.1 km of fencing with high satisfaction, while Gangkhardoong's 0.371 km protected about 4 acres and underwent minor adjustments. Toedtsho's Gomla Poktor (1 km) and Yalang's Loongkhar and Bunangzor (1 km each, requiring a three-hour trek from the road) remained functional, though the latter requested reinforcement materials.

In Trashigang's Kangpar Gewog, Drupkhang's 1 km fence secured about 10 acres after minor adjustments, while Drowachanma's fence experienced occasional primate breaches. In Dagana's Nichula Gewog, the 1.3 km installation was effective against boar, deer, and elephants, though monkeys continued to bypass the barrier.

7.1.4 Outcome

The monitoring confirmed substantial reductions in wildlife incursions across almost all sites, with several reporting zero crop loss. The introduction of PEF significantly reduced the need for night guarding, freeing farmers to focus on agricultural activities. Sites such as Tsakaling and Khotokha highlighted the long-term reliability of well-installed systems, requiring no maintenance over the monitoring period. Most maintenance issues encountered were minor, involving wire tensioning or gate fastening, and the most requested reinforcements included ring insulators, MS flat iron poles, and poly wires. While the system proved highly effective against most wildlife species, primates remained a persistent challenge in certain locations.

7.1.5 Risks and Constraints

Few high-priority sites are in remote areas that require several hours of walking from the nearest road, making the transport of materials and equipment challenging. Human activities near fences, such as tree-felling, occasionally caused system faults. Incomplete fence loops, as in Nubi Gewog, also reduced effectiveness in some areas.

7.1.6 Recommendations

To enhance the impact of PEF, priority should be given to reinforcing primate-affected sites and introducing complementary deterrents such as canopy setback and non-lethal devices. Supplying critical materials, particularly MS flat iron poles, ring insulators, and poly wires will be essential for closing gaps and completing fence loops, especially in Nubi and remote sites in Trashi Yangtse. Institutionalizing operation and maintenance through community bylaws, refresher training, and regular audits by Agriculture Extension Agents is also recommended. Furthermore, awareness campaigns should be undertaken to reduce accidental damage from activities like tree-felling near fence lines.

7.1.7 Conclusion

The 2024 – 2025 PEF monitoring results reaffirm that Portable Electric Fencing is a highly effective and sustainable tool for protecting crops and reducing human–wildlife conflict across Bhutan. In most monitored sites, fences provided consistent protection with minimal maintenance requirements, significantly reducing crop loss and labour demands for night guarding. The success in key areas, such as Tsirang and Wangdue Phodrang, and the remarkable elephant deterrence in Martshala demonstrate the potential for wider adoption of PEF as a core component of Bhutan's wildlife conflict management strategy. However, to sustain these gains, targeted reinforcement for primate-affected areas, closure of incomplete fence loops, and consistent community-led maintenance are essential. With continued material support, technical guidance, and strong local participation, PEF can remain a cornerstone of national efforts to safeguard agricultural livelihoods while fostering coexistence with wildlife.







Figure 1. Monitoring of PEF in different sites

7.2 Technical Cooperation Project (TCP) with JICA – Project for Community based Human Wildlife Conflict Management (WiCoMP)

7.2.1 Background and Rationale

Bhutan's rural farming communities face increasing human - wildlife conflict due to declining rural populations, land abandonment, and the proximity of farmlands to rich forest habitats. Wildlife incursions, primarily by wild boar, monkeys, and deer cause significant crop losses, threatening rural livelihoods and prompting migration to urban centers. While several mitigation measures, including portable electric fencing, have proven effective, gaps remain in technology adaptation, community management structures, and coordinated dissemination strategies. Recognizing the need for systematic solutions, the Government of Bhutan requested JICA's technical cooperation in August 2023 to harness Japan's extensive HWCM expertise and develop adaptable, community-driven models for Bhutan.

7.2.2 Project Objectives

The TCP seeks to:

1. Establish a national HWCM implementation structure and develop guidelines for adoption across dzongkhags.
2. Build government capacity to promote and implement HWCM effectively.
3. Develop and optimize effective HWCM technologies, particularly fencing systems suitable for Bhutan's diverse terrains and species challenges.
4. Pilot and validate community-based HWCM models at selected sites before recommending them for national dissemination.

