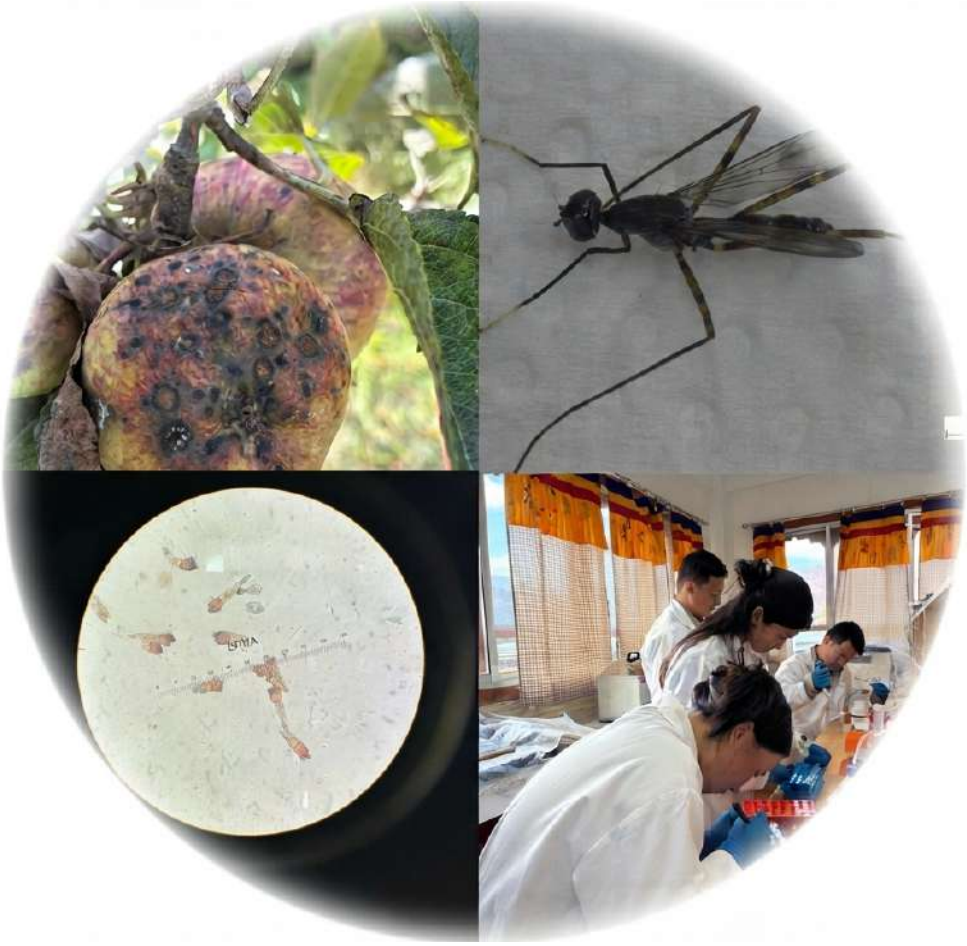
Annual Report

2025-2026



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NATIONAL PLANT PROTECTION CENTRE

DEPARTMENT OF AGRICULTURE

MINISTRY OF AGRICULTURE & LIVESTOCK

SEMTOKHA

Editors: Tshencho Dem and Dawa Dem Tamang, NPPC
Editor-in-Chief: Yeshey Dema, Program Director, NPPC

FOREWORD

The National Plant Protection Centre (NPPC) is pleased to present this annual report, detailing activities and accomplishments for the fiscal year 2025-2026. As Bhutan's principal plant health institution, the Centre continues to advance evidence-based approaches to pest and disease management, grounded in the principles of Integrated Pest Management. Our work this year has been defined by a commitment to rigorous research, systematic surveillance, and translating scientific findings into practical recommendations for the agricultural sector.

The reporting period was characterised by substantive progress across multiple domains of plant protection research. Our programs generated new knowledge on the efficacy of bio-rational compounds, refined understanding of pest population dynamics, and validated novel control strategies under local agroecological conditions. Field trials yielded critical data on treatment performance across diverse cropping systems, while laboratory investigations expanded our diagnostic capabilities and deepened insights into pathogen biology and host-pathogen interactions.

A key focus of this year's work was the evaluation of sustainable alternatives to conventional pesticides. Research on potassium phosphite demonstrated its potential as a dual-purpose tool for disease suppression in potato and garlic. At the same time, assessments of organic-compatible fungicides offered pathways for reducing synthetic chemical inputs in apple production. Concurrently, herbicide trials addressed the pressing need for effective weed management options in labour-constrained farming systems, generating recommendations that balance efficacy with environmental safety.

Surveillance activities provided essential epidemiological intelligence on emerging and endemic threats. Systematic monitoring of fall armyworm, wheat rust pathogens, and rice hopper populations established baseline data critical for forecasting and early warning systems. Investments in molecular diagnostics, including MARPLE rust analysis and wheat genome sequencing, have positioned the Centre to contribute to global surveillance networks and respond rapidly to shifts in pathogen virulence or insecticide resistance.

The achievements documented in this report reflect the collaborative efforts of our technical programs, administrative staff, and field partners. We acknowledge the contributions of the

Ministry of Agriculture and Livestock, international collaborators, and the farming communities whose participation in on-farm research has been instrumental in validating and refining our recommendations. The Centre remains committed to advancing plant protection science and delivering solutions that strengthen Bhutan's food security and agricultural resilience.

Yeshey Dema
Program Director

EXECUTIVE SUMMARY

The National Plant Protection Centre 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 2025-2026 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, Weed Science, Vertebrate Pest Management, Pest Surveillance, Biocontrol, and Plant Protection Products. Below are some of the notable achievements of these programs.

Pathology Program

1. Assessed the efficacy of Potassium Phosphite to manage Potato Bacterial wilt (*Ralstonia solanacearum*) in its second-season trial.
2. Evaluated Hexaconazole and Propiconazole for the management of Wheat Stripe Rust (*Puccinia striiformis* f. sp. *Tritici)*.
3. Assessed the efficacy of Potassium Phosphite to manage Garlic Rust (*Puccinia allii)*, comparing targeted vs. calendar-based spray schedules.
4. Evaluated organic-compatible fungicides (Copper Sulphate, Lime Sulphur, Potassium Bicarbonate) for the management of major Apple Fungal Diseases.
5. Conducted HLB screening of 284 citrus germplasm samples at the National Citrus Repository, Tsirang.
6. Provided ad-hoc disease diagnostic services for tomato, potato, ginger, wheat, and large cardamom.

Entomology Program

1. Strengthened the capacity of 727 farmers on Fall Armyworm (FAW) identification and IPM across 13 gewogs.
2. Assessed FAW incidence and population dynamics using sex pheromone traps in Rilangthang, Tsirang.
3. Monitored the population dynamics of plant and leaf hoppers in paddy fields using a light trap at ARDC Bajo.
4. Conducted a laboratory evaluation of Emamectin benzoate against FAW to establish a local LC_{50} baseline.
5. Demonstrated Koch's Postulates for *Mimegralla* sp. associated with Ginger Rhizome Rot.
6. Identified an insect infestation in Himalayan Dogwood at Motithang as a Notodontidae moth (*Deroca* spp.).
7. Identified a cicada specimen (*Cryptotympana* spp.) intercepted at Paro International Airport.

Weed Science Program

1. Evaluated the efficacy of synthetic weedicides for the management of major wetland weeds in transplanted paddy, identifying new options for *Potamogeton distinctus* and *Schoenoplectus juncoides*.
2. Evaluated the efficacy of Tembotrione 34.5% SC as a post-emergence weedicide against complex weed flora in maize.

Pest Surveillance Program

1. Strengthened the capacity of 82 agricultural officials on wheat disease surveillance and management.
2. Raised awareness on key wheat diseases for 459 farmers across 13 locations.
3. Built capacity for plant protection and research officials on barberry surveys and cereal rust diagnostics.
4. Conducted targeted surveys of barberry plants and laboratory studies to test their role in wheat rust epidemiology.
5. Established and monitored six wheat rust sentinel plots across different altitudinal zones.
6. Conducted general wheat disease surveillance across major wheat-growing dzongkhags.
7. Developed and distributed wheat disease awareness materials.
8. Disseminated weekly wheat disease early warning advisories.
9. Organised capacity building on Wheat Genome Sequencing and Bioinformatics.
10. Applied MARPLE diagnostics for molecular characterisation of wheat rust pathogens.
11. Initiated the development of an ePest Surveillance System under GEF project support.

Plant Protection Products Program

1. Procured 9,019 kg/L of fungicides, 3,872 kg/L of insecticides, 146,431 kg of herbicides, 4,772 kg/L of bio-pesticides, and 3,930 traps and lures.
2. Distributed a total of 12,821.6 kg/L of fungicides**,** 6,804.54 kg/L of insecticides, 456,181.65 kg of herbicides, 4,369.6 kg/L of bio-pesticides, and 2,586.25 traps and lures to farmers and agencies across the country.

Biocontrol Program

1. Documented the diversity and composition of natural enemies associated with FAW, with parasitoids (*Cotesia* spp.) as the dominant group.
2. Surveyed, collected, and identified predators (Earwigs, Lady Beetles) associated with FAW in major maize-growing areas.
3. Initiated mass rearing of factitious hosts (*Corcyra cephalonica* and *Sitotroga cerealella*) for egg parasitoid production.
4. Isolated and characterised native entomopathogenic fungi (*Beauveria* sp. and *Metarhizium* sp.) from FAW larvae.
5. Detected and confirmed the incidence of *Lecanicillium lecanii* infecting FAW in maize.

Vertebrate Pest Management Program

1. Successfully implemented the second year of the JICA Technical Cooperation Project (TCP), installing Universal Hybrid Fences (UHF) at three pilot sites: Dawakha (Punakha), Nyalakha (Wangdue Phodrang), and Pangserpo (Dagana), protecting over 230 acres of farmland.

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 Assessing the Efficacy of Potassium Phosphite to Manage Potato Brown Rot in Bhutan: Second-Season Report

1.1.1 Introduction

Potato (*Solanum tuberosum* L.) is a globally significant staple crop, particularly in developing countries, providing essential carbohydrates, vitamins, and minerals critical for food and nutritional security (Devaux et al., 2021; Morales-Morales et al., 2022; Tolessa, 2018; Zaheer & Akhtar, 2016). As the world's third most important food crop after rice and wheat, potato plays a vital role in sustaining growing populations, with Asia emerging as a major producer and consumer (Devaux et al., 2014; Nasir & Toth, 2022; Wijesinha-Bettoni & Mouillé, 2019). However, potato production in tropical and subtropical regions faces increasing threats from bacterial wilt, a devastating soil-borne disease that causes substantial yield losses (Jiang et al., 2017; Kurabachew & Ayana, 2017; Osdaghi, 2021).

In Bhutan, potato is a vital component of the agricultural economy and rural livelihoods, serving both as a staple food and a primary source of cash income for smallholder farmers, particularly in the high-altitude regions (Lepcha et al., 2021; Rai et al., 2022). As of 2024, roughly 28,645 rural households depend on potato cultivation—primarily in temperate zones at elevations of 1,500–3,000 masl—for food security and revenue. The recent detection of bacterial wilt caused by *Ralstonia solanacearum* in eastern Bhutan (National Plant Protection Centre, 2024) has raised serious concerns among farmers, extension agents, and policymakers, highlighting the urgent need for effective and sustainable management strategies tailored to Bhutan's unique agroecological conditions.

Ralstonia solanacearum is a soil-borne, Gram-negative bacterium with an exceptionally wide host range, capable of infecting over two hundred plant species across fifty families, including economically important Solanaceous crops such as potato, tomato, tobacco, and eggplant (Guo et al., 2021; Manda et al., 2020; Wang et al., 2023). The pathogen invades the plant vascular system through root wounds or natural openings, colonizing the xylem vessels and producing exopolysaccharides that block water transport, ultimately leading to wilting, necrosis, and plant death (Charkowski et al., 2020; Uwamahoro et al., 2020). The pathogen's remarkable adaptability, characterized by diverse races and biovars, coupled with its ability to survive for extended periods in soil, water, and asymptomatic host plants, contributes significantly to its persistence and the difficulty of its management (Ahmed et al., 2022; Farnham, 2022; Geng et al., 2022; Umrao et al., 2021). The disease causes substantial yield losses ranging from 30 to 100% under favorable conditions, leading to food shortages, economic instability, and broader food security concerns (Hayward, 1985; Waals & Krüger, 2020).

Integrated disease management is widely recognized as the most sustainable approach for controlling bacterial wilt in potatoes (Tessema & Seid, 2023; Tiwari et al., 2021). This multifaceted strategy combines various complementary approaches, including the use of resistant

or tolerant varieties, crop rotation with non-host crops, soil disinfection and solarization, strict field hygiene and sanitation practices, use of certified disease-free seed potatoes, and biological control using antagonistic microorganisms. However, in resource-constrained smallholder farming systems, many of these approaches remain either impractical, cost-prohibitive, or insufficiently effective, particularly in high-disease-pressure environments.

In recent years, potassium phosphite has emerged as a promising and environmentally friendly tool for managing bacterial wilt and other plant diseases (Bellini et al., 2023; Su, Feng, et al., 2022; Su, Qiu, et al., 2022). This compound functions primarily as a plant defense elicitor, stimulating the plant's innate immune system to produce antimicrobial compounds, including phytoalexins and pathogenesis-related proteins, while simultaneously strengthening cell walls through lignin deposition and callose accumulation (Feldman et al., 2020; Mohammadi et al., 2021; Su, Qiu, et al., 2022; Trejo-Téllez & Gómez-Merino, 2018). Additionally, phosphite has been shown to have direct antimicrobial effects, with *in vitro* studies demonstrating that potassium phosphite at a concentration of 0.05 percent weight per volume significantly inhibits the growth of *R. solanacearum* (Su, Qiu, et al., 2022). Unlike conventional fungicides and bactericides, potassium phosphite is considered environmentally safe, with low mammalian toxicity and minimal negative impacts on beneficial soil microorganisms. Importantly, phosphite has also demonstrated efficacy against *Phytophthora* species, including *Phytophthora infestans*, the causal agent of late blight, suggesting potential dual benefits for disease management in potato production systems.

The first season of this study (February to July 2025) yielded promising preliminary results, demonstrating a consistent trend toward lower pathogen loads in tubers harvested from potassium phosphite-treated plants compared to the non-treated control. While no significant differences were observed in plant growth parameters or yield between treatments, the pathogen load reduction suggested potential benefits of phosphite application for managing latent infections. These findings warranted further investigation under varying environmental conditions to confirm the efficacy and consistency of treatment effects. The current second-season study (February to July 2026) was therefore designed to build upon the first season's findings by confirming the reduction in bacterial wilt incidence and severity through potassium phosphite application, assessing treatment effects across different altitudinal gradients, and evaluating the consistency of results under varying environmental conditions. The re-establishment of the trial at the same sites in Kanglung allowed for assessment of seasonal variation in disease pressure and treatment efficacy.

Objectives

The present second-season study was designed with three primary objectives:

i. Evaluate the efficacy of foliar potassium phosphite (2 g/L) in reducing bacterial wilt incidence and severity in potatoes grown at different altitudes in Kanglung, Trashigang.

ii. Assess the impact of potassium phosphite on plant growth, tuber yield, and quality, while exploring its role within integrated disease management for sustainable potato production.

iii. Compare second-season data with first-season results to determine the consistency and reliability of treatment responses across variable environmental conditions.

1.1.2 Materials and Method

Study Design and Participatory Approach

This demonstration research was designed to engage local potato growers in the research process, fostering knowledge exchange and practical learning through participatory experimentation and field demonstration days. Building upon the successful engagement from the first season, the second season continued to involve collaborating farmers in trial management, data collection, and field day activities. This participatory approach ensures that the research findings are not only scientifically robust but also contextually relevant, increasing the likelihood of farmer adoption of effective management practices.

Experimental Sites and Layout

The demonstration trial was re-established at the same five sites in Kanglung, Trashigang, to maintain continuity with the first season and allow for assessment of seasonal variations. Kanglung was selected due to its documented history of bacterial wilt outbreaks and the presence of diverse altitudinal gradients, allowing for the assessment of treatment effects under varying environmental conditions. The five sites represented three distinct altitudinal zones. Two sites, Yonphula and Rongthoong Shingchhen, were located at higher altitudes exceeding 2500 meters above sea level. Two additional sites, Mertsham-Thragom and Maanthong, were situated at mid-altitudes ranging from 2000 to 2500 meters above sea level. The remaining site, Pangthang-Ritsangdoong, was established at a low altitude below 2000 meters above sea level.

At each site, the experiment was arranged in a randomized complete block design with two treatments and four replications. The two treatments consisted of potassium phosphite application at 2 g/L of water through foliar spray and a non-treated control receiving water spray only. Each treatment plot measured 3 m by 4 m, providing a total area of 12 m^2. Planting geometry followed a spacing of 60 cm between rows and 30 cm between plants within rows, with a buffer zone of 60 cm maintained between treatment plots to minimize cross-contamination. The planting system employed vertical planting in rows and ridges, with each plot containing 6 rows of 10 plants each, resulting in 60 plants per plot. The randomization scheme followed a systematic pattern across blocks, with each of the four blocks per site receiving alternating treatment arrangements to ensure balanced representation.

Figure 1.1. Trial setup at Yonphula, Dopung

Crop Management and Irrigation

Standard potato cultivation practices were followed, consistent with the first season's protocol, including land preparation, planting, earthing-up, and weed management as per local recommendations. Irrigation followed farmers' management practices, utilizing chlorine-treated tap water to minimize potential contamination with *R. solanacearum* from irrigation sources. Crop management practices were standardized across all sites and treatments to ensure comparability of results.

Treatment Application

Potassium phosphite was applied as a foliar spray at a concentration of 2 g/L of water, following the manufacturer's recommendations and consistent with the first season's protocol. The application was carried out using a knapsack sprayer, ensuring thorough coverage of foliage. The treatment schedule comprised three applications at critical growth stages. The first spray was applied during the vegetative growth stage, approximately 21 to 28 days after emergence. The second spray was administered at tuber initiation stage, approximately 42 to 49 days after emergence. The third and final spray was conducted during the tuber bulking stage, approximately 63 to 70 days after emergence. The control plots received an equivalent volume of water spray at the same timing to maintain consistency in application procedures.

Data Collection

Disease Assessment

Disease incidence and severity were assessed at three critical time points, consistent with the first season's protocol. The first assessment was conducted before the first spray, serving as a baseline measurement approximately three weeks after emergence. The second assessment occurred before the second spray, capturing early disease development approximately six weeks after emergence. The third assessment was conducted two weeks after the second spray, representing peak disease development approximately eight weeks after emergence. Bacterial wilt severity was evaluated

using a standard 0 -5 rating scale, where 0 indicated no symptoms, 1 represented initial wilting of 1 or 2 lower leaves, 2 indicated wilting of 25% to 50% of leaves, 3 represented wilting of 51% to 75% of leaves, 4 indicated wilting of 76% to 100% of leaves, and 5 represented plant death. Disease incidence was calculated as the percentage of symptomatic plants per plot, while disease severity was calculated using the formula that sums the product of disease score and number of plants in that score, divided by the total number of plants multiplied by the maximum score, all multiplied by one hundred.

Growth Parameters

Plant growth parameters were recorded at two time points, mid-season and pre-harvest, consistent with the first season. Plant height was measured in centimeters from the soil surface to the top of the plant. The number of leaves per plant was counted from five randomly selected plants per plot. Stem diameter was measured in millimeters at the base of the main stem using a digital caliper.

Figure 1.2. Data collection at vegetative stage

Yield Components

At harvest, several yield components were recorded. The number of tubers per plant was counted from five randomly selected plants per plot. Tuber weight per plant was recorded in kilograms. Total yield per plot and marketable yield were measured in kilograms. Tuber size distribution was classified based on tuber diameter, and tuber quality was assessed through external symptom evaluation including cracks, deformities, and rot symptoms.

Pathogen Detection and Quantification

Tuber samples were collected at harvest from each treatment plot for laboratory analysis to detect latent infections in tubers that may appear symptomless, to quantify pathogen loads using quantitative PCR to link final pathogen levels to yield outcomes, and to compare pathogen loads between the first and second seasons to assess seasonal variation. The sampling protocol involved randomly selecting approximately 10 to 15 tubers from each plot. Tubers were washed, surface-sterilized, and processed for DNA extraction using a commercial DNA extraction kit or CTAB method. Quantitative PCR was performed using species-specific primers targeting the *R. solanacearum* genome, and pathogen load was expressed as DNA concentration in nanograms per microliter or as the number of copies per gram of tuber tissue. Samples were collected on 29 June 2026 and submitted to the laboratory for analysis.

Statistical Analysis

Data from the second season will be analyzed using appropriate statistical methods. Disease incidence and severity data will be analyzed using analysis of variance for a randomized complete block design, with treatment as a fixed factor and site and block as random factors. Growth and yield data will be analyzed using analysis of variance with treatment, site, and their interaction as factors. Quantitative PCR data will be log-transformed prior to analysis and analyzed using analysis of variance. Data from both seasons will be analyzed using combined analysis of variance to assess season by treatment interactions. All statistical analyses will be performed using appropriate statistical software, and means will be separated using Tukey's honestly significant difference test at an alpha level of 0.05.

1.1.3 Results and Discussion

First-Season Summary

The first season, conducted from February to July 2025, yielded several preliminary findings. Moderate bacterial wilt pressure was observed across the trial sites, with natural disease development allowing for assessment of treatment effects. A consistent trend toward lower pathogen loads in tubers from potassium phosphite-treated plants was observed compared to the non-treated control. No significant differences were observed in plant growth parameters, including height, leaf number, and stem diameter, between treatments. Similarly, no significant differences were observed in tuber yield or quality between treatments. Quantitative PCR analysis confirmed the presence of *R. solanacearum* in harvested tubers, with treated samples showing numerically lower pathogen concentrations. These findings provided the rationale for continuing the study into the second season to confirm the observed trends.

Second-Season Disease Pressure

During the current growing season from February to July 2026, bacterial wilt pressure was unexpectedly negligible across all five experimental sites. This low disease pressure was attributed

to several factors. Unfavorable environmental conditions, including lower than average temperatures and suboptimal soil moisture conditions during the critical infection period, likely limited disease development. Seasonal variation, representing a natural fluctuation in pathogen populations that is well documented in *R. solanacearum* epidemiology, also contributed to the low disease incidence. Effective management practices adopted by collaborating farmers, including crop rotation and field hygiene, may have further suppressed pathogen populations. Additionally, the pathogen may have survived in a dormant state in the soil, with conditions unfavorable for disease development preventing active infection.

Late Blight Outbreak and Crop Failure

Although bacterial wilt was not observed, a devastating late blight infestation caused by *Phytophthora infestans* affected all trial plots during the second season. The late blight outbreak developed with remarkable rapidity during the interval between phosphite treatments, manifesting during the gap between the second and third scheduled applications. The disease spread aggressively through the foliage, causing extensive necrosis and defoliation within a matter of days. Despite the phosphite applications, the disease pressure was so intense that it overwhelmed the plants' defenses, and the potato plants were ultimately killed by the late blight infection before the crop cycle could be completed.

The premature plant death necessitated early harvest of the trial plots. However, because the plants had been killed before reaching full maturity, the tubers had not completed their development. Consequently, all sites experienced significantly smaller tuber sizes at harvest compared to the first season and compared to normal production expectations. The crop stage was not completed successfully due to the late blight outbreak, with the growing season terminated approximately three to four weeks earlier than planned. This early termination had profound implications for yield assessment, as tubers lacked sufficient time for bulking and reached only a fraction of their potential size and weight.

The infestation had several significant impacts on trial objectives. The complete plant death prevented any meaningful assessment of bacterial wilt development, as the disease of interest did not manifest and the plants were destroyed before symptoms could be evaluated. The premature harvest compromised yield data, as tubers were harvested at an immature stage, and size reduction was observed across all treatments and sites. The severe foliage damage and plant death also prevented the collection of complete growth parameter data. Furthermore, the rapid development of late blight during the treatment interval demonstrated that the current three-spray schedule, with intervals of approximately three weeks between applications, provides insufficient coverage for effective disease suppression under high disease pressure conditions. However, tuber samples were still collected for quantitative PCR analysis to assess latent pathogen populations independent of late blight damage.

Comparison of First and Second Seasons

A comparison between the two seasons reveals dramatic differences in disease pressure, crop outcome, and data completeness. The first season experienced moderate bacterial wilt pressure with low late blight pressure, allowing for completion of the crop cycle and collection of full yield data. The second season experienced negligible bacterial wilt pressure but a devastating late blight outbreak that killed the plants and necessitated early harvest, resulting in smaller tuber sizes and incomplete crop development. Treatment effects on bacterial wilt could not be fully assessed in the first season due to moderate pressure and could not be assessed at all in the second season due to plant death from late blight. Pathogen load in tubers showed a trend toward lower load in treated samples during the first season, with quantitative PCR analysis pending for the second season despite the compromised growing conditions. Growth parameters showed no significant differences in the first season, with only partial data collected in the second season due to premature plant death. Yield parameters showed no significant differences in the first season, while the second season yielded smaller tubers across all treatments due to early harvest.

Laboratory Analysis Status

Tuber samples from all trial sites were collected at the early harvest and submitted to the laboratory on 29 June 2026. Quantitative PCR analysis is currently pending. The expected outcomes of this analysis include confirmation of whether the trend observed in the first season, namely lower pathogen loads in treated tubers, is consistent across seasons despite the compromised growing conditions. The analysis will provide robust quantitative data on latent pathogen populations in immature tubers, help establish correlations between seasonal disease pressure and tuber infection rates even under abnormal growing conditions, and inform recommendations for phosphite application under varying disease pressure conditions. Laboratory results will be updated once pathogen loads are quantified using the quantitative PCR assay.

Seasonal Variation in Disease Pressure and the Emergence of Late Blight

The most significant finding of the second season was the catastrophic late blight outbreak that resulted in complete plant death and crop failure across all experimental sites. This outcome highlights the inherent unpredictability of disease development in field-based research and the complex interactions between different pathogens in potato production systems. While the bacterial wilt pressure was unexpectedly negligible, the late blight pressure was exceptionally high, demonstrating how disease pressures can shift dramatically between seasons and how multiple pathogens can threaten potato production simultaneously.

The rapid development of late blight during the interval between phosphite treatments revealed a critical vulnerability in the current experimental protocol. Late blight is notorious for its explosive epidemic potential, with lesions capable of producing hundreds of thousands of sporangia within days under favourable conditions of cool temperatures, high humidity, and rainfall. The three-week interval between phosphite applications, while aligned with the manufacturer's

recommendations for general disease suppression, proved entirely inadequate when environmental conditions favoured rapid pathogen spread. The disease established itself during the unprotected window between treatments and spread so aggressively that even subsequent phosphite applications could not halt its progression. This observation aligns with the known biology of *P. infestans*, which can complete its infection cycle in as little as four to five days under optimal conditions, meaning that a three-week interval provides multiple opportunities for the pathogen to establish and spread.

Implications of Crop Failure for Yield Assessment and Data Interpretation

The premature plant death and early harvest had profound implications for yield assessment and data interpretation. The smaller tuber sizes observed across all sites reflect the interruption of the tuber bulking process, which typically occurs during the later stages of crop development. Tubers harvested three to four weeks early would not have reached their full-size potential, resulting in a higher proportion of small and undersized tubers. This early harvest effectively eliminated the possibility of assessing true yield potential or treatment effects on yield, as the crop cycle was not completed under any treatment regime. The yield data collected from this season must therefore be interpreted with caution, as they reflect the impacts of premature harvest rather than true treatment effects on productivity.

Despite the compromised growing conditions, the collection of tuber samples for quantitative PCR analysis remains valuable. The analysis can still detect and quantify latent *R. solanacearum* infections in the immature tubers, providing insights into whether phosphite treatment influences pathogen colonization even under suboptimal growing conditions. Furthermore, the comparison of pathogen loads between the two seasons, even with the second season's abnormal conditions, may reveal patterns in disease development and pathogen survival that inform future management strategies.

The Need for Increased Spray Frequency and Integrated Management

The catastrophic late blight outbreak has provided critical insights for refining the experimental protocol. The rapid development of the disease during the interval between phosphite treatments clearly demonstrates that the current three-spray schedule is inadequate for effective disease suppression under high disease pressure conditions. The way forward must involve increasing the spray frequency while maintaining consistent intervals between applications. A modified schedule with applications at shorter intervals, such as ten to fourteen days, would ensure continuous protective coverage throughout critical growth stages and reduce the windows of vulnerability during which diseases can establish and spread.

The finding that phosphite alone, applied at the current frequency, could not prevent late blight development under exceptionally high disease pressure conditions also underscores the importance of integrating multiple management strategies. Phosphite's primary mode of action against late blight is through induced resistance rather than direct pathogen suppression, and its efficacy can

be overwhelmed when disease pressure is exceptionally high. Combining phosphite application with other management tools, including resistant varieties, targeted fungicide applications, and cultural practices, would provide more robust disease protection. Additionally, the integration of late blight monitoring and forecasting systems would enable more precise timing of interventions, allowing for proactive rather than reactive management.

Implications of First-Season Findings and the Potential for Dual Disease Management

Despite the challenges of the second season, the trend observed in the first season, namely lower pathogen loads in treated tubers, remains relevant and promising. The known mechanisms of action of potassium phosphite, including the stimulation of host defense responses and the production of antimicrobial compounds, suggest potential benefits for managing both bacterial wilt and late blight. Phosphite has demonstrated efficacy against *Phytophthora* species in various crops, raising the possibility of dual disease management benefits in potato production. If future studies confirm that increased phosphite application frequency can suppress late blight while also reducing bacterial wilt pathogen loads, the compound would represent a valuable tool for integrated disease management in Bhutan's potato production systems.

Participatory Research and Farmer Engagement

The participatory nature of this demonstration trial, which has engaged local potato growers in the research process for two consecutive seasons, provides valuable opportunities for knowledge transfer and technology adoption. Even in a season of catastrophic crop failure, the interactions with researchers allow farmers to observe disease development firsthand, understand the importance of timely interventions, learn about integrated disease management principles, and provide feedback on practical constraints and opportunities for implementation. The experience of the late blight outbreak may have been particularly instructive for farmers, reinforcing the importance of proactive disease management and the limitations of relying on a single control measure. This sustained engagement is critical for the eventual adoption of evidence-based management practices, as farmers are more likely to adopt technologies they have been involved in testing and evaluating.

Revised Protocol for Future Seasons

Based on the catastrophic outcomes of the second season, the experimental protocol will be substantially revised for future seasons. The primary modification will involve increasing the frequency of phosphite applications from the current three-spray schedule to a schedule of five to six applications at ten-to-fourteen-day intervals, ensuring continuous protective coverage throughout the crop cycle. This revised schedule would provide more consistent suppression of both late blight and bacterial wilt, reducing the windows of vulnerability during which diseases can establish and spread. The application timing will be adjusted to ensure coverage during periods of high disease risk, with particular attention to the tuber bulking stage when late blight pressure is typically highest.

Additional refinements to the protocol will include the integration of complementary disease management strategies. Future trials will evaluate the combined application of potassium phosphite with fungicides specifically targeted against late blight, allowing for assessment of additive or synergistic effects. The use of late blight-resistant potato varieties will also be considered to reduce the confounding effects of late blight on bacterial wilt assessments. Environmental monitoring will be intensified to correlate disease development with weather parameters, enabling more precise timing of phosphite applications. Finally, the inclusion of additional treatments with varying phosphite concentrations and application frequencies will allow for optimization of the protocol for Bhutanese conditions. A contingency plan for disease outbreaks will also be developed, including protocols for disease assessment even under compromised growing conditions and for salvaging data from affected trials.

1.1.4 Conclusion

This second-season study, despite the catastrophic crop failure caused by late blight, has generated critical insights that will inform future research and disease management strategies for Bhutan's potato production systems. The devastating late blight outbreak that killed the potato plants and necessitated early harvest, resulting in smaller tuber sizes across all sites, has demonstrated the vulnerability of the current experimental protocol and the need for substantial refinements. The rapid development of late blight during the interval between phosphite treatments has clearly established that the current three-spray schedule provides insufficient coverage under high disease pressure conditions. This finding has informed the primary way forward: increasing the spray frequency to ensure continuous protective coverage throughout the crop cycle. While the primary objective of confirming bacterial wilt suppression could not be achieved due to the absence of bacterial wilt pressure and the catastrophic late blight outbreak, several important conclusions can be drawn. Field-based disease management research must account for environmental variability and the potential for multiple disease outbreaks through multi-season trials and robust experimental designs that include contingencies for disease pressure fluctuations. The co-occurrence of bacterial wilt and late blight in Bhutan's potato production systems underscores the need for integrated disease management approaches that address multiple pathogens simultaneously. Participatory research provides valuable opportunities for knowledge transfer and technology adoption, even in seasons of crop failure. The preliminary trend from the first season, indicating lower pathogen loads in phosphite-treated tubers, remains promising and warrants continued investigation with a revised protocol that includes increased spray frequency and integrated management strategies. Pending the completion of quantitative PCR analyses from the 2026 season, a comprehensive assessment of phosphite effects on pathogen loads under compromised growing conditions will be possible. The combined results from both seasons, along with the lessons learned from the catastrophic late blight outbreak, will contribute significantly to the development of robust, evidence-based integrated disease management strategies for sustainable potato production in Bhutan, with the ultimate goal of improving food security and rural livelihoods.

1.1.5 References

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1.2 Evaluation of Hexaconazole and Propiconazole for Management of Wheat Stripe Rust in Bhutan: Preliminary Results from Barp, Punakha

1.2.1 Introduction

Wheat (*Triticum aestivum* L.) is a staple food for over two billion people globally, providing approximately 20% of daily calories and protein (Sharma & Sharma, 2025). In Bhutan's mountainous regions, wheat is a widely cultivated cereal ranking third after rice and maize (Tshewang et al., 2018). Stripe rust (yellow rust), caused by *Puccinia striiformis* f. sp. *tritici*, is a devastating disease that thrives in cool, moist environments (Chen, 2020). Under favorable conditions, it can cause yield losses of 10–100% by reducing green leaf area and impairing grain filling (W. Chen et al., 2014).

While host resistance is the most economical long-term strategy (Jamil et al., 2020), chemical intervention remains essential when resistance breaks down or new virulent races emerge (Carmona et al., 2020). Triazole fungicides such as propiconazole and hexaconazole have shown high efficacy against stripe rust in diverse agro-ecologies, often providing >90% control when applied as two successive sprays (Bhosale et al., 2024; Gad et al., 2020; Gamal et al., 2023). However, disease management must be tailored to local environmental conditions (Corkley et al., 2022; Wang et al., 2025), and no validated protocol currently exists for Bhutan. This study was designed to evaluate the bio-efficacy of propiconazole and hexaconazole under natural stripe rust pressure in three historical hotspots across different elevations in Bhutan. This report presents the preliminary results from the first harvested site (Barp, Punakha).

1.2.2 Materials and Method

Experimental sites

Three sites at different elevations were selected based on their strong historical records of stripe rust epidemics:

Table 1.1. Experimental sites

Site	Dzongkhag	Elevation	Status at time of reporting
Barp	Punakha	~1300 m	Harvested (data presented here)
Tang	Bumthang	~2600 m	Grain-filling stage (data pending)
Kar-tshog	Haa	~2700 m	Grain-filling stage (data pending)

All sites experience cool, moist spring conditions conducive to stripe rust. The same experimental protocol was applied at each site.

Experimental design (per site)

At each site, a randomized complete block design with four replications was used. Plot size was 20 m² (4 m × 5 m). Buffer zones of 1 m were maintained between plots, and polyethylene sheets were used during spraying to prevent chemical drift.

Figure 1.3. Trial setup at Haa

Crop management and inoculation

A local, reportedly susceptible wheat variety was sown at each site following regional planting dates (mid-November for Barp; later for higher elevations). Fertilizer was applied at recommended rates (full NPS [Nitrogen Phosphorus Sulphur] and one-third of N at sowing; remaining two-thirds N at mid-tillering). No artificial inoculation was performed. To avoid the accidental introduction of new, non-native races of *P. striiformis* f. sp. *tritici* into experimental sites, the trial relied entirely on natural infection from the existing local pathogen population.

Fungicide treatments and application

Three treatments were applied at each site; T1 (Control): Sterile water only, T2: Hexaconazole 5% EC applied at 0.05% concentration, and T3: Propiconazole 25% EC applied at 0.1% concentration (25 cm^3/100 L water, equivalent to 0.5 L/ha).

Application schedule: The protocol specified that the first spray should be applied when stripe rust severity reached the economic threshold of 5%. However, stripe rust did not appear at any of the three sites throughout the season, so the threshold was never met. Nevertheless, the sprays were applied as scheduled, i.e., at the predetermined crop growth stage (flag leaf emergence, Feekes 8–9) at each site. A second spray was applied 14 days later. All applications were made using a knapsack sprayer calibrated to deliver 200 L/ac.

Figure 1.4. Treatment application with knapsack sprayer

Data collection (Barp site only)

At the Barp site (the only site harvested at the time of this report), the following data were recorded:

- 100-grain weight: A random sample of 100 sound grains from the harvested produce of each plot was weighed using a precision balance. Values were converted to 1000-grain weight (multiplying by 10) for standard reporting.
- Disease assessment: Stripe rust severity was assessed weekly from the first spray date until harvest using the modified Cobb scale (0–100%). No uredinium were observed. At maturity, heads were inspected for other diseases; low incidences of Fusarium head blight and loose smut were noted but not quantified.
- Phytotoxicity: No visual phytotoxicity symptoms were recorded.

Statistical analysis (Barp site only)

Because the residuals were not normally distributed (Shapiro–Wilk test, $p < 0.05$) and the sample size was small ($n = 4$ blocks), a non-parametric Friedman test (the analogue of a two-way ANOVA for a randomized complete block design) was used to compare the three treatments across blocks. Post-hoc pairwise comparisons were conducted using Wilcoxon signed-rank tests with Bonferroni correction. All analyses were performed using JASP (Version 0.18.3). Statistical significance was declared at $\alpha = 0.05$.

1.2.3 Results and Discussion

Disease development at all three sites

No stripe rust was observed at any of the three sites (Barp, Tang, Kar-tshog) despite the selection of historical hotspots, and due to the absence of artificial inoculation. Weekly scouting from the flag leaf stage (Feekes 8–9) through to harvest (Barp) or through the grain-filling stage (Tang, Kar-

tshog) confirmed zero disease incidence. Consequently, disease severity ratings and per cent efficacy calculations (based on rust control) could not be performed. The primary objective of evaluating fungicide bio efficacy against stripe rust was not achieved at any site due to the absence of the target disease.

At the Barp site (harvested), low incidences of Fusarium head blight and loose smut were observed on heads at maturity, but these were not the target diseases of the fungicides and were not quantified.

1000-grain weight (Barp site only)

The mean 1000-grain weights for each treatment at Barp are presented in Table 1. The control (T1) had a mean of 60.2 g, hexaconazole (T2) 58.3 g, and propiconazole (T3) 61.2 g. The Friedman test showed no statistically significant difference among treatments ($\chi^2 = 0.50$, df = 2, p = 0.78). Pairwise Wilcoxon tests confirmed no significant contrasts after Bonferroni correction (Table 2).

Table 1.2. 1000-grain weight (g) by treatment and replication (block) – Barp site, Punakha.

Replication	Control	Hexaconazole	Propiconazole
1	63	63	62
2	61.9	53	62.9
3	60	57	57
4	56	60	63
Mean	60.2	58.3	61.2
Standard deviation	3.08	4.27	2.85

Table 1.3. Friedman test and pairwise comparisons (Barp site).

Comparison	Statistic	df	p-value (Bonferroni-corrected)
Friedman test (overall)	$\chi^2 = 0.50$	2	0.78
Control vs. Hexaconazole (Wilcoxon)	–	–	1
Control vs. Propiconazole (Wilcoxon)	–	–	1
Hexaconazole vs. Propiconazole (Wilcoxon)	–	–	1

**Note: Corrected α = 0.017. All p-values > 0.05, non-significant.*

Although not significant, the direction of the mean difference numerically favored propiconazole over hexaconazole, and the control was intermediate. In three of the four replications, hexaconazole produced lower grain weight than the control.

Complete absence of stripe rust across three elevations

The most striking outcome of this multi-site trial was the complete absence of stripe rust at all three locations (1300 m to 2700 m), each with a strong historical record of epidemics. Possible explanations include: unusually dry conditions during the tillering-to-stem-elongation period in 2025, low overwintering inoculum, a shift in local pathogen virulence away from the sown varieties (Aslam et al., 2026; W. Q. Chen et al., 2009; X. M. Chen, n.d.; Hovmøller & Justesen, 2007; Mahmoodi et al., 2024), or a regional climatic anomaly. Whatever the cause, this outcome highlights a major risk of relying solely on natural infection: even at reliable hotspots, disease can fail to appear in a given year.

The biosecurity trade-off

The decision to avoid artificial inoculation was deliberate, following national biosecurity guidelines to prevent accidental introduction of exotic rust races. This is particularly important for Bhutan, which has a unique and potentially vulnerable pathogen population. However, the trade-off is that disease pressure becomes uncontrollable. In 2026, this resulted in a failed efficacy trial for the target disease at all three sites. For future trials, a hybrid approach may be needed: either continue relying on natural infection but over multiple consecutive years (increasing the probability of an epidemic), or develop a controlled inoculation protocol using locally collected, well-characterized races under strict quarantine.

Grain weight results from Barp (preliminary)

The 1000-grain weight data from Barp show no significant difference between fungicide-treated plots and the control. In the absence of stripe rust, this is expected: the fungicides had no disease to control, and any effect on grain weight would be neutral or negative (e.g., mild phytotoxicity). The numerically lower weight in hexaconazole-treated plots might suggest a slight negative effect, but the difference was not statistically significant given the low replication (n = 4).

Crucially, these results are preliminary. The two higher-elevation sites (Tang, Kar-tshog) are still at grain filling. They may experience different environmental conditions (e.g., cooler temperatures more favorable for stripe rust, or different disease pressure). Until those sites are harvested and analyzed, no conclusion can be drawn about the effect of these fungicides on grain weight under Bhutanese conditions.

1.2.4 Conclusion (Preliminary)

Based on the first harvested site (Barp, Punakha) and the confirmed absence of stripe rust at all three locations in 2025:

- The bio efficacy of hexaconazole and propiconazole against stripe rust could not be evaluated because the target disease did not develop at any site.
- At the Barp site, neither fungicide significantly affected 1000-grain weight compared to the control under disease-free conditions.

Conclusions await the harvest and analysis of the two remaining sites (Tang, Bumthang and Kartshog, Haa). It is possible that stripe rust may develop late in the season at higher elevations, or that grain weight responses differ with altitude.

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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 is 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 in Gasa expanded garlic cultivation to about 20 acres in 2021 (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. 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 phosphorus 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).

A 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 aims to evaluate the effectiveness of potassium phosphite in managing garlic rust through a demonstration trial. It also aims to promote the adoption of potassium phosphite as a sustainable and environmentally friendly alternative to synthetic fungicides for garlic rust control.

Objectives

- Evaluate the efficacy of potassium phosphite to manage garlic rust under local conditions.
- Compare targeted spray schedule (critical growth stages: 95 and 150 DAS) versus calendar-based schedule (14-day intervals from 95 DAS) to determine which provides superior or equivalent disease control.
- Generate evidence-based recommendations for organic and low-input garlic production systems in Bhutan.

1.3.2 Materials and Method

A demonstration trial was established at three locations in Gasa Dzongkhag, specifically two sites in Khamaed and one in Khatoed. 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.

Given the unpredictable disease incidence in the farmers' field giving unreliable results in first season, an additional trial site was established on station at the centre for proper management of the research plots. The site was also known for its previous rust incidence history.

*Experimental Site and Design**:*** Three treatments were implemented in a randomized complete block design with four replications. Each replication contained 10 tagged plants, totaling 40 plants per treatment.

- T1 (Targeted schedule): Potassium phosphite (2 g/L water) applied at 95 DAS and 150 DAS.
- T2 (Calendar-based schedule): Potassium phosphite (2 g/L water) applied every 14 days from 95 DAS (sprays at 95, 109, 123, 137, 151, 165, 179, 193, 207 DAS; 9 sprays by 207 DAS).
- T3 (Untreated control): Equivalent volume of water applied.

Standard agronomic practices for planting spacing, irrigation, fertilization, and weed management were followed to ensure consistency and relevance to local farming conditions.

Each trial site was designated as: Khatoed, Khamaed 1, Khamaed 2 and On-station.

Figure 1.5: On-station trial (Left) and on-farm trial at Khatoed (Right).

Data Collection: Data were collected every 14 days from 95 to 207 DAS (9 time points). Rust severity (%) was visually assessed on each tagged plant. Rust incidence (%) was calculated as the percentage of plants with severity > 0. AUDPC (Area Under Disease Progress Curve) was calculated using the trapezoidal method (Shaner & Finney, 1977).

Data Analysis: One-way ANOVA was conducted for severity at each time point and for AUDPC. Due to heterogeneity of variances (Levene's test), non-parametric Kruskal-Wallis tests were performed, followed by Dunn's post-hoc comparisons. Significance was set at $\alpha = 0.05$. All analyses were performed using n = 40 plants per treatment.

1.3.3 Results and Discussion

On-Farm Trials at Gasa

Although no disease pressure was observed throughout the growing season at on-farm trial sites, rust symptoms appeared at harvest stage at two locations. At Khamaed 1, rust severity was recorded with notable variation among treatments (Table 1). At Khamaed 2, rust incidence was observed, but the data were homogeneous with no significant differences among treatments as all values were identical. At Khatoed, no disease was observed even at harvest stage. These findings indicate that disease pressure was minimal and inconsistent across on-farm sites this season, making field-level evaluation of treatment efficacy inconclusive.

On-Farm Trials: Disease Progression

Statistical Analysis: Khamaed 1

One-way ANOVA revealed no significant treatment effect on rust severity at Khamaed 1 ($F = 0.801$, $df = 2, 9$, $p = 0.479$). Descriptive statistics showed that T3 (control) had the highest mean severity (99.625%, SE = 0.239), followed by T2 (95.625%, SE = 3.313), while T1 had the lowest mean severity (93.875%, SE = 4.638) (Table 1). Levene's test indicated homogeneity of variances ($F = 3.850$, $p = 0.062$), satisfying the assumption for ANOVA.

Table 1.4. Rust severity (%) at harvest stage at Khamaed 1 (n = 4 replications per treatment).

Treatment	Mean (%)	SD	F-value	P-value
T1 (Targeted)	93.88	9.28	0.801	0.479
T2 (Calendar)	95.63	6.63		
T3 (Control)	99.63	0.48		

Table 1.5. Summary of On-Farm Rust Observations at Harvest

Site	Rust at harvest	Observations
Khamaed 1	Present	No significant treatment differences (p = 0.479); T1 showed numerically lowest severity (93.88%)
Khamaed 2	Present	Data homogenous; all values identical; no significant differences
Khatoed	Absent	No disease observed even at harvest stage

On-Station Trial: Disease Progression

No rust was observed in any treatment through 137 DAS, reflecting winter temperatures below the optimal range (10-15°C) required for *Puccinia allii* infection (Furuya et al., 2009; Gilles & Kennedy, 2003). First symptoms appeared in T3 (control) at 151 DAS with a mean severity of 0.125% and an incidence of 2.5% (Figures 1.6 and 1.7). Both T1 and T2 remained disease-free, confirming that a single phosphite application at 95 DAS protected 151 DAS, equivalent to four calendar-based sprays. This aligns with phosphite's systemic mobility and defense-priming properties (Saldarriaga-Gómez et al., 2025; Yáñez-Juárez et al., 2018).

At 179 DAS, first detectable rust appeared in treated plots, with T3 severity approximately 4 times higher than T1 and T2. At 193 DAS, T1 had the lowest severity (0.538%), while T3 had the highest (4.482%). T1 achieved 88% lower severity than T3 and 66% lower severity than T2. At 207 DAS, disease peaked across all treatments, with T1 maintaining the lowest severity (33.25%) and T3 the highest (45.95%). T1 had 28% lower severity than T3. The dramatic increase reflects optimal late-season conditions (15–20°C, frequent leaf wetness) typical for *Puccinia allii* (Baramidze et al., 2015; Wamser et al., 2022).

Incidence reached 100% in all treatments by 207 DAS. Critically, while final incidence was identical, severity differed dramatically. The severity-to-incidence ratio was lowest in T1 (0.3325), and highest in T3 (0.4595)—38% higher in T3 than T1. This pattern (high incidence with suppressed severity) is characteristic of induced resistance, where phosphite primes plant defenses without preventing infection (Hardy & McComb, 2008; Hunter, 2018; Machinandiarena et al.,

2012; Saldarriaga-Gómez et al., 2025). This explains why T1 and T2 achieved 88% and 65% severity reduction at 193 DAS despite comparable incidence levels.

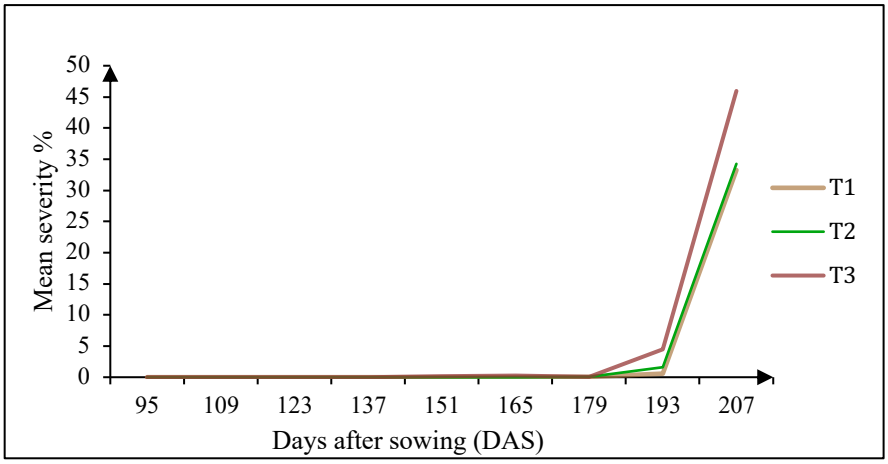

Figure 1.6: Mean rust severity (%) over time by treatment

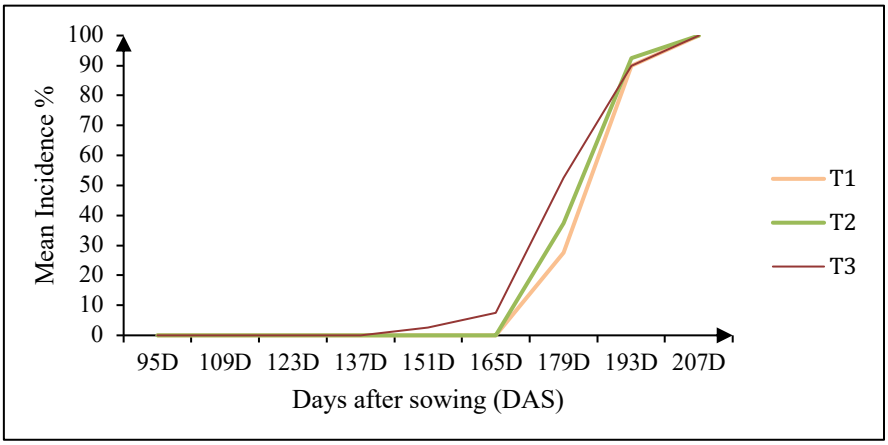

Figure 1.7: Mean rust incidence (%) over time by treatment.