7.2.3 Governance and Implementation Structure

The project is led by NPPC with the Program Director as Project Director and the Vertebrate Pest Management Program Head as Project Manager, supported by a JICA expert team comprising a Chief Advisor on HWC management, a Community Development Specialist, and a Training Coordinator. Governance will be provided through the Joint Coordination Committee (JCC), chaired by the Director, Department of Agriculture, Ministry of Agriculture and Livestock, with representation from NPPC, ARDC Bajo, the Nature Conservation Division, relevant dzongkhags, and JICA. Bhutan office.

Pilot Site Selection and Community Consultations

Field surveys and consultations identified four pilot dzongkhags:

- Punakha (Dawakha Chiwog, Toedwang Gewog): Wild boars are the dominant pest, with some monkey damage. All 52 households expressed readiness to participate in fencing projects covering the entire chiwog.
- Wangdue Phodrang (Zamding Chiwog, Ruebisa Gewog): Multiple wildlife threats including monkeys, wild boars, and deer; two candidate sites to be finalized after further consultation.
- Tsirang (Tashiyangjong Chiwog, Kilkorthang Gewog): Severe damage from wild boar, followed by monkeys and deer; proposed model block spans 20 acres with seven households.
- Dagana (Pangserpo, Drukjeygang Gewog): Heavy monkey damage to orange crops; four residential blocks identified, with the gup pledging a Nu. 1 million gewog budget to support fencing.

Figure 2. Pilot Site Selection in the field

Technology Development Focus

This initial year prioritized the conceptualization of the **"Universal Hybrid Fence"**, a prototype designed for effectiveness against multiple species, including monkeys. Common specifications include a 2.1 m post height, 1.8 m net height, integrated electric wiring, and a 30 cm underground skirt to deter digging by wild boars. Adaptations for local feasibility include the use of aluminum-taped PVC pipes, adjustable insulators, and locally available materials to ensure affordability and ease of maintenance. Community training on installation, voltage monitoring (maintaining above 5,000V), and vegetation control along fence lines will form a key component of technology deployment.

Capacity Building and Community Engagement

Community participation is central to this approach. During joint site surveys, residents actively contributed to route planning for fences and discussed roles in operation and maintenance. Training modules are being developed to cover fence installation, repair techniques, monkey deterrence measures, and cage management for livestock protection. Initial stakeholder meetings in all four dzongkhags indicated strong local buy-in and commitment to co-finance or in-kind support for the interventions.

Next Steps for 2025–2026 FY

- Finalize pilot site boundaries and secure formal community agreements.
- Procure and deliver materials for hybrid fence prototypes.
- Conduct installation training and initiate construction in first-batch sites (Punakha and Wangdue).
- Develop and test primate-specific deterrence measures.
- Establish baseline monitoring systems and conduct pre-intervention surveys.
- Prepare draft HWCM guidelines for review by stakeholders.

7.2.4 Conclusion

The first year of the JICA - NPPC Technical Cooperation Project has successfully established the foundation for a comprehensive and community driven approach to human - wildlife conflict management in Bhutan. Through careful site selection, participatory planning, and the design of context-appropriate hybrid fencing systems, the project is positioned to deliver both immediate protection for vulnerable farmlands and a replicable model for nationwide application. Strong local commitment, combined with the integration of proven Japanese techniques and Bhutanese field experience, sets the stage for the development of sustainable, scalable solutions that balance conservation priorities with the livelihoods of rural farmers.

Annexure 1. Details of samples and tests for Potato tuber rot disease diagnostics.