Statistical Analysis of Severity

At 179 DAS, Kruskal-Wallis test confirmed a significant treatment effect ($\chi^2 = 8.765$, df = 2, p = 0.012). Dunn's post-hoc test revealed that T1 was significantly different from T3 (p = 0.003), while T1 vs. T2 was not significant (p = 0.271). At 193 DAS, Kruskal-Wallis showed a significant treatment effect ($\chi^2 = 10.302$, df = 2, p = 0.006). T1 was significantly different from T3 (p = 0.001), while T1 vs. T2 approached significance (p = 0.046). At 207 DAS, Tukey's HSD showed significant treatment effect (F=3.953, df=2, p=0.022). T1 was significantly different from T3 (p = 0.034), while T1 vs. T2 was not significant (p = 0.978). These results demonstrate that both treated plots had significantly lower disease severity than the control, while the targeted and calendar-based schedules were statistically equivalent throughout the growing season.

AUDPC Analysis

AUDPC integrates disease intensity and duration over the entire growing season (Shaner & Finney, 1977). T1 had the lowest AUDPC (240.63 severity-days), followed by T2 (262.43), while T3 had the highest (391.06) (Figure 1.8). T1 reduced AUDPC by 38% compared to T3. Kruskal-Wallis test confirmed a significant treatment effect ($\chi^2 = 8.897$, df = 2, p = 0.012). Dunn's post-hoc comparisons revealed that both T1 and T2 were significantly different from T3 (T1 vs. T3: p = 0.004; T2 vs. T3: p = 0.041), while T1 vs. T2 was not significant (p = 0.391). This confirms that potassium phosphite effectively reduces cumulative disease pressure, and critically, the targeted schedule (2 sprays) provides statistically equivalent disease control to the intensive calendar-based schedule (9 sprays).

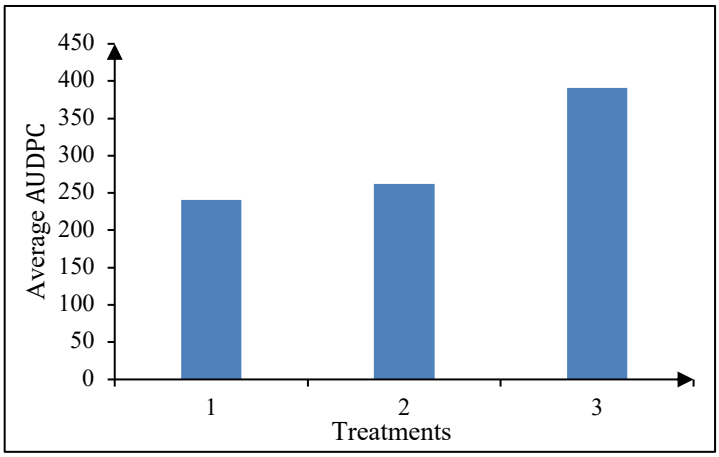

Figure 1.8: Temporal mean severity through AUDPC.

Yield Parameters across sites: Bulb Weight and Diameter

Yield data were collected from one on-station site (NPPC) and three on-farm sites (Khamaed 1, Khamaed 2, and Khatoed) in Gasa Dzongkhag. The objective was to compare bulb weight (g) and bulb diameter (mm) among three treatments: One-way ANOVA was performed for each site separately, followed by Tukey's HSD post-hoc tests for pairwise comparisons when significant differences were detected.

On-Station site

At the on-station site where disease pressure was present, one-way ANOVA revealed no significant treatment effect on bulb weight (F = 1.773, df = 2, 117, p = 0.174) or bulb diameter (F = 1.364, df = 2, 117, p = 0.260). Descriptive statistics showed that T2 recorded the highest mean bulb weight (63.86 g), followed by T1 (62.41 g), while T3 had the lowest (55.21 g). T1 and T2 produced 13% and 16% heavier bulbs, respectively, compared to the control. For bulb diameter, T2 recorded the highest mean (58.83 mm), followed by T1 (58.18 mm), while T3 had the smallest (56.00 mm). T1 and T2 produced bulbs 4% and 5% larger in diameter, respectively, compared to the control. Although not statistically significant, these agronomically meaningful trends suggest

that potassium phosphite applications may have contributed to improved bulb development through reduced disease-induced stress during the critical bulbing period (Shifa et al., 2019)

On-Farm Site: Khamaed 1

At Khamaed 1, one-way ANOVA revealed a significant treatment effect on bulb weight ($F = 5.214$, $df = 2, 117$, $p = 0.007$). Tukey's HSD post-hoc test showed that T2 (mean = 78.26 g) was significantly different from T3 (mean = 65.13 g) ($p = 0.005$). T1 (mean = 76.21 g) was not significantly different from T2 ($p = 0.686$) or T3 ($p = 0.064$). T2 produced 20% heavier bulbs than T3, while T1 produced 17% heavier bulbs than T3.

For bulb diameter, one-way ANOVA revealed a significant treatment effect ($F = 3.859$, $df = 2, 117$, $p = 0.024$). Tukey's HSD post-hoc test showed that T2 (mean = 65.42 mm) was significantly different from T3 (mean = 61.56 mm) ($p = 0.019$). T1 (mean = 64.39 mm) was not significantly different from T2 ($p = 0.771$) or T3 ($p = 0.122$). T2 produced 6% larger diameter bulbs than T3, while T1 produced 5% larger diameter bulbs than T3.

On-Farm Site: Khamaed 2

At Khamaed 2, one-way ANOVA revealed no significant treatment effect on bulb weight ($F = 1.537$, $df = 2, 117$, $p = 0.219$). Descriptive statistics showed that T2 recorded the highest mean bulb weight (98.58 g), followed by T1 (89.09 g), while T3 had the lowest (84.17 g). T2 produced 17% heavier bulbs than T3, while T1 produced 6% heavier bulbs than T3.

For bulb diameter, one-way ANOVA revealed a significant treatment effect ($F = 4.225$, $df = 2, 117$, $p = 0.017$). Tukey's HSD post-hoc test showed that T2 (mean = 68.33 mm) was significantly different from T3 (mean = 64.31 mm) ($p = 0.013$). T1 (mean = 66.62 mm) was not significantly different from T2 ($p = 0.238$) or T3 ($p = 0.147$). T2 produced 6% larger diameter bulbs than T3, while T1 produced 4% larger diameter bulbs than T3.

On-Farm Site: Khatoed

At Khatoed, there was no significant treatment effect on bulb weight ($F = 0.229$, $df = 2, 117$, $p = 0.796$) or bulb diameter ($F = 0.082$, $df = 2, 117$, $p = 0.922$). Descriptive statistics showed that T2 recorded the highest mean bulb weight (51.06 g), followed by T1 (47.27 g), while T3 had the lowest (44.16 g). T2 produced 16% heavier bulbs than T3, while T1 produced 7% heavier bulbs than T3. For bulb diameter, all treatments produced similar values (T1 = 50.96 mm, T2 = 51.37 mm, T3 = 50.56 mm)

A summary of yield parameters across all sites is presented in Table 1.6. At Khamaed 1 and Khamaed 2, significant treatment effects were observed for bulb diameter, with T2 consistently producing the largest bulbs. At Khamaed 1, significant effects were also observed for bulb weight, with T2 producing the heaviest bulbs. At the on-station site and Khatoed, no significant differences were detected, though descriptive trends consistently favored the treated plots over the control.

Table 1.6. Summary of yield parameters across all sites.

Site	Parameter	T1 (Targeted)	T2 (Calendar)	T3 (Control)	ANOVA p
On-Station	Weight (g)	62.41	63.86	55.21	0.174
	Diameter (mm)	58.18	58.83	56	0.26
Khamaed 1	Weight (g)	76.21 ab	78.26 a	65.13 b	**0.007****
	Diameter (mm)	64.39 ab	65.42 a	61.56 b	**0.024***
Khamaed 2	Weight (g)	89.09	98.58	84.17	0.219
	Diameter (mm)	66.62 ab	68.33 a	64.31 b	**0.017**
Khatoed	Weight (g)	47.27	51.06	44.16	0.796
	Diameter (mm)	50.96	51.37	50.56	0.922

**Values followed by different letters within a row are significantly different ($p < 0.05$, Tukey's HSD).*

Primary Objective: Efficacy of Potassium Phosphite

Both T1 and T2 significantly reduced disease compared to T3, as confirmed by severity analysis at 179 DAS ($p = 0.012$) and 193 DAS ($p = 0.006$), and by AUDPC analysis where both treatments differed significantly from the control. Potassium phosphite effectively controls garlic rust in on - station conditions, aligning with previous reports of phosphite efficacy against rust diseases in *Allium* crops (Huang et al., 2020; Li et al., 2025). Its dual mode of action - direct antifungal activity plus induced resistance - provides consistent protection (Guest & Grant, 1991; Saldarriaga-Gómez et al., 2025)). This finding is particularly significant for organic regions like Gasa and Gelephu Mindfulness City (GMC), where synthetic fungicides are restricted. The induced resistance mechanism is especially valuable as it does not rely on direct pathogen killing, reducing resistance risk and aligning with organic certification requirements.

Secondary Objective: Targeted vs. Calendar-Based Schedules

T1 and T2 are statistically equivalent for severity at all time points ($p > 0.05$) and for AUDPC ($p = 0.391$). Neither schedule is superior; both provide equivalent disease control. However, T1 used 78% fewer sprays (2 vs. 9 through 207 DAS) and was approximately 5-fold more efficient (6.35% vs. 1.30% severity reduction per spray). This equivalence aligns with findings in other pathosystems: targeted late blight management in potato reduced fungicide use by 30 - 66% while maintaining control (Faye Ritchie et al., 2018; Van Evert et al., 2017); targeted wheat rust applications at flag leaf emergence provided equivalent control to multiple sprays (Braithwaite et al., 1998; Conference, 2001; Joshi et al., 2017). This study provides the first such demonstration for garlic rust in Bhutan.

The targeted schedule succeeds due to precise timing. Spray 1 at 95 DAS (8 weeks before disease onset) allows systemic distribution, defense priming via PR proteins and phytoalexins, and reservoir formation in older tissue (Eshraghi et al., 2011; Mohammadi et al., 2019). Spray 2 at 150

DAS is applied exactly when rust appears (confirmed by T3 onset at 151 DAS), replenishing phosphite in expanding foliage and boosting defenses during early bulbing (Hunter, 2018; Paveley et al., 1994). In contrast, the calendar schedule suffers from suboptimal timing, dilution effects during rapid spring growth, and potential 14-day coverage gaps that allow infection (Lázaro et al., 2021; Schepers., 2004). These disadvantages explain why increasing spray frequency beyond critical timing does not proportionally improve control.

1.3.4 Conclusion

Potassium phosphite effectively controls garlic rust through induced resistance. The targeted schedule (2 sprays at 95 and 150 DAS) provides disease control statistically equivalent to the intensive calendar-based schedule (9 sprays) while using 78% fewer applications. Although not statistically significant, descriptive yield trends show that treated plots produced 13-16% heavier bulbs and 4-5% larger diameter bulbs compared to the untreated control, indicating that rust suppression translated into improved yield outcomes. The targeted schedule is recommended for October-planted garlic in temperate regions, offering optimal disease control with minimal fungicide input, reduced costs, lower environmental impact, and decreased risk of fungicide resistance development. This strategy is particularly well-suited for organic production zones like Gasa and GMC, where sustainable disease management is a priority.

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1.4 Evaluation of Organic Compatible fungicides for the Management of Apple Fungal Diseases

1.4.1 Introduction

Apple (*Malus domestica*) is one of the most important temperate fruit crops in Bhutan and serves as a major source of income for rural farming households (Choden & Shahnawaz, 2015). The crop is cultivated by more than 8,058 households and produced approximately 2,317 metric tons in 2023 (National Statistics Bureau, 2023). Despite its economic importance, sustainable apple production in Bhutan is increasingly constrained by fungal diseases that reduce canopy health, fruit yield, quality, and storage potential (Rai, 2024). Major diseases affecting Bhutanese orchards include apple scab caused by *Venturia inaequalis*, powdery mildew caused by *Podosphaera leucotricha*, apple rust caused by *Gymnosporangium* spp., and *Alternaria* leaf spot caused by *Alternaria mali* and *Alternaria alternata* (Filajdić & Sutton, 1991; Lee et al., 2011; National Plant Protection Centre [NPPC], 2017; Rai, 2024). Together, these pathogens create a season-long disease complex that weakens orchards and reduces productivity.

Apple scab remains one of the most destructive apple diseases worldwide (MacHardy et al., 1993; Bowen et al., 2010) and has been a major disease of apple in Bhutan since the 1990s (NPPC, 2017). Severe infections cause premature defoliation, fruit blemishes, and significant reductions in marketable yield. Similarly, powdery mildew reduces tree vigor through infection of young shoots and leaves, while apple rust and *Alternaria* leaf spot further contribute to foliage damage and fruit quality deterioration (NPPC, 2017; Rai, 2024). The concurrent occurrence of these diseases complicates orchard management because environmental conditions favorable for one pathogen often support the development of others throughout the growing season (Peter, 2023).

Current disease management practices in Bhutan rely mainly on orchard sanitation and scheduled fungicide applications (NPPC, 2017; Yuden, 2009). Cultural practices such as burning or burying fallen leaves, application of urea after leaf fall, and dormant-season pruning are commonly used to reduce pathogen inoculum (NPPC, 2017). Chemical control largely depends on fungicides such as hexaconazole, mancozeb, carbendazim, captan, and copper-based products. Although these fungicides provide partial disease control, continuous reliance on limited fungicide groups raises concerns regarding environmental contamination, fungicide resistance, and long-term sustainability. In particular, repeated use of copper compounds may lead to copper accumulation in soil and negatively affect beneficial microbial communities.

Bhutan's long-term vision of promoting environmentally sustainable and organic agriculture has increased the need for safer and organic-compatible disease management strategies. Among the fungicides of interest are copper sulphate, lime sulphur, and potassium bicarbonate, all of which are recognized internationally for their broad-spectrum activity and compatibility with sustainable production systems (National Organic Programme [NOP], 2019). Copper sulphate acts mainly as a protective fungicide by inhibiting fungal spore germination (Pscheidt, 2023), whereas lime sulphur possesses both preventive and curative properties, especially against overwintering

inoculum (Montag et al., 2005). Potassium bicarbonate has also shown promising activity against diseases such as apple scab and powdery mildew through disruption of fungal growth and sporulation (Ilhan et al., 2006; Speiser & Tamm, 2006). Integration of these fungicides could reduce dependence on synthetic fungicides and minimize environmental risks associated with excessive copper use.

Despite their recognized efficacy internationally, limited information is available regarding the performance of copper sulphate, lime sulphur, and potassium bicarbonate under Bhutanese orchard conditions. Important knowledge gaps remain concerning the efficacy of lime sulphur locally, reduced copper application strategies, and the role of potassium bicarbonate in integrated disease management programs. Therefore, this study aims to evaluate the in-vitro and field efficacy of copper sulphate, lime sulphur, and potassium bicarbonate against major fungal diseases of apple in Bhutan. The findings are expected to contribute to the development of sustainable and organic-compatible disease management strategies that enhance orchard resilience, reduce environmental impacts, and support long-term apple production in Bhutan.

1.4.2 Materials and Method

In-vitro Study

i. Isolation of Alternaria spp. from Naturally Infected Apple Leaves

Leaves exhibiting characteristic symptoms of Alternaria leaf spot, including dark brown to black necrotic lesions with concentric rings, were collected from apple orchards located in Thimphu, Paro, and Haa dzongkhags. Samples were collected from different parts of multiple trees to capture pathogen variability and were placed in labelled paper envelopes indicating sample code, apple variety, location, and date of collection.

Isolation of the pathogen was conducted at the National Plant Protection Referral Laboratory following standard isolation procedures with minor modifications. Small tissue sections (approximately 0.5 cm diameter) containing both diseased and healthy leaf tissue were excised using a sterile scalpel. The tissue sections were surface sterilized by immersion in 70% ethanol for 30 seconds, followed by 1% sodium hypochlorite for 1 minute, and subsequently rinsed three times with sterile distilled water.

The sterilized tissues were transferred onto water agar (WA) medium prepared at 18 g L^{-1} and incubated at 20 ± 2°C in darkness for 5-7 days. Emerging fungal colonies were observed under a stereomicroscope, and hyphal tips from individual colonies were transferred onto potato dextrose agar (PDA) plates for purification. Pure cultures were obtained through repeated sub-culturing and were identified based on colony morphology and microscopic characteristics of conidia and mycelia. Confirmed *Alternaria* spp. isolates were maintained on PDA slants at 4°C until further use.

ii. In-vitro Fungicidal Assay against Alternaria spp.

The efficacy of lime sulphur, copper sulphate, captan, and mancozeb against *Alternaria* spp. was evaluated using the poisoned food technique described by Nene and Thapliyal (1979) with minor modifications. Captan and mancozeb were included as synthetic fungicide controls, while non-amended PDA served as the untreated control.

Stock solutions of each fungicide were prepared in sterile distilled water according to the manufacturer's recommended concentration. Appropriate volumes of each stock solution were mixed thoroughly with molten PDA cooled to approximately 45-50°C to obtain the desired fungicide-amended media. The amended media were poured into sterile 90 mm Petri plates and allowed to solidify under aseptic conditions.

Mycelial discs (10 mm diameter) were cut from the actively growing margins of 5–7day old *Alternaria* cultures and placed upside down at the center of each fungicide-amended plate. Plates containing PDA without fungicide served as untreated controls. Each treatment was replicated five times.

The inoculated plates were incubated at 20 ± 2°C in darkness for 8 days. Plates were arranged in a completely randomized design and rotated periodically within the incubator to minimize positional effects. Colony diameter was measured daily along two perpendicular directions until the fungal growth in the control plates reached the edge of the Petri dish.

The percentage inhibition of mycelial growth over the untreated control was calculated using the following formula:

$$Percent\ Inhibition\ (\%) = \frac{(C - T)}{C} \times 100$$

Where:

- **C** = average radial growth of the fungus in the untreated control
- **T** = average radial growth of the fungus in fungicide-amended plates

The experiment was repeated independently to ensure reproducibility of the results.

Field Study

i. Experimental Site

The field experiment was conducted in apple orchards located at the National Centre for Organic Agriculture, Yusipang, and in two farmer orchards located at Maedwang Gewog, Thimphu, and Dokar Gewog, Paro. The selected orchards represented major apple-growing environments and were maintained under natural disease pressure conditions.

ii. Experimental Design

The field experiment was laid out in a Randomized Complete Block Design consisting of six treatments with four replications (blocks). Each experimental unit consisted of three contiguous apple trees per treatment plot. To minimize spray drift and cross-contamination between adjacent plots, one untreated buffer tree was maintained between treatment plots within each block. Buffer trees were excluded from disease assessment and statistical analysis.

Treatments were randomly assigned within each block to ensure unbiased comparison among treatments.

Figure 1.9. Orchard layout and treatment application at NCOA

iii. Treatments

The study consisted of six treatments, as presented in Table 1.7. The treatments were applied using a power knapsack sprayer. The fungicides were applied at the following concentrations: lime sulphur at 2 ml per litre of water, copper sulphate at 1 g per litre of water, and potassium bicarbonate at 5 g per litre of water.

Table 1.7. Treatment list and application timing

Apple growth stages	Treatments					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Dormant	*Lime sulphur*	-	-	*Lime sulphur*	-	*(Untreated control)*
Silver tip-green tip	-	*Copper Sulphate*	*Copper Sulphate*	-	*Copper Sulphate*	-
Tight cluster	-	-	*Potassium Bicarbonate*	*Potassium Bicarbonate*	*Potassium Bicarbonate*	-
Petal Fall	-	-	*Potassium Bicarbonate*	*Potassium Bicarbonate*	*Potassium Bicarbonate*	-
Early fruit set	-	-	*Potassium Bicarbonate*	*Potassium Bicarbonate*	*Potassium Bicarbonate*	-

Data Collection

Disease Severity Assessment

Disease severity assessments were conducted for apple scab, apple rust, and alternaria leaf blotch at approximately three-week intervals throughout the growing season. Approximately four assessments were made in total.

For leaf assessments, 100 leaves per plot (approximately 33 leaves per tree) were randomly selected from different canopy levels and visually evaluated for disease severity. Severity was estimated as the percentage of diseased leaf area affected by each pathogen.

For fruit assessments, 50 fruits per plot were randomly sampled and visually assessed for disease symptoms and severity.

The AUDPC was calculated for each disease using the following formula:

$$AUSPC = \sum_{i=1}^{N_i-1} (\frac{y_i + y_{i+1}}{2})(t_{i+1} - t_i)$$

Where:

- y_i= disease severity at the i^{th}assessment
- t_i= time at the i^{th}assessment

- n= total number of assessments

Fruit disease severity was further evaluated using the Townsend–Heuberger formula:

$$S(\%) = \frac{\sum (n_i v_i)}{NV} 100$$

Where:

- n_i= disease severity rating
- v_i= number of fruits in each category
- V= total number of fruits assessed
- N= highest disease rating

Disease ratings were categorized as:

- 0 = no visible symptoms
- 1 = mild infection
- 2 = moderate infection
- 3 = severe infection

The final disease assessment was conducted four weeks after the last fungicide application.

Disease Incidence

Disease incidence for apple scab, rust, and Alternaria leaf blotch was recorded separately on leaves and fruits.

For leaf incidence, 100 leaves per tree were randomly selected across different canopy levels and examined for the presence or absence of disease symptoms.

Disease incidence was calculated using the formula:

$$Incidence\ (\%) = \left(\frac{Number\ of\ infected\ leaves}{Total\ number\ of\ leaves\ examined}\right) \times 100$$

For fruit incidence, 50 fruits per tree were randomly selected and evaluated similarly using the formula:

$$Incidence\ (\%) = \left(\frac{Number\ of\ infected\ fruits}{Total\ number\ of\ fruits\ examined}\right) \times 100$$

Data Analysis

a. In-vitro Study

Radial growth data obtained from the poisoned food assay were used to calculate the Percent Inhibition of Mycelial Growth (PIMG). The data violated assumptions of normality (Shapiro-Wilk test, $p < 0.001$) and homogeneity of variances (Levene's test, $p = 0.043$). Therefore, a non-

parametric Aligned Rank Transform (ART) ANOVA was employed to analyze the effects of Treatment (four fungicides: Captan 50% WP, Copper sulphate 50% WP, Lime sulphur 22% SC, and Mancozeb 75% WP) and Concentration

(100, 250, 500, and 1000 ppm) on mycelial inhibition (%). The ART procedure ranks the data after aligning them to remove main effects and interactions, allowing for a robust analysis of factorial designs without assuming normality. Post-hoc pairwise comparisons were conducted using

Tukey's Honestly Significant Difference (HSD) test with ART-derived marginal means. All tests were considered significant at $\alpha = 0.05$.

b. Field Study

Field data on disease incidence, disease severity, and AUDPC values for apple scab, rust, and alternaria leaf blotch were analyzed using Analysis of Variance (ANOVA) in R statistical software version 4.4.2 (R Core Team, 2024).

Repeated measures ANOVA using mixed-effect models was performed to evaluate disease progression across multiple assessment dates. Whenever treatment effects were significant, mean comparisons among treatments were conducted using Bonferroni-adjusted post-hoc tests through the “lsmeans” package (Lenth & Lenth, 2018).

All statistical analyses were performed at a 5% level of significance.

1.4.3 Results and Discussion

In-Vitro efficacy of fungicides on radial growth of Alternaria spp.

Mycelial growth of *Alternaria* spp. was significantly affected by fungicide treatment, concentration, and their interaction (ART ANOVA; $p < 0.001$), indicating that fungicide efficacy varied with concentration.

Across both isolates (DK101 and SS101), mycelial growth inhibition generally increased with increasing fungicide concentration. Captan and mancozeb were the most effective fungicides, producing the highest levels of inhibition across concentrations. Complete inhibition of isolate DK101 was achieved by both fungicides at 1000 ppm, whereas for isolate SS101, complete inhibition was observed only with captan at 1000 ppm; mancozeb achieved 92.4% inhibition at the same concentration.

copper sulphate exhibited moderate antifungal activity, with inhibition increasing progressively with concentration and reaching complete inhibition at 1000 ppm for both isolates. In contrast, lime sulphur consistently showed the lowest efficacy, with maximum inhibition of 64.9% and 56.6% against DK101 and SS101, respectively, at 1000 ppm.

Overall, captan and mancozeb were the most effective fungicides against *Alternaria* spp., followed by copper sulphate, while lime sulphur was significantly less effective. The results demonstrate a clear concentration-dependent response for all fungicides tested.

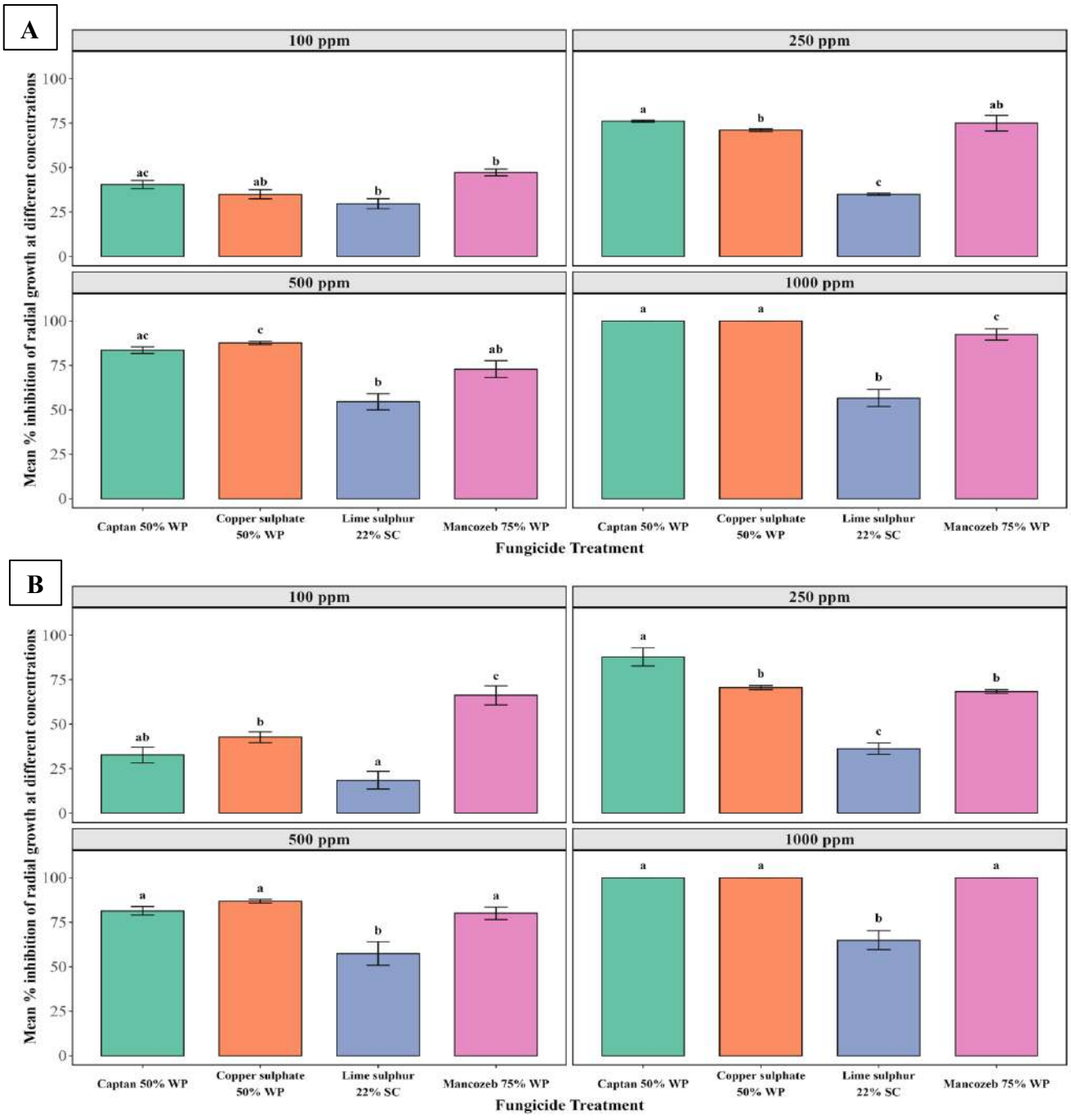

Figure 1.10. Effect of fungicide treatments at different concentrations on radial growth inhibition of *Alternaria* spp. isolates DK101 (A) and SS101 (B).

Field efficacy of fungicides on fungal pathogens

The apple fungal disease management trial was established at NCOA-Yusipang, Sisinang, and Dawakha to evaluate the efficacy of selected fungicides against apple scab, powdery mildew, rust, and Alternaria leaf blotch. During the reporting period, two rounds of disease assessments were completed following fungicide applications.

Preliminary observations indicated variation in disease incidence and severity among treatments across the trial sites. In general, treated plots showed lower disease development compared to untreated controls, suggesting a positive response to fungicide application. However, disease progression varied among locations and diseases, likely due to differences in environmental conditions and disease pressure.

As the trial is still ongoing, the current observations represent only a portion of the seasonal disease development. Additional assessments on leaves and fruits will be conducted during the remaining growing season to capture the full impact of treatments on disease management and fruit protection. Final statistical analysis and interpretation will be undertaken upon completion of all scheduled observations.

1.4.4 Conclusion

The study demonstrated that fungicide treatment and concentration significantly influenced the mycelial growth of *Alternaria* spp. isolates DK101 and SS101. Among the fungicides evaluated, the reference fungicides captan and mancozeb exhibited the highest inhibitory activity, confirming their effectiveness against *Alternaria* spp. under in vitro conditions. copper sulphate also showed substantial antifungal activity, achieving complete inhibition at the highest concentration tested, while lime sulphur was comparatively less effective. Inhibition generally increased with increasing fungicide concentration, indicating a dose-dependent response. These findings suggest that copper sulphate has potential for the management of *Alternaria* spp. and warrants further evaluation under field conditions. However, the efficacy of fungicides under orchard conditions should be confirmed through ongoing field trials before management recommendations are made.

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1.5 HLB Screening of Citrus Germplasm at the National Citrus Repository, Tsirang

1.5.1 Introduction

During FY 2025-2026, a total of 284 citrus samples were collected from the National Citrus Repository (NCR) and subjected to confirmatory testing for Huanglongbing (HLB). The primary objective of HLB testing is to support the production and maintenance of disease-free planting materials in Bhutan. This testing program was initiated in the FY 2017-2018 following the recommendations of the New Citrus Nursery Development Proposal meeting held on 3rd May 2017. All samples were processed using standard laboratory protocols, including DNA extraction followed by detection of HLB-associated pathogens using real-time qPCR. The test results also contribute to the national HLB surveillance and support efforts to prevent and manage the spread of diseases in citrus growing regions.

Based on the qPCR test results generated by the National Plant Protection Centre (NPPC), the National Citrus Repository is mandated to rogue all HLB-positive plants to maintain disease-free mother plants. Healthy mother plants are subsequently used to produce disease-free seedlings, which are supplied to the National Seed Centre for mass multiplication. This integrated system strengthens HLB surveillance, safeguards the health of citrus germplasm and supports the production of certified disease-free planting materials in Bhutan.

1.5.2 Materials and Method

Sampling Method

Citrus plants exhibiting characteristic symptoms of HLB, including yellowing, blotchy mottling and lopsided fruits, were selected for sampling. Samples were collected during two major flushing periods to maximize the likelihood of pathogen detection. From each selected plant, one leaf sample and one bark sample were collected. The leaf sample comprised approximately 10-16 leaves depending on leaf size, while the bark sample consisted of 5 to 7 strips measuring approximately 1-2 cm in width and 3 to 5 cm in length, taken from the main trunk of the tree.

Immediately after collection, all samples were placed in sterile sample bags containing desiccant to preserve sample quality and were subsequently stored under refrigerated conditions until further processing. Each sample was carefully labelled with a plant identification number assigned in the laboratory.

Figure 1.11. Samples collected from ARDSC-Tsirang

Molecular detection

DNA was extracted from all samples using the CTAB method. Real-time PCR was conducted using HLB-specific primers and probes (Li et al., 2006). An internal control plant mitochondrial DNA (COX gene) was also included to verify DNA quality and reaction performance.

- HLBas: 5'- TCG AGC GCG TAT GCG AAT ACG-3'
- HLBr: 5'-GCG TTA TCC CGT AGA AAA AGG TAG-3'
- HLB probe: 5'-(6FAM) AGA CGG GTG AGT AAC GCG (BHQ1)-3'

COX internal control (plant mitochondrial DNA)

- COXf: 5'-GGTATGCCACGTCGCATTCCAGA-3'
- COXr: 5'-GAATGCCCTTAGCAGTTTTGGC-3'
- COX probe: 5'-ATCCAGATGCTTACGCTGG-3'

Figure 1.12. DNA extraction from the citrus samples

Real-time PCR result interpretation

qPCR results were interpreted based on cycle threshold (Ct) values. Samples with a Ct value of 0 or greater than 36 were considered negative (not detected**)**, while samples with a Ct values greater than 0 and less than or equal to 36 were considered positive for HLB. Plants that remained positive after a third qPCR test were retained as confirmed positive samples for future assay calibration and as positive controls for HLB diagnostics.

1.5.3 Results and Discussion

A total of 284 citrus samples were analyzed using qPCR for HLB detection. Samples with Ct values of 0 or greater than 36 were classified as negative, while those with Ct values greater than 0 and less than or equal to 36 were considered positive for HLB. Although all samples were successfully processed, the results were inconclusive due to inconsistent Ct values, likely associated with primer performance issues, which limited reliable classification of HLB status. Therefore, confirmatory testing during the next citrus flushing season is required. Final classification of plants will depend on the outcome of repeat testing, where only consistent and reproducible results will be used to give recommendations regarding mass propagation of disease-free planting material or removal of infected plants from the repository.

1.5.4 Conclusion

The qPCR analysis was completed for all 284 citrus samples; however, the results were considered inconclusive and could not be used to reliably determine the HLB status of the tested plants. The inconclusive results are due to inconsistent Ct values and a possible primer-related issue affecting amplification reliability. Consequently, the current dataset was insufficient to confidently classify the tested plants as either HLB positive or HLB free.

Given the uncertainty in the results, confirmatory testing is required before any management decisions are made. A second round of sampling and qPCR analysis during the next citrus flushing season is recommended to validate the infection status of the plants. Only upon obtaining consistent and reproducible results will the plants be approved for mass propagation as disease-free planting material, or recommended for removal from the repository if confirmed positive for HLB.

1.5.5 References

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1.6 Ad-Hoc Activities of the Pathology Program: Disease Diagnostics

1.6.1 Introduction

During the Fiscal Year 2025-2026, the Pathology Program under the National Plant Protection Centre conducted several ad-hoc disease diagnostic investigations in response to emerging disease outbreaks and farmer reports received from different Dzongkhags. The diagnostic activities focused on important crops including tomato, potato, ginger, wheat, and large cardamom, with samples submitted by farmers, research centres, and extension agencies for laboratory confirmation and management recommendations.

Laboratory investigations involved a combination of visual symptom assessment, wet tissue incubation, pathogen isolation on artificial growth media, microscopic examination, rapid diagnostic kits, and gram staining techniques. These analyses enabled the identification of diverse disease-causing agents, including fungal, oomycetous, and bacterial pathogens associated with seedling mortality, rhizome rot, tuber soft rot, head blight, leaf blight, and anthracnose diseases. Several cases also highlighted the role of environmental conditions, poor drainage, and insect infestation in aggravating disease development and crop losses.

Major findings from the diagnostic investigations confirmed the occurrence of damping-off in tomato caused by *Rhizoctonia* spp., bacterial soft rot in potato associated with Gram-negative bacterial pathogens, ginger rhizome rot caused by a disease complex involving *Pythium* spp. and *Fusarium* spp., Fusarium Head Blight in wheat, and co-infection of *Curvularia* spp. and *Colletotrichum* spp. in large cardamom plantations. These findings demonstrated the increasing complexity of plant disease problems affecting different cropping systems across the country.

The disease diagnostic services provided timely technical recommendations to farmers and stakeholders through integrated disease management approaches emphasising sanitation, crop rotation, healthy planting materials, drainage improvement, balanced nutrient management, and judicious fungicide use where necessary. Overall, the ad-hoc diagnostic activities strengthened rapid response capacity for emerging plant health issues and contributed to informed disease management decisions aimed at minimising crop losses and improving agricultural productivity.

1.6.2 Tomato Seedling Disease Diagnosis – Paro

During the reporting period, the Pathology Program received tomato seedling samples from a semi-commercial farm in Sharpa (Shaba) Gewog, Paro, after farmers reported severe wilting and mortality in both nursery and transplanted seedlings. The affected plants showed characteristic symptoms including complete wilting and dark brown to black discoloration around the collar region just above the soil line. The disease persisted despite the use of mancozeb root dip treatment during transplanting, raising concerns regarding the presence of a soil-borne pathogen affecting seedling establishment and field survival.

Figure 1.13. Dark brown to black discolouration on the stem, often at or above the soil line

Diagnostic Methods and Findings

The submitted samples were examined through visual observation, wet tissue incubation, isolation on Potato Dextrose Agar, Gram staining, and Pythium rapid test kits. Wet tissue incubation did not show any visible microbial growth; however, isolation on PDA resulted in the development of both fungal and bacterial colonies. Microscopic observation of the fungal culture revealed hyphal structures consistent with *Rhizoctonia* spp., a common soil-borne pathogen associated with damping-off disease in vegetables. Gram staining indicated the presence of Gram-negative bacteria, while the Pythium diagnostic kit tested negative, ruling out *Pythium* as the primary causal agent.

Diagnosis and Interpretation

Based on laboratory observations and cultural characteristics, the disease was diagnosed as damping-off caused primarily by *Rhizoctonia* spp. The pathogen is known to survive in soil and infected plant debris, particularly under conditions of high soil moisture, poor drainage, and continuous cropping. The occurrence of disease both in nursery and field conditions indicated that the pathogen was likely present in the growing medium or field soil prior to transplanting. Secondary bacterial association was also suspected in some infected tissues.

Figure 1.14. Hyphae with septum and spores of *Rhizoctonia* spp

Recommendations and Follow-up

To minimize future outbreaks, farmers were advised to use certified disease-free seeds and sterilized nursery media, practice crop rotation with non-host crops, and improve field drainage to reduce soil moisture buildup. Recommendations also included prompt removal of infected seedlings, maintenance of proper plant spacing, and disinfection of tools and nursery equipment. Judicious use of fungicides such as carbendazim or hexaconazole as seed treatment or soil drench was also recommended during early crop establishment stages. Regular monitoring and awareness creation among growers were emphasized to support early detection and management of damping-off diseases in tomato nurseries.

1.6.3 Diagnosis of Potato Tuber Rot Samples from NSC Phobjikha

Background

The National Plant Protection Centre received potato tuber samples of the varieties Khangma Kaap and Desiree from the National Seed Centre (NSC), Phobjikha, following reports of severe tuber rotting during harvest. The affected seed lots were Generation-1 tubers produced from Generation-0 microtubers under greenhouse conditions. Significant post-harvest losses and rapid tuber deterioration raised concerns regarding the presence of bacterial soft rot pathogens affecting seed potato quality and storage potential.

Figure 1.15. Potato tuber samples. Khangma Kaap (Left) & Desiree (Right)

Diagnostic Methods and Findings

Laboratory diagnosis involved visual examination, bacterial streaming tests, isolation on Potato Dextrose Agar, and Gram staining. The tubers exhibited symptoms of common scab on the surface together with extensive internal soft rot characterised by mushy tissue breakdown, foul odour, and cream-colored bacterial ooze. Bacterial streaming tests produced milky-white threads from infected tissues, confirming high bacterial populations within the tubers. Isolation on PDA consistently produced mucoid cream-colored bacterial colonies without fungal growth, strongly indicating a bacterial aetiology. Microscopic examination following Gram staining confirmed the bacteria to be Gram-negative rod-shaped cells.

Figure 1.16. Brown discolouration inside and on the surface of the tuber (Desiree (Top left) & Khangma Kaap (Top right)). Severe rotting of the tubers progressing towards the pit of the tubers (Bottom Left). The semi-liquid, cream-colored ooze characteristic of bacterial soft rot, expressed from decayed potato tissue (Bottom right).

Diagnosis and Interpretation

The disease was diagnosed as bacterial soft rot, most likely caused by *Pectobacterium* spp. or *Dickeya* spp. The investigation further suggested that common scab lesions caused by *Streptomyces scabies* may have predisposed the tubers to secondary infection by soft rot bacteria through wounds and cracks on the tuber surface. The disease complex resulted in rapid tissue maceration and severe post-harvest losses, particularly under moist handling and storage conditions.

Recommendations and Follow-up

Recommendations focused on strict post-harvest sanitation and improved storage management. Infected and damaged tubers were advised to be removed immediately to reduce inoculum buildup during storage. Proper curing of harvested tubers before storage was recommended to facilitate wound healing and reduce bacterial entry. Improved ventilation, low-temperature storage, careful handling to minimise injuries, and field sanitation were also emphasised. Long-term recommendations included crop rotation, balanced nutrient management, use of healthy seed tubers, and prevention of excessive soil moisture to reduce disease incidence in future seed production cycles.

1.6.4 Ginger Rhizome Rot Diagnosis – Samdrup Jongkhar

Background

During the 2025 cropping season, widespread rhizome rot was reported in ginger-growing areas of Martshala and Orong Gewogs under Samdrup Jongkhar Dzongkhag. The disease caused substantial crop losses, affecting farmer livelihoods in one of the important ginger-producing regions of the country. Symptoms observed in the field included yellowing and drying of leaves, wilting of shoots, pseudostem collapse, and soft decaying rhizomes with foul odour. A total of 44 samples were submitted by ARDC Samtenling for laboratory diagnosis.

Diagnostic Methods and Findings

The samples underwent visual observation, pathogen isolation on PDA, microscopic examination, and rapid diagnostic testing using commercial *Pythium* kits. Laboratory isolation consistently yielded two distinct fungal colony types. One isolate produced rapidly growing cottony white colonies with broad aseptate hyphae and sporangial structures characteristic of *Pythium* spp. The second isolate produced pale pink colonies with septate hyphae and multicellular curved spores typical of *Fusarium* spp. The *Pythium* diagnostic kit further confirmed the presence of *Pythium* antigens in representative samples from all locations.

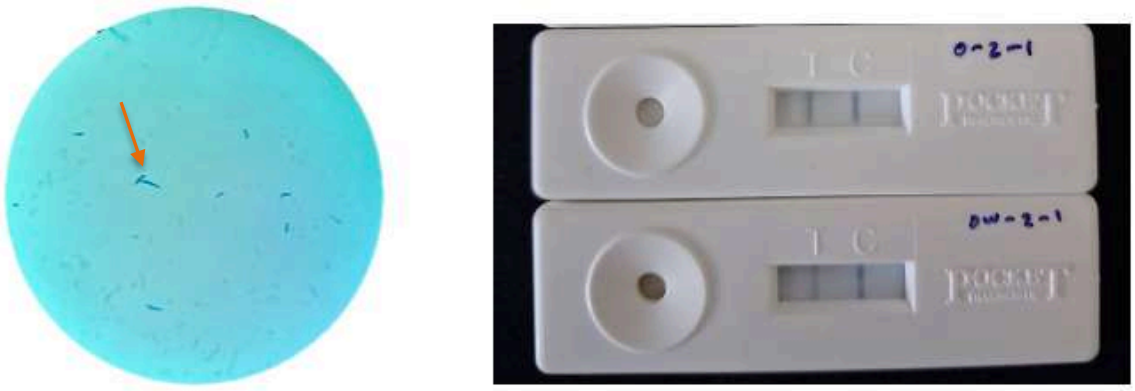

Figure 1.17. Fusarium spores (left) and Pythium diagnostic test kit showing positive test results (right)Diagnosis and Interpretation

The disease was diagnosed as a rhizome rot complex involving both *Pythium* spp. and *Fusarium* spp. The study also indicated that insect infestation and tunneling damage may have facilitated secondary infection and accelerated disease progression in affected rhizomes. Favorable environmental conditions such as poor drainage, waterlogging, continuous monocropping, and use of infected planting materials likely contributed to the severity of the outbreak.

Recommendations and Follow-up

Integrated disease management strategies were recommended for subsequent cropping seasons. Farmers were advised to improve drainage through raised beds, deep ploughing, and incorporation of organic matter to improve soil structure. The use of healthy rhizomes, warm water treatment before planting, crop rotation with non-host crops, and proper field sanitation were strongly emphasised. Recommendations also included balanced fertilizer application, drip irrigation, and use of biological or botanical products where possible. For severe outbreaks under conventional farming systems, targeted fungicide applications and rhizome treatments were suggested under regulated use conditions.

1.6.5 Wheat Head Blight Diagnosis – Bumthang

Background

A severe disease outbreak affecting wheat panicles was reported from Chhumig Gewog, Bumthang during crop maturity. The affected field exhibited extensive pinkish-grey discolouration of wheat heads, which later turned dark brown and appeared burnt from a distance. Farmers also reported a distinct fishy odour associated with infected panicles. The disease affected a newly introduced local wheat variety cultivated under riverside conditions, potentially favouring disease development under humid environmental conditions.

Figure 1.18. Symptoms observed in the field

Diagnostic Methods and Findings

The samples were subjected to wet filter paper incubation and pathogen isolation on PDA. Significant fungal growth was observed under both methods within three days of incubation. Microscopic examination revealed elongated sickle-shaped multi-septate macroconidia, branched septate hyphae, and chlamydospore-like structures characteristic of *Fusarium* species. These structures were consistently isolated from infected panicles and cultured tissues.

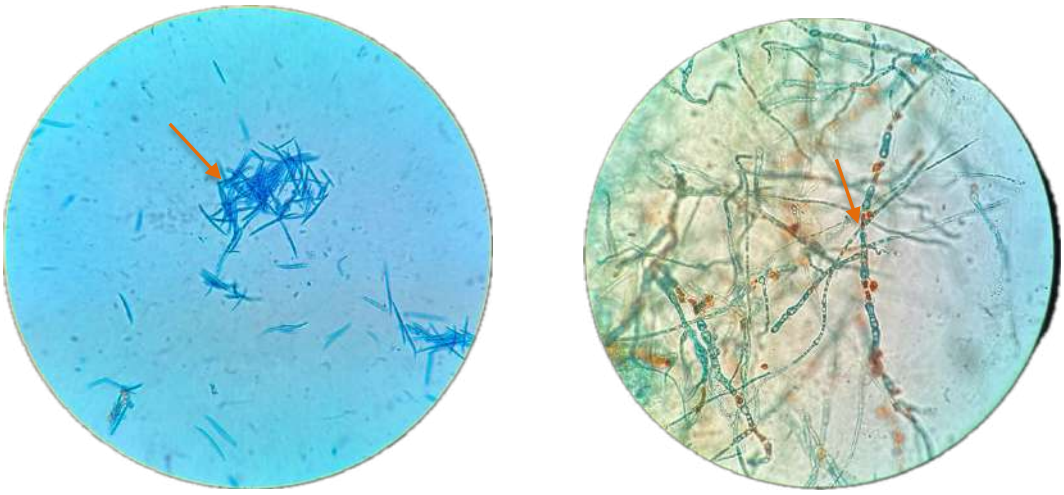

Figure 1.19. Macroconidia of *Fusarium* sp. (40×) (Left); Hyphae of *Fusarium* sp. (40×) (Right)

Diagnosis and Interpretation

Based on the observed symptoms and fungal morphology, the disease was diagnosed as Fusarium Head Blight (FHB), likely caused by *Fusarium graminearum*. The disease is known to thrive under humid conditions during flowering and grain filling stages and can significantly reduce grain quality and yield. The proximity of the field to riverside conditions and possible introduction of infected planting material may have contributed to disease establishment and spread.

Recommendations and Follow-up

Integrated disease management measures were recommended to reduce inoculum levels and prevent future outbreaks. Farmers were advised to avoid cereal-to-cereal rotation, particularly following maize or wheat crops, and instead rotate with legumes or non-host crops. Use of certified disease-free seed and seed treatment with fungicides such as carbendazim were recommended for the next planting season. Regular field monitoring, removal of infected crop residues, and proper weed management were also emphasized to minimize disease carryover and reduce future infection risk.

1.6.6 Diagnosis of Large Cardamom Disease Samples from Sarpang

Background

The NPPC laboratory received large cardamom samples from Chhudzom Gewog, Sarpang, following reports of severe leaf blight, leaf spot, stem lesions, and rhizome rot in plantations. The submitted samples also showed maggot infestation, indicating a possible interaction between pest damage and disease development. The disease incidence was reported from multiple locations and raised concerns regarding widespread fungal infection affecting plantation productivity.

Diagnostic Methods and Findings

Disease diagnosis involved visual observation, isolation on PDA, and microscopic examination of fungal structures. Laboratory cultures revealed dark pigmented fungal colonies with concentric zonation typical of *Curvularia* spp. microscopic examination showed curved, melanised, multi-septate conidia characteristic of *Curvularia eragrostidis*. In addition, hyaline cylindrical conidia and septate hyphae typical of *Colletotrichum* spp. were also identified from infected tissues.

Diagnosis and Interpretation

The disease was diagnosed as a co-infection of *Curvularia* leaf blight and *Colletotrichum*-associated anthracnose. The simultaneous occurrence of both fungal pathogens explained the complexity of symptoms observed in the field, including leaf blight, necrotic spots, stem lesions, and rhizome rot. High humidity, dense canopy growth, poor field sanitation, and insect infestation were considered major contributing factors favoring disease spread and establishment.

Recommendations and Follow-up

An integrated management approach was recommended, combining sanitation, cultural management, biological control, and selective fungicide use. Immediate removal and destruction of infected plant debris, pruning of old pseudostems, and improved airflow within plantations were advised to reduce inoculum buildup. Preventive use of Bordeaux mixture and neem-based products was recommended for disease suppression under organic systems. For severe infections under conventional systems, fungicides such as mancozeb, propiconazole, and carbendazim were recommended with caution and rotation strategies to prevent fungicide resistance development. Use of healthy planting materials and improved irrigation management were also emphasized for long-term disease prevention.