Sample	Bacterial ooze test	Wet tissue culture		Media (PDA)		Media (CA)		RT-PCR (Plant)	RT-PCR (from PDA)
	Bacteria	Bacteria	Fungus	Bacteria	Fungus	Bacteria	Fungus		
1	Detected	Detected	No	No	Detected	Detected	Detected	Detected	NA
2	Detected	Detected	No	Detected	Detected	Detected	No	Detected	NA
3	No	Detected	No	Detected	No	Detected	Detected	Detected	Detected
4	Detected	Detected	No	Detected	No	Detected	No	Detected	NA
5	Detected	Detected	No	Detected	No	Detected	No	Detected	Detected
6	No	Detected	No	Detected	No	Detected	Detected	Detected	Detected
7	No	No	No	Detected	No	Detected	No	Detected	Detected
8	No	Detected	Detected	Detected	Detected	Detected	No	Detected	NA
9	No	Detected	No	Detected	No	Detected	Detected	Detected	Detected
10	No	Detected	No	Detected	No	Detected	Detected	Detected	Detected
11	No	Detected	Detected	Detected	Detected	No	Detected	Detected	NA
12	No	No	No	Detected	No	No	No	Detected	NA
13	Detected	Detected	No	No	No	Detected	Detected	Detected	NA
14	Detected	No	No	No	No	Detected	Detected	Detected	NA
15	No	Detected	No	Detected	No	Detected	Detected	Detected	NA
16	No	Detected	No	No	No	Detected	No	Detected	NA
17	Detected	Detected	Detected	No	Detected	Detected	No	Detected	NA
18	Detected	Detected	Detected	No	No	Detected	Detected	Detected	NA

*NA: qPCR not performed as there was no growth of bacteria on PDA

Annexure 2. Details of the Quinoa insect pests survey.

...wog	GPS coordinates	Date of survey	Crop Stage	Agro-Ecosystem	Variety	Pests Observed (Common Name)	Scientific Name(s)	Feeding T... Sucking, D... leaf...
...ntey,	27.4602°N, 90.1728°E	30-Oct-2024	Grain Filling and Maturation	Cool Temperate	*Amarilla marangani*	No insect pests observed	-	-
...usa	27.4515°N, 90.1748°E	30-Oct-2024	Grain Filling and Maturation	Cool Temperate	*Amarilla marangani*	Caterpillars		Defoliator
	26.8265°N, 89.4577°E	05-Nov-2024	Germination and Emergence	Warm Subtropical	*Amarilla marangani*	No insect pests observed	-	-
...rla,	26.8003°N, 89.6199°E	07-Nov-2024	Germination and Emergence	Warm Subtropical	*Amarilla marangani*	No insect pests observed	-	-

rla,	26.8020°N, 89.6213°E	07-Nov-2024	Germination and Emergence	Warm Subtropical	*Amarilla marangani*	No insect pests observed	-	-
ngo,	27.0443°N, 89.6060°E	07-Nov-2024	Flowering Stage	Warm Temperate	*Amarilla marangani*	No insect pests observed	-	-
	27.1963°N, 89.5570°E	08-Nov-2024	Flowering Stage	Warm Temperate	*Amarilla marangani*	Black bean aphids	*Aphis fabae*	Piercing-Suck
	27.1952°N, 89.5440°E	08-Nov-2024	Flowering Stage	Warm Temperate	*Amarilla marangani*	Black bean aphids	*Aphis fabae*	Piercing-Suck
wog,	27.4911°N, 89.8994°E	28-Nov-2024	Flowering Stage	Warm Subtropical	*Amarilla marangani*	No insect pests observed	-	-
RDC	27.4909°N, 89.8991°E	28-Nov-2024	Flowering Stage	Warm Subtropical	*Amarilla marangani*	Leaf miners	*Liriomyza spp.*	Leaf miner
c	27.4911°N, 89.8994°E	28-Nov-2024	Flowering Stage	Warm Temperate	*Amarilla marangani*	Black bean aphids	*Aphis fabae*	Piercing-Suck
rkar,	27.1838°N, 90.0061°E	21-Feb-2025	Vegetative Stage	Dry Subtropical	*Amarilla marangani*	Leaf miners and green peach aphids	*Liriomyza spp.*, *Myzus persicae*	Leaf miner, P
kar,	27.1834°N, 89.9961°E	21-Feb-2025	Germination and Emergence	Dry Subtropical	*Amarilla marangani*	Leaf miners, Black bean aphids, green peach aphids	*Liriomyza spp.*, *Aphis fabae*, *Myzus persicae*	Leaf miner, P
kar,	27.1763°N, 89.9870°E	21-Feb-2025	Germination and Emergence	Dry Subtropical	*Amarilla marangani*	Leaf miners, Black bean aphids, green peach aphids	*Liriomyza spp.*, *Aphis fabae*, *Myzus persicae*	Leaf miner, P
kar,	27.2239°N, 90.0260°E	22-Feb-2025	Vegetative Stage	Dry Subtropical	*Amarilla marangani*	Leaf miners, Green Peach aphids	*Liriomyza spp.*, *Myzus persicae*	Leaf miner, P
kar, ayzu	27.2241°N, 90.0312°E	22-Feb-2025	Germination and Emergence	Dry Subtropical	*Amarilla marangani*	No insect pests observed	-	-
kar, nsa	27.2383°N, 90.0528°E	22-Feb-2025	Vegetative Stage	Warm Subtropical	*Amarilla marangani*	Leaf miners, Black bean aphids, green peach aphids	*Liriomyza spp.*, *Aphis fabae*, *Myzus persicae*	Leaf miner, P
	26.8415°N, 89.4234°E	04-Mar-2025	Grain Filling and Maturation	Warm Subtropical	*Amarilla marangani*	Shield bugs, Loopers	*Nezara viridula*, *Trichoplusia ni*	Piercing-Suck
ewog, faed	27.1184°N, 90.1258°E	04-Mar-2025	Grain Filling and Maturation	Warm Subtropical	*Amarilla marangani*	Leaf miners, Black bean aphids, Lygus bugs	*Liriomyza spp.*, *Aphis fabae*, *Lygus spp.*	Leaf miner, P