2 Entomology Program

2.1 Capacity Development of Farmers on Fall Armyworm Identification and Integrated Pest Management in Maize

2.1.1 Introduction

Fall armyworm (*Spodoptera frugiperda*) remains one of the most destructive invasive insect pests affecting maize production in Bhutan. Since its introduction into the country, the pest has spread rapidly across maize-growing regions owing to its high reproductive capacity, migratory behaviour, and adaptability to diverse agroecological conditions. Severe infestations result in extensive foliar damage, destruction of maize whorls and cobs, and significant yield losses, posing a serious threat to food security and the livelihoods of maize-growing communities.

Despite the availability of recommended management practices, effective field management continues to be constrained by limited farmer knowledge on pest identification, field scouting, economic threshold-based decision-making, and integrated pest management (IPM). Farmers often rely on calendar-based or indiscriminate pesticide applications, resulting in increased production costs, reduced pesticide efficacy, and adverse impacts on human health, beneficial organisms, and the environment.

To strengthen farmers' knowledge and capacity for sustainable management of fall armyworm, the National Plant Protection Centre (NPPC), through the AFACI PMP+ Project funded by the Rural Development Administration (RDA), Republic of Korea, implemented a series of farmers' training programmes on fall armyworm identification and integrated pest management during February–March 2026 in collaboration with Agriculture Research and Development Centres (ARCDs)

Objectives

The training programme aimed to:

- Enhance farmers' capacity to correctly identify fall armyworm
- Improve understanding of the pest's biology, life cycle, damage symptoms, and field ecology.
- Promote regular field scouting and early detection for timely intervention.
- Demonstrate integrated pest management strategies, including cultural, mechanical, biological, behavioural, and chemical control measures.
- Encourage the safe, rational, and need-based use of insecticides.
- Strengthen farmers' capacity to sustainably manage fall armyworm

2.1.1 Programme Implementation

The training programme was implemented across 13 gewogs in six dzongkhags, covering major maize-producing areas of the country. Training sessions were conducted in Sergithang gewog (Tsirang); Lauri and Gomdar Gewogs (Samdrup Jongkhar); Yangnyer Gewog, Udzorong, and Kanglung gewogs (Trashigang); Toedtsho and Khamdang gewogs (Trashi Yangtse); Chhaling and Chagsakhar gewogs (Monggar); and Nangkor, Bardo, and Goshing gewogs (Zhemgang).

A participatory extension approach was adopted, combining classroom lectures, practical field demonstrations, question-and-answer sessions, and farmer experience sharing. Training was delivered in local dialects to facilitate effective communication and encourage active participation. Technical sessions focused on field identification of fall armyworm, scouting and monitoring techniques, damage assessment, pheromone trap installation, conservation of natural enemies, safe pesticide handling, and integrated pest management practices.

2.1.2 Coverage and Participation

A total of 727 farmers, comprising 292 men (40.2%) and 435 women (59.8%), participated in the programme, demonstrating strong engagement of farming communities and the significant involvement of women in maize production and plant protection activities.

Table 2.1. Participation in Fall Armyworm Farmers' Training Programme

Dzongkhag	**Gewog(s) Covered**	**Farmers Trained**
Tsirang	Sergithang	15
Samdrup Jongkhar	Lauri and Gomdar	108
Trashigang	Yangnyer, Udzorong and Kanglung	158
Trashiyangtse	Toedtsho and Khamdang	94
Monggar	Chhaling and Chagsakhar	156
Zhemgang	Nangkor, Bardo and Goshing	196
Total	**13 Gewogs**	**727**

Figure 2.1. Group photo of farmers and trainers during the FAW management training at Yangnyer, Trashigang.

2.1.3 Major Technical Areas Covered

The training program emphasised practical knowledge and field-based skills required for sustainable management of fall armyworm in maize. Major topics included:

- Biology, ecology, and global spread of fall armyworm.
- Identification of different life stages and distinguishing characteristics from other maize caterpillars.
- Identification of damage symptoms and infestation levels.
- Field scouting techniques and monitoring for early detection.
- Installation and use of pheromone traps for pest surveillance.
- Cultural, mechanical, biological, and chemical management options.
- Conservation of natural enemies and promotion of biological control.
- Safe handling, mixing, and application of recommended insecticides.
- Principles and implementation of Integrated Pest Management (IPM).

Hands-on demonstrations enabled participants to identify characteristic morphological features of fall armyworm larvae, install pheromone traps, and correctly prepare pesticide solutions for field application.

Figure 2.2. Trainers explaining the key morphological features of Fall Armyworm larvae to farmers during the training session at Khamdang, Trashiyangtse

Figure 2.3. Trainer explaining the damage symptoms of Fall Armyworm larvae to farmers during the training session at Lauri, Samdrup Jongkhar

Figure 2.4. Hands-on demonstration of pheromone trap placement by trainer (left) and participant (right) during FAW training

Figure 2.5. Practical demonstration: Participant preparing recommended pesticide solution under supervision, ensuring correct dosage.

2.1.4 Key Achievements

The programme successfully strengthened the technical capacity of maize farmers in managing fall armyworm through integrated pest management approaches. Major achievements included:

- Training was delivered across 13 gewogs in six dzongkhags, covering key maize-growing regions of Bhutan.
- 727 farmers equipped with practical knowledge and skills on fall armyworm identification and management.
- Improved farmer capacity to distinguish fall armyworm from other maize caterpillars using key diagnostic characteristics.
- Enhanced understanding of field scouting, monitoring techniques, and early intervention strategies.
- Increased awareness of integrated pest management, emphasising reduced dependence on chemical pesticides.
- Improved knowledge of safe pesticide handling, dosage calculation, sprayer calibration, and appropriate application techniques.
- Strengthened collaboration between NPPC, ARDCs, extension officers, and farming communities in promoting sustainable pest management.

2.1.5 Outcomes and Impact

The training programme contributed significantly to improving farmers' awareness and preparedness for managing fall armyworm outbreaks. Participants demonstrated increased confidence in identifying the pest, recognising infestation symptoms, and selecting management interventions based on field observations instead of relying exclusively on pesticide applications.

Practical demonstrations enhanced farmers' understanding of scouting methods, pheromone trap use, pesticide calibration, and integrated pest management practices. Farmers also showed greater appreciation for conserving natural enemies and adopting preventive cultural practices as part of sustainable pest management.

The programme strengthened linkages between researchers, extension personnel, and farming communities, laying the foundation for continued technical support, surveillance, and dissemination of improved pest management technologies. Although standardised pre- and post-training assessments were not conducted across all training sites, participant feedback and field observations indicated substantial improvements in knowledge, awareness, and confidence in managing fall armyworm.

2.1.6 Challenges

Several challenges were identified during programme implementation:

- Limited baseline knowledge of fall armyworm identification among farmers.
- Low awareness of economic threshold-based decision-making and biological control options.
- Need for continued technical backstopping and follow-up support during the maize growing season.

2.1.7 Lessons Learned

The programme demonstrated that participatory and field-based learning approaches are highly effective in enhancing farmer understanding and encouraging adoption of improved pest management practices. Practical demonstrations, interactive discussions, and farmer-to-farmer knowledge sharing were more effective than classroom lectures alone. Conducting training before the cropping season also improved knowledge retention and facilitated immediate application of the recommended practices under field conditions.

2.1.8 Way Forward

Building on the success of the programme, NPPC will continue to strengthen farmer capacity by expanding training to other maize-growing areas, establishing field demonstration plots, promoting biological control and conservation of natural enemies, improving access to pheromone traps and recommended pest management inputs, and introducing standardised pre- and post-training evaluations to measure programme impact. Continued collaboration with ARDCs, extension officers and local communities will further enhance early detection, surveillance, and sustainable management of fall armyworm in Bhutan.

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

2.2.1 Introduction

The fall armyworm (*Spodoptera frugiperda* J.E. Smith) is a highly destructive polyphagous pest native to the tropical and subtropical regions of the Americas and has emerged as a major threat to agricultural production worldwide following its rapid invasion of Africa and Asia (Goergen et al., 2016). Since its first detection in Bhutan in 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) because it provides the data needed for forecasting pest outbreaks, determining optimal timing for control measures, and reducing unnecessary pesticide applications (Boni et al., 2026). Among available monitoring tools, sex pheromone traps have been widely used for early detection and surveillance of *Spodoptera frugiperda* male moth populations worldwide (Tingle & Mitchell, 1975). These traps utilise synthetic female sex pheromones to attract males, enabling estimation of adult population dynamics and assessment of potential infestation risks in the field (Van Den Berg et al., 2021).

In Bhutan, studies on fall armyworm have so far focused mainly on documenting its occurrence, host plant, and farmer perceptions (Zangpo et al., 2024), with limited quantitative data on seasonal population patterns. Understanding the incidence and fluctuations of the FAW population 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 fall armyworm proliferation (Mahat et al., 2021). This study aims to assess fall armyworm 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 Method

Study Area

The study was conducted at Rilangthang under Sergithang Gewog, Tsirang Dzongkhag, during the 2026 maize-growing season. The gewog is one of the major maize-producing areas in the country and is characterised by a warm subtropical climate that provides favourable conditions for maize cultivation as well as the establishment and proliferation of Fall Armyworm (*Spodoptera frugiperda*). The study site comprised approximately 14 acres of maize field selected for continuous monitoring of adult FAW populations throughout the cropping season. Fourteen

monitoring locations were established across the study area to ensure adequate spatial coverage and representative sampling of FAW populations. The geographical coordinates of each trap location were recorded using the My Altitude application and are presented in Table 2.2.

Table 2.2. GPS coordinates of pheromone trap locations used for Fall Armyworm monitoring in Rilangthang, Sergithang Gewog, Tsirang Dzongkhag

Sl. No	Trap ID	GPS coordinates
1	1	27° 7′ 44″ N 90° 4′ 8″ E
2	2	27° 7′ 43″ N 90° 4′ 9″ E
3	3	27° 7′ 41″ N 90° 4′ 12″ E
4	4	27° 7′ 40″ N 90° 4′ 14″ E
5	5	27° 7′ 43″ N 90° 4′ 17″ E
6	6	27° 7′ 44″ N 90° 4′ 18″ E
7	7	27° 7′ 46″ N 90° 4′ 19″ E
8	8	27° 7′ 46″ N 90° 4′ 17″ E
9	9	27° 7′ 48″ N 90° 4′ 16″ E
10	10	27° 7′ 50″ N 90° 4′ 15″ E
11	11	27° 7′ 48″ N 90° 4′ 14″ E
12	12	27° 7′ 49″ N 90° 4′ 11″ E
13	13	27° 7′ 46″ N 90° 4′ 11″ E
14	14	27° 7′ 45″ N 90° 4′ 13″ E

Figure 2.6. Study area and distribution of pheromone trap locations used for monitoring fall armyworm populations in Rilangthang, Sergithang Gewog, Tsirang Dzongkhag

Study Design and Duration

A longitudinal field study was conducted from March to June 2026 to monitor the population dynamics of Fall Armyworm (FAW) using sex pheromone traps. The monitoring period encompassed the major maize-growing season and was designed to capture changes in adult moth abundance across different crop growth stages. Repeated observations were made throughout the study period to assess temporal fluctuations in FAW populations and to generate baseline information on seasonal pest incidence in the study area. Biweekly monitoring was undertaken to ensure consistent data collection and to facilitate the identification of population peaks and trends over time.

Pheromone Trap Design and Deployment

Adult male FAW moths were monitored using funnel-shaped Phero-Sensor™ traps, which are specifically designed for the capture and retention of lepidopteran pests. These traps were selected due to their durability, trapping efficiency, and ability to minimise specimen damage, thereby facilitating accurate identification of captured insects. A total of fourteen traps were deployed across approximately 14 acres of maize cultivation area, maintaining a density of one trap per acre.

Figure 2.7. Sex pheromone trap used for monitoring *Spodoptera frugiperda* (left) and installation of the trap in the field (right)

Traps were strategically positioned within the maize fields to ensure representative sampling of moth populations while minimising potential edge effects. To avoid overlap and interference among pheromone plumes, a minimum distance of 50 m was maintained between adjacent traps. Trap height was maintained at approximately 1.5–2.0 m above ground level and was adjusted periodically throughout the growing season to remain approximately 20 cm above the maize canopy. This ensured optimal exposure of the pheromone plume and maximised trapping efficiency as the crop developed. Trap installation, placement, and maintenance procedures were carried out in accordance with the protocols for FAW pheromone-based monitoring (Sisay et al., 2024a).

Pheromone Lure

Each trap was baited with a commercially available synthetic sex pheromone lure supplied by PHEROMONE CHEMICALS, India. The lure formulation consisted of (Z)-9-tetradecenyl acetate (Z9-14Ac) (87.0%), (Z)-11-hexadecenyl acetate (Z11-16Ac) (12.5%), and (Z)-7-dodecenyl acetate (Z7-12Ac) (0.5%). These compounds mimic the natural sex pheromone released by female FAW moths and are highly attractive to adult males, making them suitable for population monitoring and early detection purposes.

To maintain lure effectiveness, all lures were handled with disposable gloves to prevent contamination and stored under refrigeration before field deployment. Pheromone lures were replaced every 45 days in accordance with the manufacturer's recommendations. Details of lure installation and replacement dates were systematically recorded to ensure consistency throughout the monitoring period.

Data Collection and Laboratory Identification

Insect samples were collected from all pheromone traps on a biweekly basis throughout the study period. During each sampling event, captured insects were carefully removed from the traps using soft brushes and forceps to prevent damage to specimens. The collected insects were then transferred into labelled collection bottles indicating the trap identification number and date of collection. Samples were transported to the laboratory in insulated cool boxes to preserve specimen quality and prevent deterioration during transport.

In addition to insect collection, detailed field observations were recorded during each monitoring visit. Information collected included trap identification number, date of observation, geographical coordinates of trap locations, crop growth stage, and the number of insects captured per trap. Environmental parameters including temperature, relative humidity, and wind speed were also documented using data obtained from the AccuWeather application. These variables were recorded to facilitate assessment of potential relationships between environmental conditions and FAW population dynamics.

Figure 2.8. Bi-weekly collection of insect samples from sex pheromone traps at Rilangthang

Upon arrival at the laboratory, all collected specimens were sorted and examined systematically. Insects were first separated into FAW and non-target insect groups. The total number of FAW moths and non-target insects captured in each trap was recorded for every observation period. Adult FAW moths were identified under a stereomicroscope based on standard morphological characteristics, particularly the distinctive forewing markings and wing patterns described in published identification keys. Careful identification was undertaken to ensure the accuracy and reliability of monitoring data and to minimise errors arising from the presence of morphologically similar species. All field and laboratory data were subsequently compiled and entered into a database for analysis.


Figure 2.9. Laboratory sorting and identification of samples from the sex pheromone traps

Data Analysis

Data collected throughout the monitoring period were organised and analysed using descriptive statistical methods. The abundance of Fall Armyworm (FAW) and non-target insect species captured in pheromone traps was summarised for each observation period. Mean FAW catch per trap was calculated to assess changes in moth populations over time and to facilitate comparisons among sampling dates. Temporal trends in FAW abundance were examined using tables, graphical representations, and summary statistics to identify seasonal fluctuations and periods of peak moth activity, thereby providing insights into the population dynamics of FAW throughout the maize-growing season.

Ethical Considerations

The study involved the monitoring and collection of insect specimens using pheromone traps and did not involve human participants, vertebrate animals, or activities posing significant environmental risks. All monitoring activities were conducted with the knowledge and consent of the field owners. Trap deployment, sampling, and specimen handling procedures were undertaken in a manner that minimised disturbance to the crop and surrounding environment. Collection, handling, and disposal of specimens were carried out in accordance with standard entomological research practices and institutional guidelines.

2.2.3 Results and Discussion

Population Dynamics of Fall Armyworm

A total of seven adult fall armyworm moths were captured in sex pheromone traps during the monitoring period, with moth activity recorded only on 23rd April and 21st May, 2026, while no captures were observed on the remaining monitoring dates. These findings indicate that the adult FAW population remained low throughout the study period, with only brief periods of activity. The absence of moths during late March and early April may be attributed to low population establishment, limited availability of suitable host plants, and unfavourable environmental conditions. In contrast, the increase in trap catches during late April and May coincided with the vegetative growth stage of maize, which provides suitable conditions for oviposition and larval development. Similar seasonal fluctuations in FAW populations have been reported in other studies, where crop phenology, climatic conditions, and the availability of host plants strongly influenced adult abundance (Sisay et al., 2024a; Thu Phuong et al., 2024).

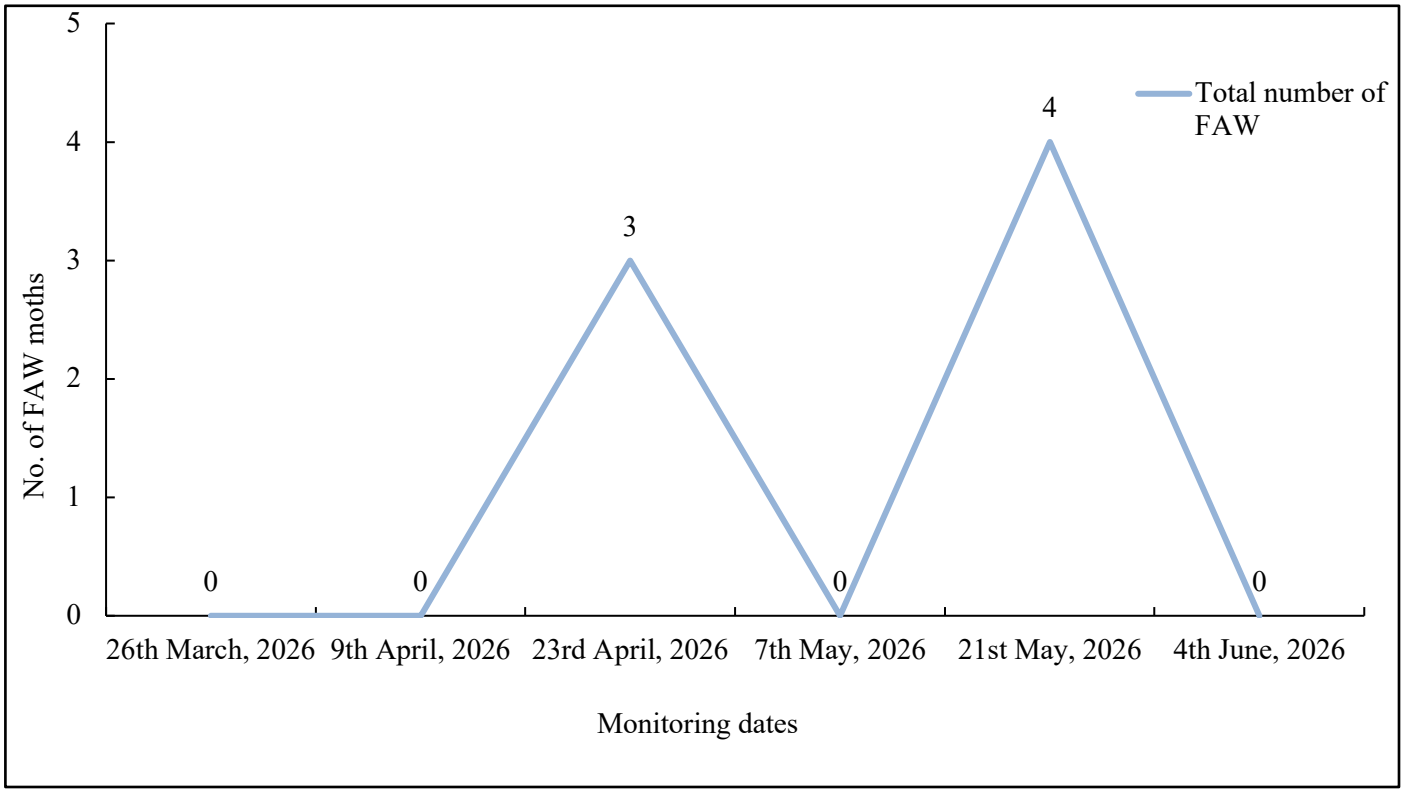

Figure 2.10. Temporal population dynamics of FAW moths captured in sex pheromone traps

Population Dynamics of Non-target Species

A total of 841 non-target insect individuals were captured in the sex pheromone traps during the monitoring period. The highest number of non-target species was recorded on 23rd April (369 individuals), followed by 26th March (228 individuals), 7th May (122 individuals), and 9th April (116 individuals). In contrast, only three individuals were captured on both 21st May and 4th June, indicating a sharp decline in non-target captures toward the end of the monitoring period. This temporal variation suggests that the abundance of non-target insects was influenced by seasonal changes in environmental conditions, insect emergence patterns, and the availability of food and habitat resources. Although sex pheromone traps are designed to attract male fall armyworm, they may inadvertently capture other insect taxa due to incidental attraction, trap design, or increased insect activity during certain periods (Sisay et al., 2024a). Previous studies have similarly reported that the composition and abundance of non-target insects captured in pheromone traps vary considerably over time and are influenced by habitat characteristics, seasonal phenology, and climatic factors (Sisay et al., 2024b; Witzgall et al., 2010). These findings indicate that while pheromone traps are highly valuable for monitoring FAW populations, they also provide useful information on the temporal dynamics of non-target insect communities and their seasonal fluctuations.

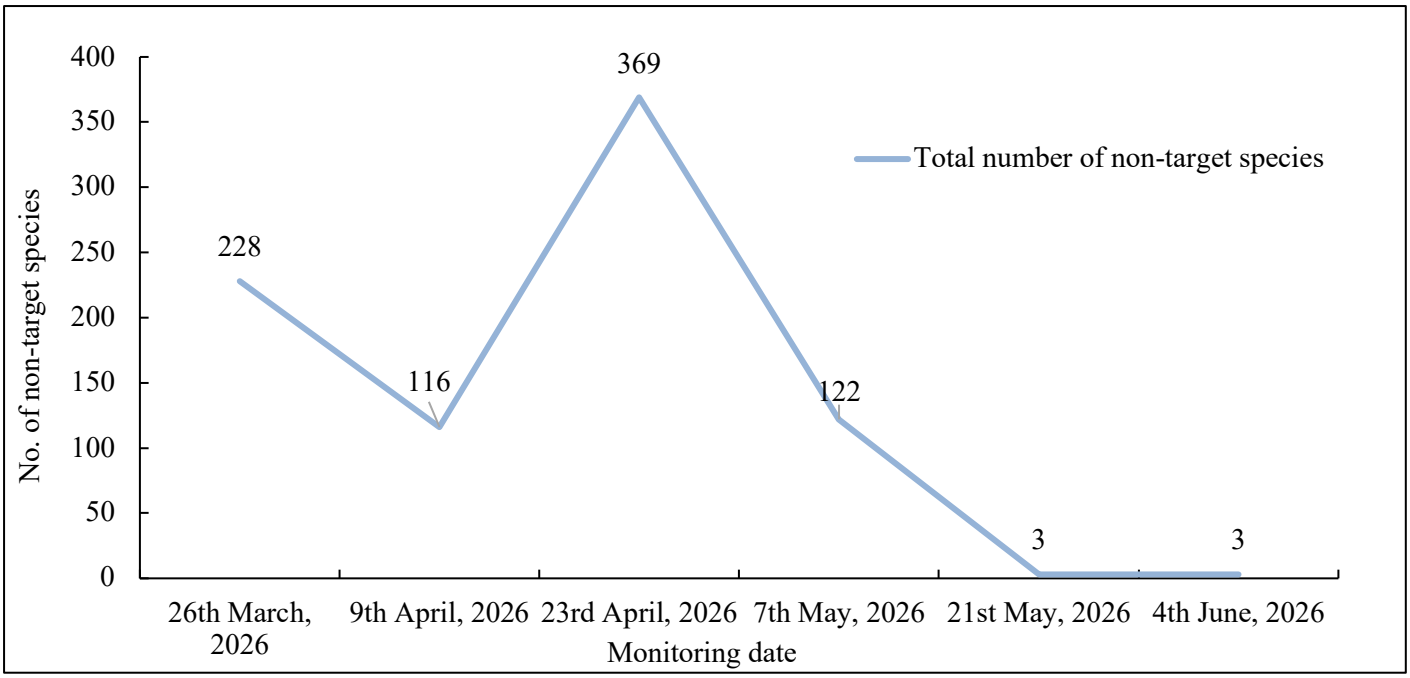

Figure 2.11. Temporal population dynamics of non-target species captured in sex pheromone traps

Relationship Between Environmental Variables and Insect Abundance

Spearman's rank correlation analysis showed that fall armyworm (FAW) abundance was not significantly correlated with any of the environmental variables measured. FAW abundance exhibited very weak positive correlations with temperature ($\rho = 0.051$, $p = 0.923$) and wind speed ($\rho = 0.189$, $p = 0.720$), and a negligible negative correlation with relative humidity ($\rho = –0.017$, $p = 0.974$). Similarly, non-target species abundance showed weak, non-significant correlations with temperature ($\rho = 0.103$, $p = 0.846$) and relative humidity ($\rho = –0.338$, $p = 0.512$). However, a very strong positive and statistically significant correlation was observed between non-target species abundance and wind speed ($\rho = 0.956$, $p = 0.003$).

The absence of significant relationships between FAW abundance and the measured environmental variables suggests that adult FAW populations during the study period were likely influenced by factors other than temperature, relative humidity, and wind speed alone, such as crop phenology, host plant availability, and seasonal migration (Early et al., 2018). In contrast, the significant positive correlation between non-target species abundance and wind speed indicates that wind may have influenced the movement of flying insects or the efficiency of pheromone traps in capturing non-target species. Previous studies have shown that wind conditions can substantially affect insect flight behaviour and pheromone plume structure, thereby influencing trap catches and capture efficiency (Östrand et al., 2001; Reardon et al., 2006). However, because the present analysis was based on a limited number of observations, these findings should be interpreted with caution, and additional studies with larger datasets are required to confirm the observed relationship.

2.2.4 Conclusion

The present study demonstrated that the population of fall armyworm (*Spodoptera frugiperda*) remained low in Rilangthang, Sergithang Gewog, Tsirang, during the 2026 maize-growing season, with adult moth activity detected only during late April and May. The observed population peaks coincided with the vegetative stage of maize, indicating that crop phenology may play a greater role in influencing FAW occurrence than the environmental variables measured in this study. Although temperature, relative humidity, and wind speed did not show significant relationships with FAW abundance, a strong positive correlation was observed between wind speed and non-target insect captures, suggesting that environmental conditions may influence the movement and trapping efficiency of non-target insects.

Overall, the findings provide valuable baseline information on the seasonal occurrence of FAW in one of Bhutan's important maize-growing areas and highlight the effectiveness of sex pheromone traps as a reliable tool for early detection and population monitoring. Despite the limited number of FAW captures and the relatively short monitoring period, the study contributes to the understanding of FAW population dynamics in Bhutan and supports the integration of pheromone-based surveillance into integrated pest management (IPM) programmes. Future studies involving

longer monitoring periods, multiple agroecological zones, and additional environmental and agronomic factors are recommended to strengthen pest forecasting and improve sustainable FAW management strategies.

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2.3 Monitoring the population dynamics of plant and leaf hoppers in paddy fields at ARDC Bajo, Wangdue Phodrang using the light trap

2.3.1 Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops in the world (Hu et al., 2017; Kumar et al., 2022). It provides a major source of daily calories for nearly half of the global population (Abdelsalam et al., 2025). Because of its importance for food security, stable rice production is essential for livelihoods, especially in developing countries where rice is a primary food source (YAO et al., 2020). However, rice production is continuously affected by many insect pests that reduce both yield and grain quality (Skawsang et al., 2019). Understanding these pest problems is important for improving rice productivity and ensuring food security.

Among the different insect pests of rice, sap-sucking insects such as planthoppers and leafhoppers are among the most highly destructive pests of rice (Haider et al., 2021). The major species include the Brown Planthopper (*Nilaparvata lugens*), White-Backed Planthopper (*Sogatella furcifera*), Zig Zag Leaf Hopper (*Recilia dorsalis),* and Green Leafhoppers (*Nephotettix virescens*). These insects feed on the phloem sap of rice plants, which weakens the plant and disrupts nutrient transport. This feeding leads to symptoms such as yellowing, stunting, reduced tillering, and, in severe cases, "hopper burn." In addition to direct damage, these insects also act as vectors of important viral diseases such as rice tungro, ragged stunt, and grassy stunt, which can cause severe yield losses. Their strong migratory ability allows them to spread quickly and cause sudden outbreaks in rice-growing regions.

In Bhutan, rice is a key crop for food security and rural livelihoods. Most farmers depend on rice cultivation for household consumption and income. Despite its importance, information on how planthopper and leafhopper populations change during the rice-growing season is still limited. Most pest management decisions are based on general regional data, which may not fully represent Bhutan's local agro-ecological conditions. These conditions vary across valleys and altitudes, which can influence pest development and abundance. As a result, pest control measures may not always be applied at the right time, leading to reduced effectiveness and unnecessary pesticide use.

Monitoring insect populations is an important part of integrated pest management (IPM). It helps in understanding when pest populations increase and which species are dominant during different crop stages. Light traps are widely used for monitoring sap-sucking insects because they attract insects using light and allow continuous and standardised collection of adult populations. Compared to traditional methods such as field scouting and sweep netting, light traps are easier to use and provide more consistent long-term data. They are also useful for identifying peak activity periods and seasonal changes in pest populations.

Despite their usefulness, there is still limited information on the seasonal patterns and species composition of rice planthoppers and leafhoppers across different rice growth stages in Bhutan. In particular, there is a need to understand how these pest populations change from the early vegetative stage to maturity under local field conditions. Therefore, this study was conducted at

the paddy field of the Agriculture Research and Development Centre (ARDC) Bajo, Wangdue Phodrang, using light traps to investigate the population dynamics and species composition of rice planthoppers and leafhoppers. Specifically, the study aims to (i) examine changes in hopper populations across rice phenological stages and (ii) identify the dominant species and their seasonal variation. The findings are expected to support better timing of pest management and improve site-specific integrated pest management strategies for rice production in Bhutan.

2.3.2 Materials and Method

Study Design

This study employed a quantitative research design to assess the population dynamics and abundance of rice planthoppers and leafhoppers using light-trap-based monitoring at the Agriculture Research Development Centre (ARDC) Bajo paddy field in Wangdue Phodrang.

Monitoring Sites and Period

A Light trap was installed on 11 July 2025 at:

• ARDC Bajo On-station (27°29'24.84" N, 89°53'54.69" E, 1187 m asl), Thedtsho Gewog, Wangdue Phodrang Dzongkhag

Monitoring was conducted from July (vegetative stage of rice) through November (maturity stage of rice) 2025, corresponding to the complete rice growing season in the region. This temporal window was selected to capture pest dynamics across all the crop phenological stages.

Participants and Stakeholders

This study was conducted under the AFACI (Asian Food and Agriculture Cooperation Initiative) (PMP+) project. Field operations were carried out with the cooperation and support of management and regional plant protection officers of ARDC Bajo, who assisted with trap operation, insect collection, and preliminary field observations. Laboratory identification and analysis of hopper specimens were performed by staff of the Entomology and Biocontrol program at the National Plant Protection Centre (NPPC), Department of Agriculture, Ministry of Agriculture and Livestock, Bhutan.

Instruments and Equipment

Light Trap Design:

Light traps were constructed from green-painted mild steel with the following specifications: overall dimensions of 40 cm × 40 cm × 25 cm, a funnel measuring 30 cm in length and 5 cm in diameter, and a 20 W incandescent bulb positioned 20 cm above the trap roof to optimize insect attraction. A collection box containing removable trays was positioned at the bottom of the funnel

to hold trapped specimens. Traps were installed at the edge of paddy fields and sheltered to prevent rain interference.

Data Collection Procedures

Operation and Specimen Collection:

Light traps were operated daily during the monitoring period, switched on at dusk (18:00 hours) and off at dawn (06:00 hours). This daily operation was performed manually by staff and the regional plant protection officers at ARDC Bajo. Trapped insects were collected every two weeks starting two weeks after trap installation (to allow for initial population establishment) and continuing at fortnightly intervals until crop maturity.

Hopper Identification

Trapped hoppers were transported to the laboratory at NPPC, where they were segregated and morphologically identified. Four target hopper species were recorded and counted: (1) Green Leafhoppers (GLH) comprising *Nephotettix virescens*, (2) Brown Planthopper (BPH) - *Nilaparvata lugens*, (3) White-Backed Planthopper (WBPH) - *Sogatella furcifera*, and (4) Zigzag Leafhopper (ZZLH) - *Recilia dorsalis*. Adult hoppers were counted and recorded separately by species; immature stages were not included in this analysis due to difficulties in reliable field-level identification.

Crop Phenological Stages

Rice phenological stages were categorised according to Zangpo et al. (2025) the following: tillering (hopper catches in August), booting (September), grain filling (October), and maturity (November).

Data Analysis

Hopper population data were analysed using descriptive statistical methods based on bi-weekly count data collected throughout the monitoring period. Population dynamics were examined using time-series line graphs to illustrate temporal trends across rice crop growth stages. For each hopper species, peak abundance and the timing of peak occurrence were identified. Species composition was determined by calculating the total abundance of each species recorded over the study period. All data cleaning and organisation, analysis, and graphical visualisation were performed using Microsoft Excel (version 2021).

Ethical Considerations and Permissions

This study was conducted with formal permission from the Program Director, ARDC Bajo. As the research involved monitoring insect populations without human or vertebrate subjects, formal ethical approval was not required. No pesticides or other chemical interventions were applied in the monitored plots during the study period, ensuring that observations reflected natural pest dynamics. All insect specimens were collected solely for identification and research purposes.

2.3.3 Results and Discussion

Hopper Population Dynamics Across Rice Phenological Stages

The temporal dynamics of hopper populations across different rice growth stages are presented in Figure 2.12. Hopper populations were at zero during the initial Tillering stage and stayed relatively low through the early vegetative stages (*Stem Elongation* and *Panicle Initiation*), with populations remaining below 5 individuals for all observed species. Population began to increase significantly from the booting stage, with a highly pronounced primary peak occurring during the heading-flowering stage, which aligns with the findings of Padala et al. (2024) stating the heading-flowering stage of the paddy has a peak hopper population.

All four hopper species reached their maximum seasonal abundance during the Heading-Flowering stage. White-Backed Planthopper (WBPH) exhibited the highest peak with 113 hoppers, followed by Brown Planthopper (BPH) with 38 hoppers, Green Leafhopper (GLH) with 18 hoppers, and Zigzag Leafhopper (ZZLH) with 11 hoppers. Following this initial peak, populations experienced a sharp decline during the Milk stage, where WBPH dropped to 31 hoppers, and all other species fell below 12 individuals. A distinct secondary resurgence was observed during the Dough stage, most notably for WBPH, which rose to a secondary peak of 62 hoppers, and BPH, which increased to 22 hoppers. Populations then fell drastically by the mature stage, with all species reaching minimal counts near crop maturity.

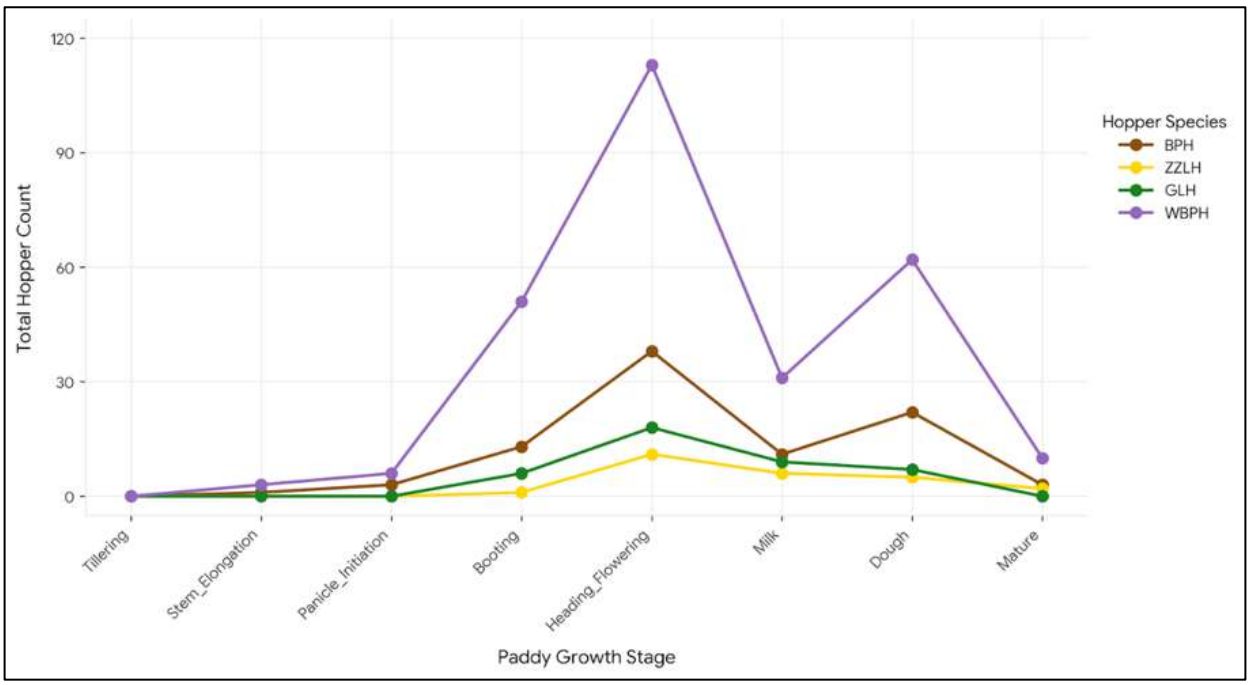

Figure 2.12. Hopper Population Dynamics Across Rice Phenological Stages

Figure 2.13. From left to right: WBPH, ZZLH, BPH, and GLH

Species Composition Across Rice Phenological Stages

The proportional distribution of hopper species varied considerably across different rice growth stages. No hoppers were recorded during the initial Tillering stage. During the early vegetative growth periods (*Stem Elongation* and *Panicle Initiation*), the hopper population was shared exclusively between the White-Backed Planthopper (WBPH) and the Brown Planthopper (BPH). WBPH was the dominant species, followed by the BPH and then the GLH.

As rice plants progressed toward reproductive stages, the hopper populations increased for all four hopper species, which aligns with the study Zhong-Xian et al. (2006) stating that hopper populations in the paddy usually peak during the reproductive stage of the rice. During the Booting stage, WBPH continued to dominate at 71%, followed by BPH at 19%, GLH at 8%, and a minor presence of ZZLH at 2%. At the Heading-Flowering stage, WBPH comprised 63% of the hopper population, while BPH stabilized at 21%, GLH reached 10%, and ZZLH increased to 6%.

This multi-species presence persisted through the grain-filling period. During the Milk and Dough stages, WBPH remained the primary species (60% and 65%, respectively). ZZLH peaked during the milk stage at 10% before dipping slightly to 5% at the dough stage. Conversely, GLH contributed 11% at the milk stage and 5% at the dough stage, while BPH consistently held a substantial share of 19-24% across both stages.

By the Mature stage, the community population shifted significantly. GLH populations completely disappeared (0%), while BPH maintained a strong presence at 20%. Notably, ZZLH reached its highest proportional abundance of the entire crop cycle at 13%, while WBPH constituted the remaining 67% of the total hopper assemblage at crop maturity.

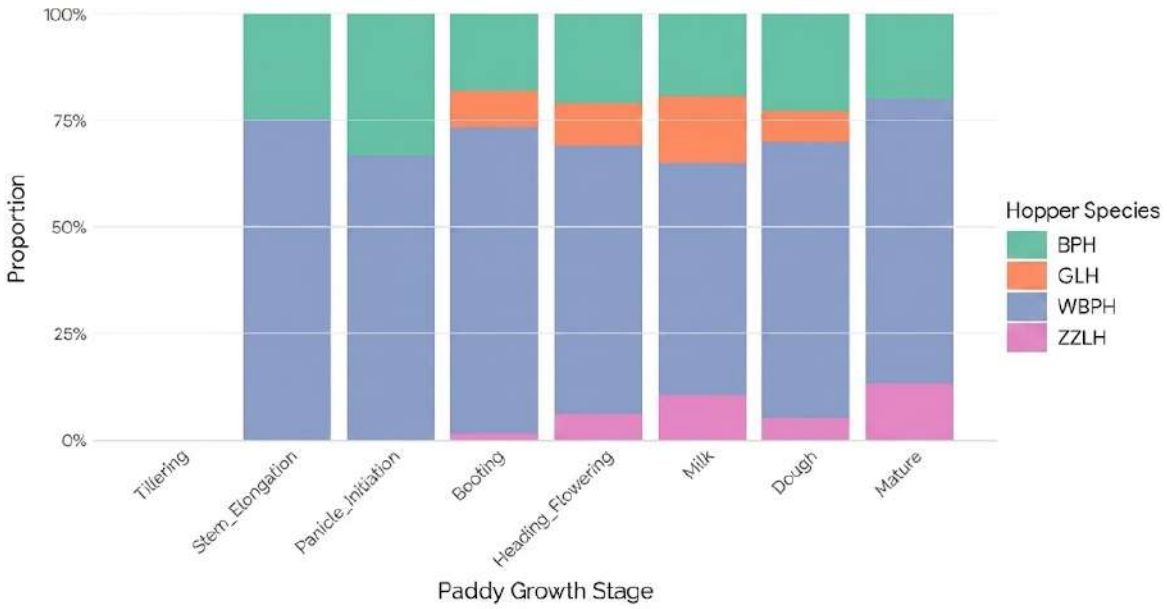

Figure 2.14. Hopper Species Composition Across Rice Phenological Stages

2.3.4 Conclusion

This study successfully monitored the population dynamics and species composition of rice planthoppers and leafhoppers in the paddy fields of ARDC Bajo, Wangdue Phodrang, throughout the 2025 rice-growing season using light traps. The findings demonstrated that hopper populations varied considerably across different rice phenological stages. Populations remained absent or very low during the early vegetative stages but increased rapidly as the crop entered the reproductive phase, reaching their highest abundance during the heading-flowering stage before declining towards crop maturity. A smaller secondary increase was also observed during the dough stage, particularly for White-Backed Planthopper and Brown Planthopper.

Among the four monitored species, the White-Backed Planthopper (*Sogatella furcifera*) was consistently the dominant species throughout the monitoring period, accounting for the largest proportion of the hopper community across nearly all crop stages. The Brown Planthopper (*Nilaparvata lugens*) was the second most abundant species, whereas Green Leafhopper (*Nephotettix virescens*) and Zigzag Leafhopper (*Recilia dorsalis*) occurred at comparatively lower densities. The study also revealed shifts in species composition as the rice crop matured, with Green Leafhopper disappearing at the maturity stage while Zigzag Leafhopper increased in relative abundance.

The observed seasonal patterns indicate that the reproductive stages of rice, particularly the heading–flowering stage, represent the most critical period for hopper infestation under the agroecological conditions of ARDC Bajo. These findings provide valuable baseline information on the seasonal occurrence of major hopper pests in Bhutan and highlight the usefulness of light traps as an effective tool for long-term monitoring and early detection of pest population build-up.

The information generated from this study can support the development of timely, evidence-based integrated pest management (IPM) strategies by identifying periods of hopper burn risk and improving the timing of surveillance and control measures. Continued multi-season monitoring across different rice-growing regions of Bhutan, together with the integration of climatic variables and disease incidence, is recommended to strengthen pest forecasting systems and enhance sustainable rice production.

2.3.5 References

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2.4 Laboratory evaluation of different concentrations of Emamectin benzoate 5% SG against the Fall Armyworm (*Spodoptera frugiperda*) in Bhutan

2.4.1 Introduction

In Bhutan, maize (*Zea mays* L.) is the second most produced cereal crop after paddy, cultivated by 36,613 farmers on 22,849 acres, with a total production of 27,636 MT in 2024 (NSB, 2024). Beyond serving as a staple food, maize provides an important source of household income for many farming communities (Manda et al., 2018; Thanh et al., 2004). This dependence on maize for both food security and livelihoods makes the crop highly vulnerable to production losses caused by insect pests, a concern that has intensified across South Asia following the rapid spread of several invasive lepidopteran species (Deshmukh et al., 2020; Divya et al., 2021)

Among these invasive pests, the fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith, has emerged as one of the most destructive threats to maize production worldwide. Native to the Americas, FAW has rapidly expanded across Africa and Asia since 2016 (Kenis et al., 2023; WAN et al., 2021). The species is highly polyphagous, feeding on more than 350 plant species belonging to over 76 families (Ajmal et al., 2024; Altaf et al., 2022; G.K. & Krishnan, 2025; Togola et al., 2025). However, maize is considered its most preferred host, with larvae capable of damaging plants throughout all growth stages, from seedling emergence to reproductive development. Severe infestations can result in substantial yield losses, and states that the absence of effective control measures could lead to economic losses of up to 8.3-20.6 million tonnes annually, equivalent to 21-53% of production (Kumar et al., 2022). In Bhutan, FAW was first recorded in Guma Gewog, Punakha, in September 2019 and has subsequently established itself as one of the major insect pests of maize production (Mahat et al., 2021).

The widespread establishment of FAW has made effective management a priority for maize production in Bhutan. Among the available integrated pest management (IPM) strategies, chemical control remains one of the principal approaches, with Chlorantraniliprole 18.5% SC and Emamectin benzoate 5% SG currently recommended for FAW management in Bhutan. Emamectin benzoate, an avermectin-group insecticide, acts primarily on glutamate-gated chloride channels (GluCls) within the insect nervous system, enhancing chloride ion influx and disrupting neurotransmission, ultimately causing paralysis and death (Chen et al., 2025; S. Patil et al., 2024).

Despite its widespread use and demonstrated efficacy, concentration-specific dose-response information for Emamectin benzoate against FAW under Bhutanese laboratory conditions remains unavailable. In particular, locally derived estimates of the median lethal concentration (LC_{50}), the concentration required to kill 50% of a test population, were lacking. Establishing such baseline susceptibility data is important because it enables the optimisation of field application rates, provides a quantitative benchmark for future insecticide resistance monitoring, and supports evidence-based IPM recommendations for maize growers. In the absence of locally generated dose-response data, recommendations for pesticide application must rely largely on manufacturer

guidelines or studies conducted under different agroecological conditions, which may not accurately reflect the susceptibility of Bhutanese FAW populations.

The present study was therefore conducted to evaluate the potency of a concentration gradient of Emamectin benzoate 5% SG against third-instar FAW larvae under controlled laboratory conditions in Bhutan. Survival analysis and Probit regression were conducted to characterise the dose-mortality relationship over time. Specifically, the study aimed to (i) determine larval survival and mortality patterns across a range of Emamectin benzoate concentrations at successive observation intervals, (ii) establish the dose-response relationship and estimate LC_{50} values at 6, 12, 24, 48, 72, and 96 hours after treatment, and (iii) generate a baseline toxicological reference that can support dose optimisation and future resistance management strategies for FAW control in Bhutan. It was hypothesised that larval mortality would increase in both a concentration- and time-dependent manner, with higher concentrations producing more rapid knockdown and mortality.

2.4.2 Materials and Method

Fall Armyworm Culture and Maize Plants

The Fall Armyworm larvae used in this study originated from the office colony at the National Plant Protection Centre (NPPC), Semtokha (27°26′26″ N, 89°39′49″ E, 2,269 m asl). FAW larvae were reared on an artificial diet inside an incubator held at a constant 27 °C and 70% relative humidity (Abbas et al., 2022; Ouda, 2024; Rindiani et al., 2024) to allow adult emergence, mating, and oviposition. Progeny from this colony were subsequently reared through to the third larval instar (L3), the stage selected for bioassay because L3 larvae were able to be handled readily and fed vigorously, which helped ensure uniform exposure to the treatments. Fresh maize leaves used in the bioassay were sourced from pesticide-free maize plants (local variety) of the NPPC greenhouse, providing an untreated host substrate for larval feeding trials.

Test Insecticide and Preparation of Concentrations

Emamectin benzoate 5% SG was selected for evaluation as it is one of the principal pesticides recommended for FAW management in Bhutan (*Maize - Pests of Bhutan*, n.d.). A concentration gradient was prepared through serial dilution, a laboratory technique used to generate a series of solutions with systematically decreasing concentrations from a single stock solution, ensuring high accuracy, minimal preparation error, and a scientifically standardized dose gradient suitable for establishing a dose-response relationship and calculating the LC_{50} (Kusano et al., 2024; Kushwaha, 2022; Long et al., 2023; R. G. Patil & Tayde, 2022).

A master stock solution of 100 mg a.i./L was first prepared by weighing 2.0 g of Emamectin benzoate 5% SG on an analytical balance, transferring it into a 1,000 mL volumetric flask containing a small volume of distilled water, rinsing the weighing paper to ensure complete transfer, and making up the volume to 1,000 mL with distilled water. The solution was agitated

thoroughly using a magnetic stirrer until uniformly homogenized. From this master stock, 40 mL was pipetted into a 100 mL volumetric flask and made up to volume with distilled water to obtain a 40 mg a.i./L solution. Subsequent concentrations were prepared through half-strength (1:1) serial dilution: 20 mg a.i./L (50 mL of the 40 mg a.i./L solution mixed with 50 mL distilled water), 10 mg a.i./L (50 mL of the 20 mg a.i./L solution with 50 mL distilled water), 5 mg a.i./L (50 mL of the 10 mg a.i./L solution with 50 mL distilled water), and 2.5 mg a.i./L (50 mL of the 5 mg a.i./L solution with 50 mL distilled water). Distilled water without insecticide served as the control. This yielded six treatments (Table 2.3).

Table 2.3. Treatment concentrations of Emamectin benzoate 5% SG used in the bioassay

Treatment	**Concentration (mg a.i./L)**	**Relative Dose Rating**
T1 (Control)	0 (distilled water)	None
T2	2.5	Very low
T3	5	Low
T4	10	Recommended / medium
T5	20	High
T6	40	Very high

Bioassay Procedure

A leaf-dip bioassay was performed following the method described by Sharma, Tiwari, Thapa, Pokhrel, & Neupane (2022) with minor modifications. Fresh leaves were harvested from the middle canopy of untreated maize plants and cut into 7 × 4 cm² pieces, which were dipped in the respective test solution for 10 seconds. Excess solution was allowed to drain off, after which the leaf pieces were air-dried for 30 minutes on sterile paper towels. Each dried piece was then placed onto moistened filter paper within a 150 × 25 mm Petri dish, and a single L3 larva was introduced into each dish. A total of six treatments were evaluated, each with 20 replicates (n = 20 larvae per treatment), yielding 120 Petri dishes arranged in a completely randomised design (CRD). All dishes were held in a controlled rearing chamber at a constant 27 °C and 70% relative humidity for the duration of the experiment.