ewog, bed	27.1249°N, 90.1249°E	04-Mar-2025	Grain Filling and Maturation	Warm Subtropical	*Amarilla marangani*	Leaf miners, Black bean aphids	*Liriomyza spp.*, *Aphis fabae*	Leaf miner, P
	26.8265°N, 89.4576°E	04-Mar-2025	Grain Filling and Maturation	Warm Subtropical	*Amarilla marangani*	No insect pests observed	-	-
	27.0945°N, 90.1335°E	04-Mar-2025	Vegetative Stage	Warm Subtropical	*Amarilla marangani*	Leaf miners, Black bean aphids, Lygus bugs	*Liriomyza spp.*, *Aphis fabae*, *Lygus spp.*	Leaf miner, P
ewog, faed	27.1241°N, 90.1275°E	04-Mar-2025	Grain Filling and Maturation	Warm Subtropical	*Amarilla marangani*	Leaf miners, Black bean aphids	*Liriomyza spp.*, *Aphis fabae*	Leaf miner, P
ewog,	27.0961°N, 90.1251°E	04-Mar-2025	Grain Filling and Maturation	Warm Subtropical	*Amarilla marangani*	Leaf miners, Black bean aphids, Lygus bugs	*Liriomyza spp.*, *Aphis fabae*, *Lygus spp.*	Leaf miner, P
	26.8269°N, 89.4736°E	04-Mar-2025	Grain Filling and Maturation	Warm Subtropical	*Amarilla marangani*	Leaf miners, shield bugs, loopers	*Liriomyza spp.*, *Nezara viridula*, *Trichoplusia ni*	Leaf miner, P Defoliator
ewog,	27.0918°N, 90.1401°E	05-Mar-2025	Vegetative Stage	Warm Subtropical	*Amarilla marangani*	Leaf miners, black bean aphids	*Liriomyza spp.*, *Aphis fabae*	Leaf miner, P
ewog,	27.0894°N, 90.1391°E	05-Mar-2025	Vegetative Stage	Warm Subtropical	*Amarilla marangani*	Leaf miners, Black bean aphids, green peach aphids, Lygus bugs	*Liriomyza spp.*, *Aphis fabae*, *Myzus persicae*, *Lygus spp.*	Leaf miner, P
ewog,	27.0935°N, 90.1398°E	05-Mar-2025	Vegetative Stage	Warm Subtropical	*Amarilla marangani*	Leaf miners, Black bean aphids, Lygus bugs	*Liriomyza spp.*, *Aphis fabae*, *Lygus spp.*	Leaf miner, P
	27.0980°N, 90.1312°E	05-Mar-2025	Vegetative Stage	Warm Subtropical	*Amarilla marangani*	Leaf miners, Black bean aphids, Lygus bugs	*Liriomyza spp.*, *Aphis fabae*, *Lygus spp.*	Leaf miner, P
ewog,	27.0967°N, 90.1330°E	05-Mar-2025	Vegetative Stage	Warm Subtropical	*Amarilla marangani*	Leaf miner, black bean aphids	*Liriomyza spp.*, *Aphis fabae*	Leaf miner, P
, ang II	26.9958°N, 90.1234°E	31-Mar-2025	Grain Filling and Maturation	Dry Subtropical	*Amarilla marangani*	Leaf miners, Black bean aphids, Lygus bugs	*Liriomyza spp.*, *Aphis fabae*, *Lygus spp.*	Leaf miner, P
, SC	26.9956°N, 90.1223°E	31-Mar-2025	Grain Filling and Maturation	Dry Subtropical	*Amarilla marangani*	Leaf miners, Black bean aphids, Lygus	*Liriomyza spp.*, *Aphis fabae*, *Lygus*	Leaf miner, P