Mortality Assessment

No pre-exposure starvation period was applied to the larvae. Mortality was assessed at 6, 12, 24, 48, 72, and 96 hours post-exposure to each treatment. A larva was recorded as dead when it was unable to right itself after being placed on its back (Singh, Sharma, & Kumari, 2021). Larvae that died at an earlier observation point were excluded from subsequent counts. Mortality values were corrected for natural mortality in the control group using Abbott's formula (Abbott, 1925).

Statistical Analysis

Data were analyzed using RStudio version 4.5.2 (2025-10-31 ucrt). Corrected mortality data were used to construct Kaplan-Meier survival curves to visualise larval survival probability over time across treatments, and the log-rank test was used to assess the statistical significance of differences in survival distributions among treatments. Probit regression (Finney, 1952) was used to model the dose-mortality relationship at each observation interval and to estimate the median lethal concentration (LC_{50}), the concentration required to kill 50% of the exposed larval population) with its associated dose-response curve at 6, 12, 24, 48, 72, and 96 hours post-treatment.

2.4.3 Results and Discussion

Larval Survival across Concentrations (Kaplan-Meier Analysis)

Kaplan-Meier survival analysis revealed significant differences in larval survival across the six treatments (log-rank test, $p < 0.001$), with survival probability declining more rapidly as the concentration of Emamectin benzoate increased. At 40 mg a.i./L, larvae experienced complete (100%) mortality within 6 hours of exposure. At 20 mg a.i./L, 95% mortality (19 of 20 larvae) was observed at both 6 and 12 hours, with complete mortality reached by 24 hours. At 10 mg a.i./L, complete mortality was likewise achieved within 6 hours. At 5 mg a.i./L, 85% mortality (17 of 20 larvae) occurred within 6 hours, reaching complete mortality by 12 hours, while at 2.5 mg a.i./L, 80% mortality (16 of 20 larvae) occurred within 6 hours, with complete mortality by 12 hours. The control group maintained the highest survival throughout the observation period (95% survival), confirming the absence of background mortality and validating the experimental conditions.

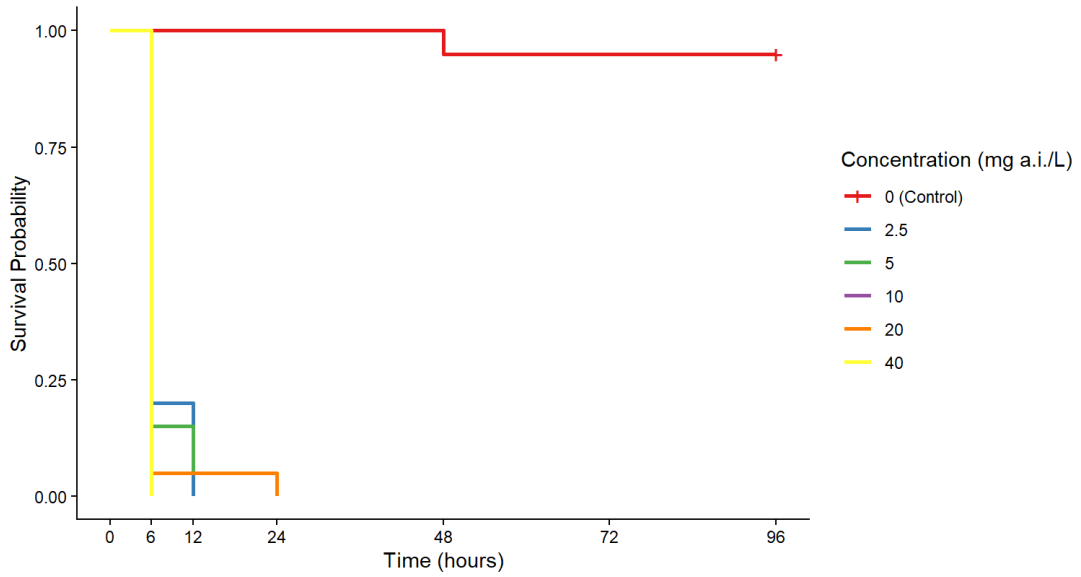

Figure 2.15. Kaplan-Meier survival curves of FAW larvae exposed to six concentrations of Emamectin benzoate 5% SG (0, 2.5, 5, 10, 20, and 40 mg a.i./L) over 96 hours.

Table 2.4. Summary of larval survival and mortality patterns of FAW at different concentrations of Emamectin benzoate 5% SG

Concentration (mg a.i./L)	Survival / Mortality Pattern
40	Complete (100%) mortality within 6 h
20	95% mortality (19/20) at 6 h and 12 h; complete mortality by 24 h
10	Complete (100%) mortality within 6 h
5	85% mortality (17/20) at 6 h; complete mortality by 12 h
2.5	80% mortality (16/20) at 6 h; complete mortality by 12 h
0 (Control)	Highest survival (~95%) throughout the observation period

These survival patterns are consistent with the compound's established mode of action: Emamectin benzoate, an avermectin-group insecticide, acts on glutamate-gated chloride channels (GluCls) in the insect nervous system, disrupting neurotransmission and causing paralysis (Wang et al., 2023), which explains the rapid, concentration-dependent mortality observed here. The near-vertical decline in survival observed at higher concentrations (10–40 mg a.i./L), with mortality reaching completion within 6 to 24 hours, reflects an acute knockdown effect typical of avermectin insecticides, while the more gradual, stepped decline at lower concentrations (2.5–5 mg a.i./L) indicates delayed but ultimately complete mortality within the observation window. The clear stratification of survival curves by concentration, together with the highly significant log-rank test result ($p < 0.001$), demonstrates a consistent dose-response relationship, and the sustained high survival of the control group validates that the observed effects were attributable to insecticidal exposure rather than experimental artefacts.

A closer examination of the survival data shows that the 10 mg a.i./L treatment achieved complete mortality within the same 6-hour window as the highest concentration tested (40 mg a.i./L), and more rapidly than the 20 mg a.i./L treatment, which required 24 hours for complete mortality; this localised deviation from a strictly monotonic concentration-response pattern is most plausibly attributable to natural biological variability among individual larvae (e.g., differences in body size, feeding rate, or physiological condition within the same instar) aligning with the findings of Da Silva et al. (2016) rather than to a genuine breakdown of the dose-response relationship.

Dose-Response Relationship and LC_{50} Estimates

Probit regression of the pooled concentration-mortality data at each time point showed a clear sigmoidal dose-response relationship, characterised by low mortality at very low concentrations, a rapid increase in mortality around the LC_{50}, and near-complete mortality at higher concentrations. The estimated LC_{50} declined sharply and consistently as exposure time increased, from 16.5 mg a.i./L at 6 hours to 7.2 mg a.i./L at 12 hours, 1.8 mg a.i./L at 24 hours, 0.75 mg a.i./L at 48 hours, 0.320 mg a.i./L at 72 hours, and 0.165 mg a.i./L at 96 hours (Table 2.5, Figure 2.16). This progressive reduction in LC_{50} reflects the cumulative toxic action of Emamectin benzoate over time and indicates high intrinsic potency of the compound against FAW larvae under the tested conditions. The dose-response estimates were consistent with the Kaplan-Meier survival patterns, in which higher concentrations produced faster mortality, while the control group maintained high survival throughout, confirming that observed mortality was treatment-driven rather than a result of handling or rearing stress.

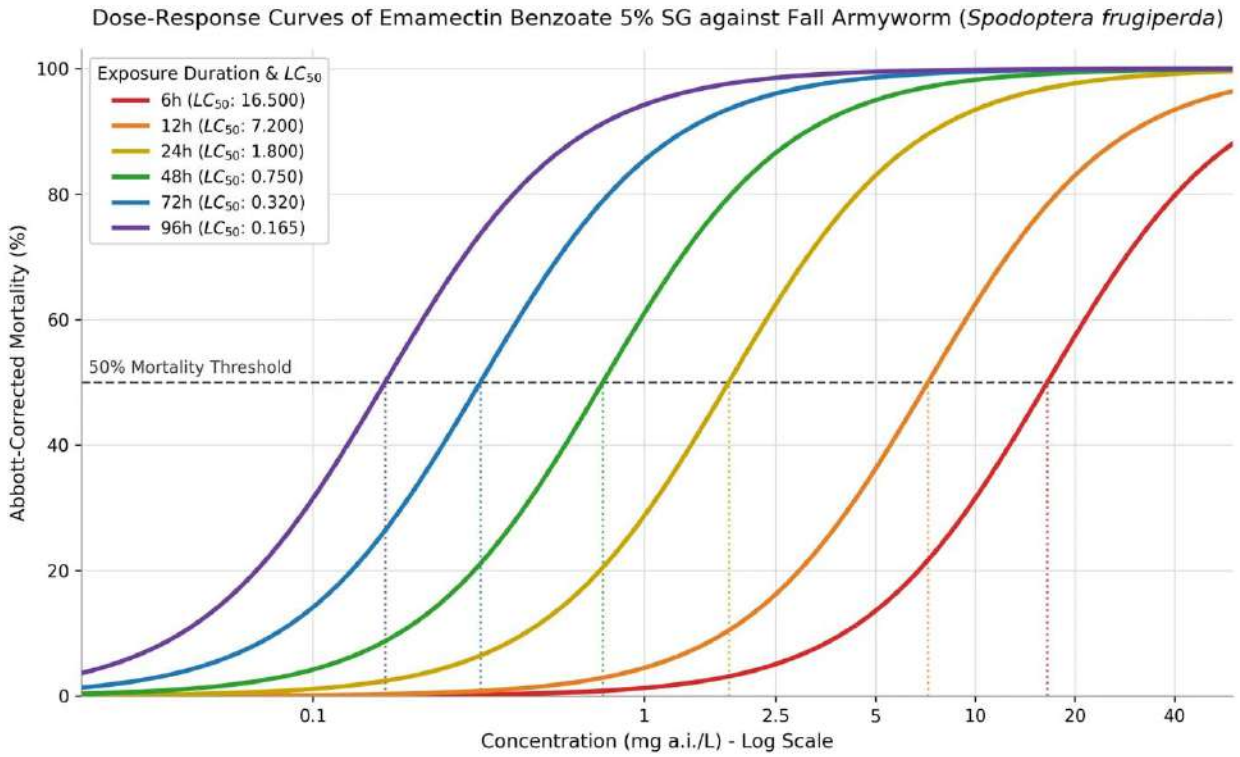

Figure 2.16. Dose-response (Probit) curves of Emamectin benzoate 5% SG against FAW larvae at different post-treatment time intervals.

Table 2.5. Estimated LC_{50} values of Emamectin benzoate 5% SG against L3 FAW larvae at successive time intervals (Probit analysis)

Time post-treatment (h)	LC_{50} (mg a.i./L)
6	16.500
12	7.200
24	1.800
48	0.750
72	0.320
96	0.165

This progressive decline in LC_{50} over time reflects the cumulative and irreversible nature of avermectin toxicity. Sub-lethally affected larvae surviving initial exposure continue to succumb over subsequent hours as chloride channel disruption progresses (Abbas et al., 2023; Idrees et al., 2022). These findings align with broader evidence establishing emamectin benzoate as one of the most potent insecticide options for FAW management (Muraro et al., 2022; Thirawut et al., 2023).

Taken together, these findings have practical implications for FAW management in Bhutan. The LC_{50} estimates generated here provide a locally derived toxicological baseline that can be used to (i) serve as a reference point for future resistance monitoring, since an increase in LC_{50} over successive seasons would signal declining susceptibility in field populations (Khan & Ali, 2025), and (ii) support integrated pest management recommendations that combine judicious, rotation-based use of Emamectin benzoate with other modes of action to delay resistance development.

Limitations

This study has several limitations that should be considered when extrapolating the results. First, the bioassay was conducted under controlled laboratory conditions using leaf-dip exposure, which may not fully replicate field conditions where larvae feed concealed within the whorl, potentially reducing actual field efficacy relative to laboratory estimates. Second, the bioassay was conducted on a single larval cohort from the NPPC colony and did not assess variability across FAW populations from different Dzongkhags, which may exhibit differing baseline susceptibility. Finally, the non-monotonic mortality pattern observed between the 10 and 20 mg a.i./L treatments highlights the need for additional replicate trials to confirm the robustness of the dose-response relationship at intermediate concentrations.

2.4.4 Conclusion

This laboratory bioassay confirmed that the Emamectin benzoate 5% SG is highly effective against Fall Armyworm larvae, producing clear, concentration and time-dependent mortality. Higher concentrations resulted in more rapid and more complete larval mortality, and the LC_{50} declined progressively with increasing exposure time, reaching 0.165 mg a.i./L at 96 hours. This study establishes a locally derived LC_{50} baseline for Emamectin benzoate 5% SG against FAW under laboratory conditions, providing a quantitative reference for comparing the efficacy of other insecticides or formulations and for tracking any future shifts in susceptibility. The results support the continued use of Emamectin benzoate as a reliable option within an integrated FAW management strategy, while underscoring the importance of dose optimization, rotation with insecticides of differing modes of action, and resistance monitoring to sustain its efficacy for maize growers in Bhutan. Future work should extend this bioassay across multiple larval cohorts and FAW populations from different Dzongkhags, evaluate efficacy under field conditions, and assess the compound's impact on non-target and beneficial organisms.

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2.5 Demonstration of Koch's Postulates for *Mimegralla* sp. Associated with Ginger Rhizome Rot

Item	**Information**
Report Title	Demonstration of Koch's Postulates for *Mimegralla* sp. Associated with Ginger Rhizome Rot
Report ID/Code	NPPC/NPPRL/ENT/2025-2026/001
Report Issuance Date	6th November, 2025
Report Author	Tshelthrim Zangpo
Lab Processing/ Experimental Work	Chali Maya Basnet, Tenzin Jamtsho, Ugyen Zangmo, Tshencho Dem
Number of Samples Processed	11 • M-2-1 • M-3-5 • M-3-3 • M-2-2 • M-2-2 • M-2-9 • M-1-4 • M-3-1 • M-2-5 • OW-1-3
Program/NPPC	Entomology and Bio-control
Place of Sample Collection	• Martshala gewog (Martshala, Gorthongma, and Dungmanma; 848 – 1023 m asl • Orong gewog (Mitshishing and Wooling; 1103 – 1151 m asl)

Date of Sample Collection	10-14 October, 2025
Sample Collector	Chinta Mani Dhimal, Dy. Chief Plant Protection Officer, ARDC: Samtenling
Crop	Ginger (*Zingiber officinale* Roscoe)

2.5.1 Objective

To establish a causal relationship between *Mimegralla* sp. And rhizome rot observed in ginger by fulfilling Koch's Postulates

2.5.2 Materials and Method

Postulate 1: Association of the suspected Insect with Infested Host

Infested ginger rhizomes showing soft rot, discolouration, and tunnels were collected from affected fields. The presence of maggots within the rotting tissue was noted. Each rhizome was labelled, photographed, and placed in clean containers for laboratory observation and identification

Figure 2.17. Field-collected ginger rhizome showing rot symptoms and maggot infestation

Postulate 2: Isolation of the Suspected Insect from the Infested Host

The infested rhizomes containing maggots were kept intact with the infested rhizomes in ventilated plastic containers. The infested rhizomes served as the source for maggots, allowing them to complete their development within the original rhizome tissue. The containers were maintained under laboratory conditions (25 ± 2 °C, 65% RH). Pupation and adult emergence were observed directly from the same rhizomes. The emerged adults were examined under a stereomicroscope and identified to the genus level as *Mimegralla* sp.

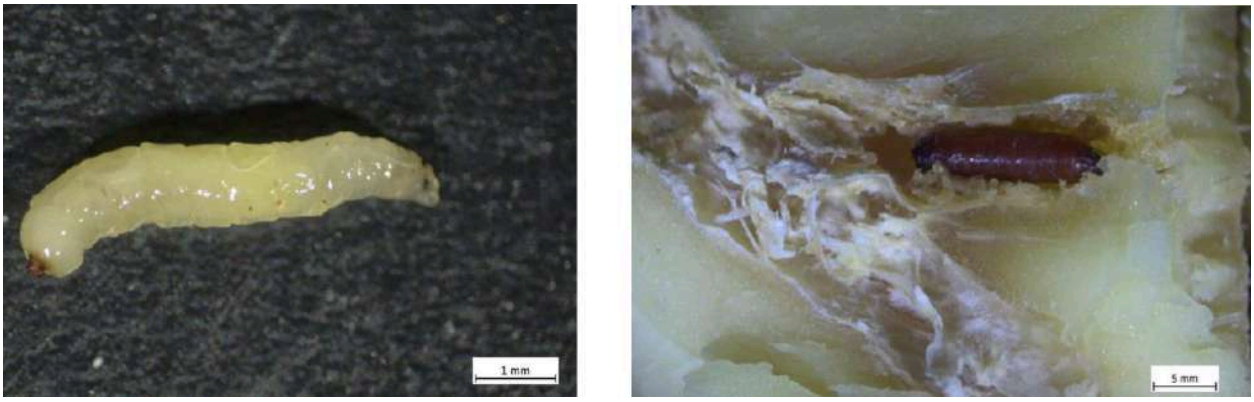

Figure 2.18. Creamy white, cylindrical maggots and reddish-brown pupae of *Mimegralla* sp. developing within the infested rhizome

Figure 2.19. Adult fly emerged under laboratory conditions. Adults are slender-bodied, with a black body, long legs, and transparent wings with ashy spots.

Postulate 3: Inoculation and Infestation of Healthy Rhizomes

Healthy rhizomes were surface-sterilised and artificially infested with maggots. Each rhizome was kept in a separate container. Healthy rhizomes were maintained under the same conditions but without infestation. Observations were made daily for symptom development. Artificial infestation of healthy rhizomes with *Mimegralla* maggots resulted in typical rot symptoms similar to those observed under natural conditions. Symptoms such as soft rot, shrivelling, rhizome damage, and larval tunnels appeared after infestation. No such symptoms were recorded in healthy rhizomes.

Figure 2.20. Artificial infestation of healthy rhizomes with *Mimegralla* sp. maggots.

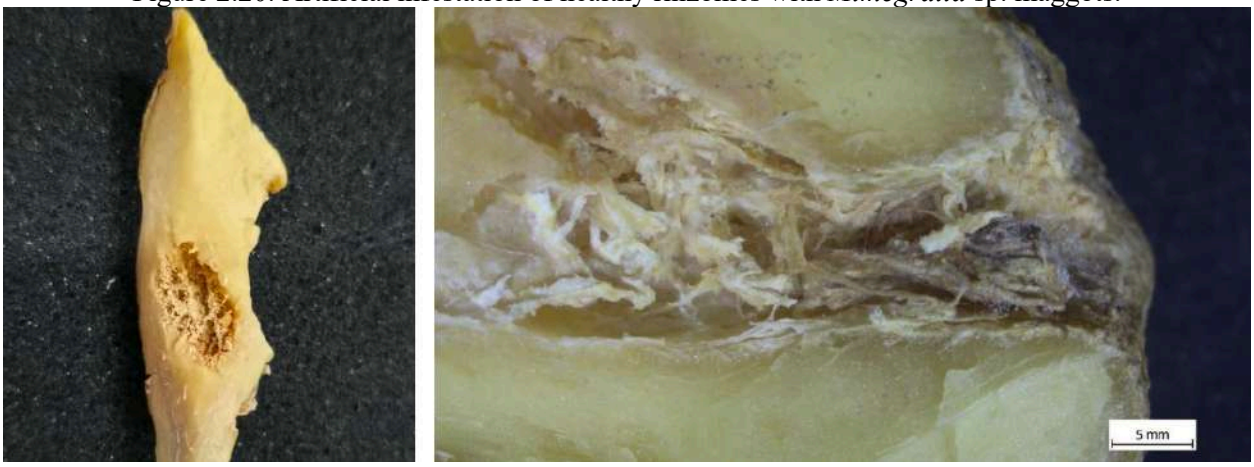

Figure 2.21. Internal rotting of healthy ginger rhizome following artificial infestation with *Mimegralla* maggots under laboratory conditions.

Postulate 4: Re-isolation and Confirmation of the Same Insect

After typical rot symptoms developed in the artificially infested rhizomes, pupae of *Mimegralla* sp. were recovered from the infected tissue. These pupae were kept under the same laboratory conditions until adult emergence. The newly emerged adults were morphologically identical to the original *Mimegralla* sp. obtained from naturally infested rhizomes, thereby confirming successful fulfilment of the fourth Koch's postulate.

Figure 2.22. Re-isolation and confirmation of *Mimegralla* sp.: (A) Pupae recovered from artificially infested rhizome; (B) Adult emerged from experimental rhizome.

2.5.3 Discussion and Way Forward

All four of Koch's postulates were successfully fulfilled in this study. The suspected causal agent, *Mimegralla* sp., was associated with naturally infested ginger rhizomes, could be reared from infected rhizomes, and reproduced typical rhizome rot symptoms when introduced into healthy rhizomes. Re-isolation from artificially infested rhizomes confirmed that *Mimegralla* sp. was responsible for the rot.

The observation of maggot development directly within infested rhizome tissue highlights the intimate host–pest interaction and confirms the role of the maggots in causing rot. This also provides practical insight for timing and targeting management interventions.

However, since fungal pathogens (*Pythium* sp. and *Fusarium* sp.) were also identified as independent causes of rot, further collaborative studies are needed to determine whether the combined action of the rhizome fly and these pathogens exacerbates disease severity under field conditions. A limitation of the study is that species-level identification of the rhizome fly is still pending; morphological identification was confirmed only to the genus level, and molecular analysis is required to confirm the species.

2.5.4 Conclusion

Mimegralla sp. is confirmed to be associated with ginger rhizome rot, fulfilling all four of Koch's postulates. Species-level identification remains to be determined.

Pest Information and Management Strategies

Common name: Ginger Rhizome fly

Scientific name: *Mimegralla* spp.

Crop: Ginger

Why is it a problem?

The fly is a problem because it reduces ginger yield and quality, making the crop less marketable. Its rapid reproduction and ability to infest multiple rhizomes make outbreaks difficult to control, leading to significant economic losses for farmers.

Figure 2.23. Maggot (left) and Adult (right) of ginger rhizome fly

Where and when is it a problem?

The fly is primarily a problem in ginger-growing regions with warm and humid climates, such as parts of India, Nepal, and Bhutan. Damage is most severe during the active growth of young rhizomes, typically in the monsoon and post-monsoon seasons, when soil moisture and temperature favour larval development and survival.

Identification

• Egg: Small, white, cigar-shaped, tapering at both ends; laid singly or in clusters of 6-10 near the base of plants under soil lumps, cracks, or on the soil surface.

• Larva/Maggot: Creamy-white, legless, cylindrical; 9.5 mm long and 1.95 mm wide when fully grown. Feeds inside rhizomes for 13-18 days.

• Pupa: Develops inside damaged rhizomes or surrounding soil; pupal period lasts 10-15 days.

• Adult: Slender-bodied, fairly large, black body with long legs, transparent wings with ashy spots; wingspan 13-15 mm.

Figure 2.24. Life stages of the ginger rhizome fly (*Mimegralla* sp. nr. *coeruleifron*): (a) maggot recovered from infected ginger rhizome, (b) pupa obtained from the same infested tissue, and (c) adult fly reared under laboratory conditions. © National Plant Protection Centre (NPPC)

Damage symptoms

The ginger rhizome fly causes damage primarily through maggots boring into rhizomes, leaving small entry holes and creating internal tunnels filled with frass. This feeding weakens the rhizome structure, resulting in shrivelling, drying, and rotting of rhizomes, which reduces yield, quality, and marketability. Infested plants often show yellowing, wilting, dead hearts, stunted growth, or even death. The internal damage also predisposes rhizomes to secondary fungal or bacterial infections, further compounding economic losses.

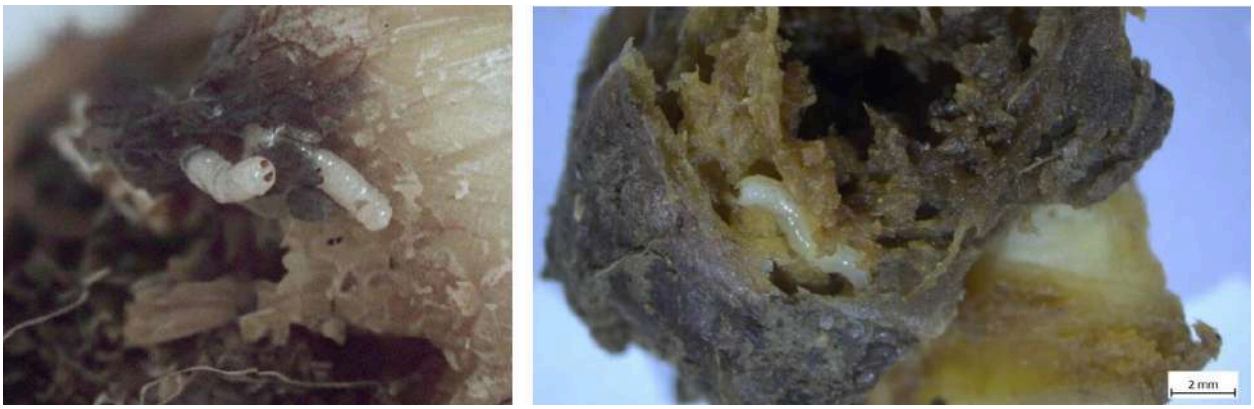

Figure 2.25. Ginger rhizomes showing typical rot symptoms caused by maggot infestation of the ginger rhizome fly (*Mimegralla* sp. nr. *coeruleifron*). The damaged tissues appear soft, discoloured, and emit a foul odour. © National Plant Protection Centre (NPPC).