)						bugs	*spp.*	
g, g	26.9524°N, 89.9657°E	01-Apr-2025	Grain Filling and Maturation	Warm Subtropical	*Amarilla marangani*	Lygus bugs, green peach aphids	*Lygus spp.*, *Myzus persicae*	Piercing-Suck
g, ng II	26.9538°N, 89.9658°E	01-Apr-2025	Grain Filling and Maturation	Warm Subtropical	*Amarilla marangani*	Leaf miners, Green Peach aphids, Lygus bugs, stem borer	*Liriomyza spp.*, *Myzus persicae*, *Lygus spp.*,	Leaf miner, P Borer
g, g III	26.9497°N, 89.9618°E	01-Apr-2025	Grain Filling and Maturation	Warm Subtropical	*Amarilla marangani*	Leaf miners, Lygus bugs	*Liriomyza spp.*, *Lygus spp.*	Leaf miner, P
g, g IV	26.9481°N, 89.9601°E	01-Apr-2025	Grain Filling and Maturation	Warm Subtropical	*Amarilla marangani*	No insect pests observed	-	-
bisa oh	27.0623°N, 89.9256°E	02-Apr-2025	Grain Filling and Maturation	Dry Subtropical	*Amarilla marangani*	Leaf miners, green peach aphids, black bean aphids	*Liriomyza spp.*, *Myzus persicae*, *Aphis fabae*	Leaf miner, P

Annexure 3. Details of the quinoa weeds survey

Site name	GPS location	Weeds observed	Growth stage	Weed De
Pangzhing, Tading, Samtse	26.876871, 89.247677 1134 masl	*Galinsoga parviflora, Galinsoga quadriradiata, Ageratum conyzoides, Bidens Pilosa, Persicaria nepalensis*	Vegetative & Flowering	Moderate
Pangzhing gongma, Tading, Samtse	26.877063, 89.24132 1205 masl	*Persicaria runcinata, Galinsoga quadriradiata, Drymaria cordata, Siegesbeckia orientalis, Bidens pilosa*	Vegetative & Flowering	Moderate
Ranthaling, Tendruk, Samtse	27.095944, 88.894635 1250 masl	*Galinsoga quadriradiata, Bidens pilosa, Persicaria runcinata, Persicaria nepalensis, Ageratina adenophora*	Vegetative & Flowering	Moderate
Charmaling, Tendruk, Samtse	27.144813, 88.861251 975 masl	*Polygonum aviculare, Bidens pilosa, Ageratum conyzoides, Crassocephalum crepidioides, Conyza canadensis, Persicaria nepalensis, Acmella uliginosa,*	Vegetative & Flowering	Moderate
Nimalung , Ugyentse, Samtse	26.972009, 89.007818 729 masl	*Chenopodium album, Ageratum conyzoides, Alternanthera sessilis, Persicaria nepalensis, Crassocephalum crepidioides, Cynodon dactylon, Galinsoga quadriradiata, Galinsoga parviflora, Bidens pilosa, Solanum khasianum*	Vegetative & Flowering	Moderate
Chargari, Samphelling, Chhukha	26.826485, 89.4576533, 406 masl	*Ageratum conyzoides, Bidens pilosa, Cynodon dactylon, Commelina diffusa, Synedrella nodiflora, Cyperus rotundus*	Flowering	Moderate
Bjabcho , Chhukh a	27.079057, 89.580517 2400 masl	*Galinsoga quadriradiata, Galinsoga parviflora, Bidens Pilosa, Persicaria nepalensis, Crassocephalum crepidioides, Persicaria runcinate, Drymaria cordata*	Vegetative & Flowering	Moderate