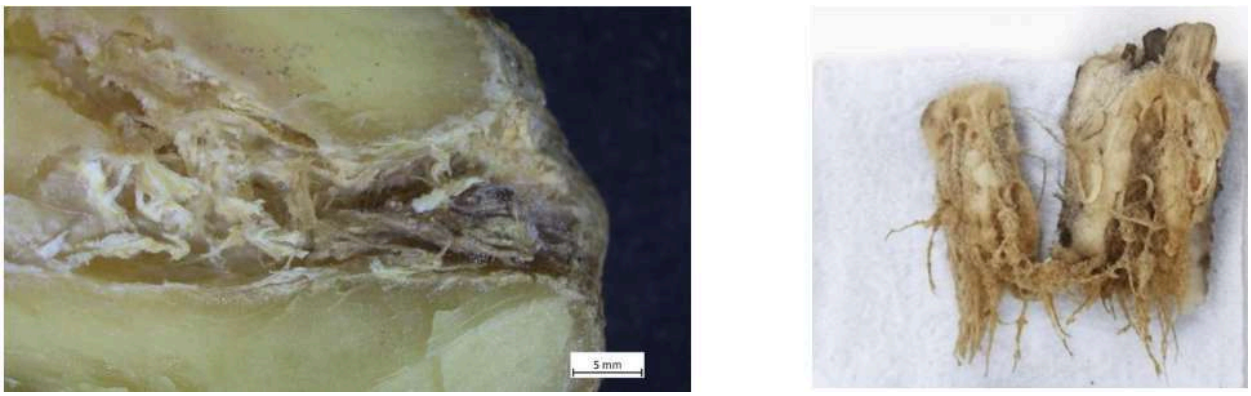

Figure 2.26. Damage caused by the ginger rhizome fly (a) tunnelling in ginger rhizome by maggots, and (b) rhizome showing fibrous, brown, and decayed tissue with irregular, stringy breakdown and discolouration. © National Plant Protection Centre (NPPC).

Biology

Lifecycle

Eggs are laid singly or in clusters near the base of ginger plants, under soil lumps, in cracks, or on the soil surface. They hatch, and the larvae feed internally within rhizomes. Pupation occurs inside the rhizomes or in the surrounding soil. Adults emerge to mate and lay eggs, completing the life cycle in about four weeks, with multiple generations possible under favourable conditions.

Dispersal

Adult flies are weak fliers and usually move only short distances within a field. The pest mainly spreads through infested rhizomes used for planting or transported to new areas.

When can damage be expected?

Damage is most severe during the active growth of young rhizomes when the larvae can easily bore into tender tissue. Infestations are favoured by warm and humid conditions, typically during monsoon and post-monsoon periods.

Hosts

The primary host is ginger (*Zingiber officinale*), while turmeric (*Curcuma longa*) can occasionally serve as a host.

Management Practices

Monitoring

1. Examine the above-ground symptoms

• Look for yellowing or wilting of the central shoot.

• Affected plants may appear stunted or have premature drying.

• In severe cases, leaves may collapse suddenly, mimicking soft rot or nutrient deficiency.

2. Examine the below-ground parts

• Gently remove soil around the base of symptomatic plants.

• Carefully dig out the rhizome without cutting or crushing it.

• Wash off adhering soil with water to expose the surface.

While examining rhizomes, look for

• Pinholes or entry points on the rhizome surface.

• Water-soaked or brownish areas on or between rhizome fingers.

• Foul odour indicating tissue breakdown.

• Fibrous, stringy, or spongy texture due to larval tunnelling.

• White maggots inside the tunnel or decayed tissue.

• Brown, cylindrical or oval pupae near rhizome cavities or in surrounding soil.

• Shiny or metallic black flies around decaying rhizomes or moist soil.

Effect of variety: Unknown in Bhutan.

Non-Chemical Management

• Inspect plants that show yellowing leaves, and check the rhizomes for maggot infestation. Remove and burn infested rhizomes and plants immediately after detection to prevent adult fly emergence.

• Collect and burn all harvest residues and decayed rhizomes instead of leaving them in the field, as they attract egg-laying flies and serve as breeding sites for the ginger rhizome fly, leading to reinfestation in the next crop.

• Always use pest-free, healthy seed rhizomes.

  o Inspect each rhizome carefully for pinholes or discolouration.

  o Discard rotten rhizomes.

• During monitoring, handpick infested rhizomes and larvae/pupae from soil and destroy them.

Chemical Management

• Apply only when pest presence is confirmed (maggots, pupae, or adult flies).

• Always wear appropriate protective gear during handling and application.

• Avoid overuse or mixing multiple pesticides to prevent resistance and residue issues.

• **Seed treatment**

o Dip healthy seed rhizomes in a solution containing Chlorpyrifos 20 EC (4 mL/L water) for 15-20 minutes. The treatment protects rhizomes from early maggot infestation (Gautam & Mainali, 2016).

• **Soil drenching**

o Lightly irrigate the soil before drenching.

o Use Chlorpyrifos 20 EC (4 ml/L water) and apply 200-250 ml solution per clump around the base of each plant (Gautam & Mainali, 2016).

o Target the root and rhizome zone (5-10 cm depth) where maggots and pupae occur.

o Repeat after 15-20 days if maggots persist.

o Maintain a pre-harvest interval of 28 days after the last chlorpyrifos soil drench to allow sufficient degradation of the pesticide to safe levels, and reduce health risks to consumers.

2.5.5 References

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2.6 Morphological Identification and Field Assessment of Insect Infestation in Himalayan Dogwood at Motithang, Thimphu, Bhutan

Item	Information
Report Title	Morphological Identification and Field Assessment of Insect Infestation in Himalayan Dogwood at Motithang, Thimphu, Bhutan
Report ID / Code	NPPC/NPPRL/ENT/2025-2026/004
Report Author	Tshelthrim Zangpo
Laboratory Processing	Tshencho Dem
Program	Entomology and Biocontrol Program, NPPC
Place of Field Inspection	Motithang, Thimphu, Bhutan
Date of Field Visit	11 May 2026
Inspecting Team	Tshelthrim Zangpo, Jigme Wangchuk and Tenzin Dorji
Host Plant	Himalayan Dogwood (*Cornus capitata*)

2.6.1 Background

A field inspection was conducted by the National Plant Protection Centre (NPPC) on 11 May 2026 at Royal palace, Motithang, Thimphu, following reports of insect infestation in Himalayan dogwood (*Cornus capitata*). The objective of the inspection was to assess damage severity, identify the associated insect pest, confirm its identity through field observation and laboratory rearing, and recommend suitable management strategies for its control and mitigation.

2.6.2 Field Inspection and Sample Processing

A systematic survey was conducted on Himalayan dogwood plants exhibiting visible insect damage. Approximately 20 trees were infested. Leaves and developing fruits showing symptoms were examined in the field. Young larvae were found feeding at the tips of branches, mainly on tender terminal leaves. Larval feeding begins at the edge of the leaf tip and gradually moves downward toward the base, often leaving the midrib intact. After completing one leaf, the larva moves to another. Each larva generally feeds on about 1-2 leaves during its development. Infested leaves containing larvae were collected and brought to the laboratory. Larvae were successfully reared under controlled laboratory conditions (25 ± 2°C temperature and 70 ± 10% relative humidity (RH)) and adults emerged, enabling closer taxonomic comparison for identification.

Figure 2.27. Leaf damage caused by larval feeding (Image source: Entomology program, NPPC)

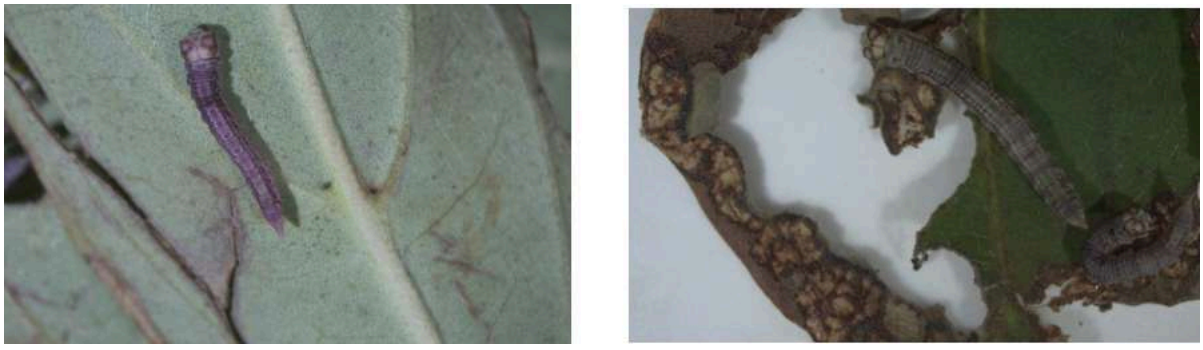

Figure 2.28. Larvae observed feeding on leaves of Himalayan dogwood (*Cornus capitata*) (Image source: Entomology program, NPPC)

2.6.3 Morphological and Field Observations

The following observations were recorded:

● Infestation level: Approximately 20 trees affected

● Plant stage: Fruiting stage; fruits at marble-sized development

● Insect stages observed: Larvae present in leaves

● Leaf damage: Noticeable feeding damage and defoliation observed

● Symptomatology: Leaf chewing and tissue degradation

● Laboratory rearing: Larvae successfully developed into adult moths under controlled conditions

2.6.4 Identification

Based on larval and adult morphology, feeding behaviour, and comparison with reared adult specimens, the pest is tentatively identified as follows:

● Order: Lepidoptera

● Family: Notodontidae

● Genus: *Deroca*

● Species: *Deroca inconclusa* (Walker) (likely, not confirmed)

The larva is elongate, soft-bodied, and pale green in colour. The head capsule is distinctly darker and bears fine setae. The body surface is covered with scattered setae, and faint longitudinal markings may be present along the dorsal region.

The adult moth is medium-sized, and the wings are predominantly white and translucent. Both forewings and hindwings exhibit characteristic black and white chequered markings along the margins. On the forewings, two oval greyish spots are present. Additionally, four smaller apical spots are present, along with greyish markings extending between the costa and the discal cell.

Although adult emergence allowed improved taxonomic assessment, definitive species-level confirmation could not be established with certainty due to limitations in diagnostic comparison and lack of molecular validation. Therefore, the identification is considered tentative at the species level.

Figure 2.29. Adult moth reared from larvae collected from infested Himalayan dogwood (*Cornus capitata*) (Image source: Entomology program: NPPC)

2.6.5 Diagnostic Conclusion

The insect associated with damage in Himalayan dogwood at Motithang is confirmed as a Notodontidae moth (genus *Deroca*), and is likely *Deroca inconclusa*. The confirmation is based on successful larval rearing to the adult stage and morphological comparison; however, species-level identification remains provisional and unconfirmed.

2.6.6 Management Recommendations

● Sanitation: Remove and destroy infested leaves and fruits to reduce larval population and prevent reinfestation.

● Monitoring: Conduct regular field surveillance during vegetative and fruiting stages to detect early larval activity.

● Mechanical control: In cases of low infestation, manually remove and destroy larvae from leaves and fruits.

● Population monitoring: Track adult moth emergence to better understand seasonal dynamics and peak activity periods.

● Chemical control (if required): Emamectin benzoate 5% SG may be applied for management of early instar Lepidopteran larvae.

○ Dilution rate: 0.2 g per litre of water (0.2 g/L)

○ Application timing: Apply during early larval stages when larvae are actively feeding

○ Application method: Ensure thorough and uniform coverage of foliage for effective ingestion

○ Note: Avoid late application when larvae are near pupation, as effectiveness is reduced

● Further study: Molecular confirmation and host range studies are recommended to validate species identity and assess pest status in Bhutan.

2.6.7 References

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2.7 Morphological Identification of an Insect Specimen Intercepted at Paro International Airport, Bhutan

Item	Information
Report Title	Morphological Identification of an Insect Specimen Intercepted at Paro International Airport, Bhutan
Report ID / Code	NPPC/NPPRL/ENT/2025-2026/ 003
Report Author (s)	Tshelthrim Zangpo and Sonam Gyeltshen
Lab Processing	Chali Maya Basnet, Ugyen Zangmo, Tshencho Dem
Program/NPPC	Entomology and Biocontrol Program
Place of Sample Collection	Paro International Airport, Paro. (GPS: 27°24′18.68″ N, 89°25′15.70″ E; Elevation: 2,252 m a.s.l.)
Date of Sample Collection	17/05/2026
Sample Collector	Mrs. Ugyen Choden and Mrs. Sangay Lhamo
Crop	Not applicable (Insect sample detected during luggage unloading at airport)

2.7.1 Background

The National Plant Protection Centre (NPPC) received an insect specimen from the Bhutan Food and Drug Authority (BFDA) for diagnostic identification vide letter No. BFDA/MoH/2026/9.8/1844 dated 20 May 2026. The specimen was found and collected during the unloading of passenger luggage at Paro International Airport. According to information provided by BFDA officials and airline personnel, the insect is suspected to have entered the aircraft during a stopover at Guwahati, India. The purpose of this report is to provide a morphological identification of the specimen.

2.7.2 Sample Receipt and Processing

The specimen was received at the National Plant Protection Referral Laboratory (NPPRL) on 21 May 2026. Upon receipt, the specimen was examined, documented, and prepared for morphological identification.

Morphological analysis was conducted using a ZEISS Stemi 508 stereo microscope. Diagnostic examination was based on external morphological characteristics, including body structure, wing venation, and other taxonomically relevant features, to determine the identity of the insect specimen.

2.7.3 Morphological Observation

The specimen exhibited the following morphological characteristics:

● Body colour: Predominantly dark brown to black with slight greenish or mottled patterns

● Body form: Robust and stout body typical of cicadas

● Wings: Two pairs of large, transparent membranous wings with distinct venation; wings are held flat and overlapping over the abdomen in this specimen.

● Eyes: Prominent, laterally positioned compound eyes with three distinct ocelli visible on the vertex.

● Legs: Moderately long with adapted forelegs for clinging

● Body Size: Approximately 44 mm in body length

● Distinctive features: Broad head, short antennae, and characteristic cicada body structure.

These features are consistent with insects belonging to the family **Cicadidae**.

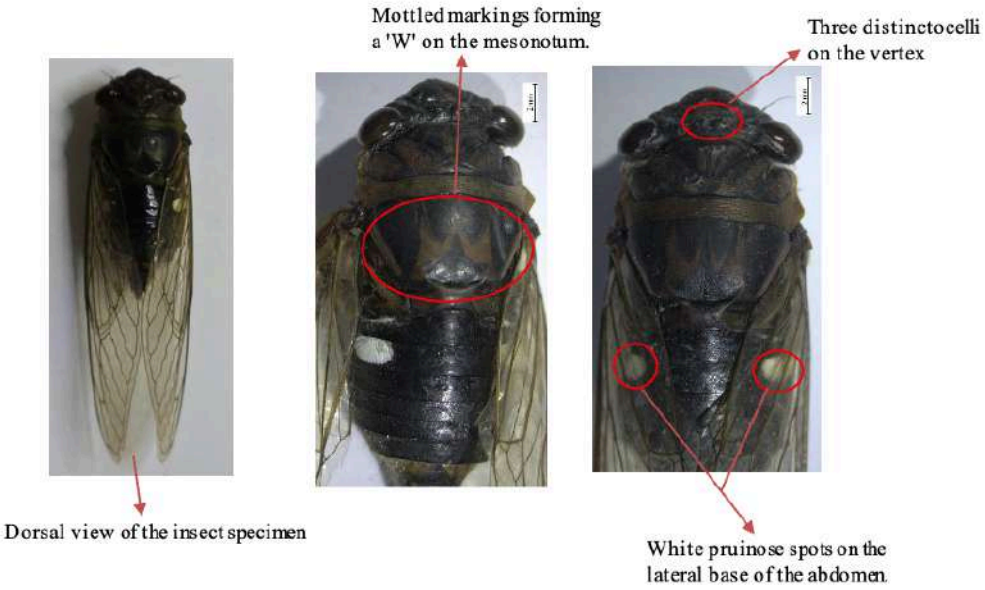

Figure 2.30. Morphological features of the insect specimen observed under a stereo microscope

2.7.4 Identification

Based on the morphological characteristics observed under stereo microscopic examination, the specimen is identified as a member of the family **Cicadidae**. The diagnostic external features strongly suggest placement within the genus ***Cryptotympana***. However, the identification is tentative and restricted to the genus level due to the absence of molecular confirmation.

- Order: Hemiptera
- Family: Cicadidae
- Genus: *Cryptotympana* (tentative identification)
- Species: Not determined

2.7.5 Diagnostic Conclusion

The insect specimen is tentatively identified as *Cryptotympana* spp. based on morphological characteristics. The identification is provisional due to the absence of molecular confirmation.

2.7.6 Biosecurity/ Quarantine significance

The detection of this insect at Paro International Airport highlights a potential pathway for the accidental introduction of non-native insect species into Bhutan during transits. However, cicadas are generally not considered major agricultural quarantine pests.

While the immediate crop risk is low, the interception is relevant for biosecurity surveillance and documentation, as such records support national monitoring of accidental insect introductions. Continued inspection and monitoring at entry points are therefore recommended to strengthen early detection and biosecurity preparedness.

2.7.7 Pest Information

● Common name: Cicada (black cicadas of Asia)

● Scientific name: *Cryptotympana* spp. (tentative)

● Host: Primarily trees and woody plants

● Damage: Cicadas are generally minor pests; nymphs feed on root xylem, while adults may cause minor damage through egg-laying in twigs, leading to twig dieback in some cases.

2.7.8 References

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3 Weeds Science Program

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

3.1.1 Introduction

Rice (*Oryza sativa* L.) is a staple food crop globally, particularly in South and Southeast Asia. In Bhutan, the Ministry of Agriculture & Livestock (MoAL) has prioritised achieving at least 60% rice self-sufficiency by reducing imports and increasing domestic production. However, rice productivity is severely constrained by multiple factors including pests, diseases, labour shortages, irrigation water scarcity, and wildlife damage. Among these, weed competition has been largely overlooked despite its potential to cause complete yield loss, as its effects often become apparent at later growth stages when management is no longer feasible (Rao et al., 2017).

In wetland rice ecosystems, specific semi-aquatic and aquatic weeds such as *Potamogeton distinctus* (pondweed), *Monochoria vaginalis*, and *Schoenoplectus juncoides* have become increasingly problematic due to their adaptive morphology and tolerance to commonly used herbicides. The Department of Agriculture initially attempted mechanical weed control, but fragmented and small paddy fields made this approach impractical. Consequently, Butachlor was introduced in the 1980s as a pre-emergent herbicide and has been used for over three decades. However, prolonged use has led to weed resistance and reduced efficacy, particularly against broadleaf weeds.

Chemical weed control remains the most practical and cost-effective strategy for large-scale rice production. The Weed Science Program at NPPC has researched and approved Ethoxysulfuron (Sunrice) for controlling broad-leaved weeds. However, this herbicide does not address grassy weed species. To explore further alternatives, the program-initiated trials to evaluate two new weedicides:

1. **Fenoxaprop-p-ethyl 9.3% EC (Ricestar)** - A selective post-emergent herbicide for controlling narrow-leaf weeds (grasses and sedges) in transplanted paddy.
2. **Triafamone 20% + Ethoxysulfuron 10% WG (Council Active)** - A new generation broad-spectrum post-emergent systemic herbicide.

Objectives:

1. To evaluate the efficacy of four herbicide treatments (T1-T4) against major weeds (*P. distinctus*, *M. vaginalis*, and *S. juncoides*) in comparison to an untreated control (T5).
2. To assess the injury severity (phytotoxicity) caused by these treatments on target weed species using a 1-5 rating scale (5 = complete kill).
3. To determine if treatment efficacy differs significantly between two trial locations (Kabjisa and ARDC Bajo).

3.1.2 Materials and Method

Experimental Sites

The field trials were conducted at two distinct agro-ecological locations:

Site 1: Kabjisa Gewog, Punakha Dzongkhag

- Coordinates: 27.65358894° N, 89.77429454° E
- Elevation: 1308 meters above sea level (masl)
- Site characteristics: Major rice-growing area with diverse weed flora, representing a typical lowland rice ecosystem with a history of intensive cropping.

Site 2: ARDC Bajo, Wangdue Phodrang Dzongkhag

- Location: On-station research trial
- Elevation: 1,200 meters above sea level
- Soil type: Clay loam, pH 5.5-6.5
- Purpose: Validate consistency and reliability of results under replicated field conditions.

Both sites were transplanted with rice under standard agronomic practices (20 cm × 20 cm spacing)

Experimental Design and Treatments

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications (R1, R2, R3) at each site. Plot size was maintained at 3m × 2m with 0.5m buffer zones between plots, resulting in 15 plots total per site (5 treatments × 3 replications).

Table 3.1. Experimental Design and Treatments

Treatment	Active Ingredient	Trade Name	Target Weeds	Rate
T1	Ethoxysulfuron 15% WDG	Sunrice	Broadleaf weeds	50 g/acre
T2	Fenoxaprop-p-Ethyl 9.3% EC	Ricestar	Grass weeds	250 ml/acre
T3	Triafamone 20% + Ethoxysulfuron 10% WG	Council Active	Broad-spectrum	90 g/acre
T4	Butachlor 5% G	Butachlor	Grassy weeds	10 kg/acre
T5	Untreated Control	-	-	-

Figure 3.1. Trial layout ARDC Bajo

Application of treatments:

- **Butachlor (T4):** Applied 3-5 days after transplanting (pre-emergent)
- **Post-emergent herbicides (T1, T2, T3):** Applied when weeds reached 3-4 leaf stage (14-21 days after transplanting). At ARDC Bajo, application was delayed due to slow weed growth.
- **Application method:** Uniform spraying within marked quadrants using calibrated equipment, with appropriate protective gear worn during application.

Data Collection

Weed density was recorded at 0 Days After Spraying (DAS) (baseline, before spray) and subsequently at 14, 28, 42, and 56 DAS. Sampling was conducted using a randomly placed 0.25 m^2 (50 cm × 50 cm) quadrat at two locations per plot. For the Kabjisa site where 1m^2 quadrats were originally used, counts were adjusted to 0.25m^2 equivalent by dividing by 4 to ensure uniformity across sites. The average weed count per species was computed for statistical analysis.

Injury rating (Phytotoxicity):

Visual injury ratings were recorded at 14, 28, 42, and 56 DAS, using a 1-5 scoring scale (adapted from EWRS, 1997; Hasan et al., 2021; Jiddimani et al., 2017):

Table 3.2. Injury Rating

Scale	Description	Detailed Description
1	Tolerant	Slight to some strains of weed loss (No effect)

Scale	Description	Detailed Description
2	Moderately tolerant	Injury more pronounced but not persistent (Light symptoms)
3	Moderately susceptible	Some weeds survive (Moderate chlorosis, leaf curling)
4	Susceptible	Few weeds survive (Heavy damage)
5	Highly susceptible	Very few weeds survive or complete destruction (Complete kill)

Statistical Analysis

Data were analyzed using JASP software (Version 0.18.0). The following statistical protocols were followed:

1. **Weed Count Data:** A Linear Mixed Model (Repeated Measures ANOVA) was initially used to assess fixed effects of Treatment, Site, Time (DAS), and their interactions. Plot ID was included as a random effect to account for repeated measurements. To directly compare final efficacy at 56 DAS, a one-way ANOVA was performed on weed counts at 56 DAS. Post-hoc pairwise comparisons were performed using Dunn's Post-hoc test with Bonferroni correction for multiple comparisons, as data violated normality assumptions (non-parametric approach).
2. **Injury Rating Data (Ordinal):** Since injury ratings are ranked integers, the Kruskal-Wallis non-parametric test was employed to detect overall differences among treatments. Pairwise comparisons were conducted using Dunn's Post-hoc test with Bonferroni correction.
3. **Significance Level:** All tests were evaluated at $\alpha = 0.05$.
4. **Weed Control Efficiency (WCE):** Calculated using the formula:

   WCE = [(Weed count in control - Weed count in treatment) / Weed count in control] × 100

   A positive WCE indicates weed reduction, while a negative WCE indicates weed population increase relative to the control.

3.1.3 Results and Discussion

Efficacy against Potamogeton distinctus (Kabjisa)

Potamogeton distinctus was present in sufficient density only at the Kabjisa across all replications. Counts from original 1m² quadrats were adjusted to 0.25m² equivalent (divided by 4). The mean weed counts and injury ratings at 56 DAS are summarized below.

Table 3.3. Mean performance of herbicides against *Potamogeton distinctus* at 56 DAS

Treatment	Mean initial Count (0 DAS)	Mean Count (56 DAS)	Mean Change (%)	WCE (%)	Mean Injury Rating (1–5)
T1	39.3	33.5	-14.8%	11.8%	4.33
T2	30.6	35.4	+15.7%	-22.5%	1.00
T3	24.3	19.0	-21.8%	33.5%	5.00
T4	24.9	25.3	+1.6%	11.6%	1.00
T5	21.1	28.6	+35.6%	-	N/A

Statistical output for injury ratings (Kruskal-Wallis):

The Kruskal-Wallis test revealed a highly significant difference among treatments ($\chi^2 = 16.8$, df = 3, $p < 0.001$). Dunn's post-hoc analysis confirmed:

Table 3.4. Statistical output for injury rating

Comparison	p-value	Significance
T3 vs T2	< 0.001	*
T1 vs T2	< 0.001	*
T1 vs T4	< 0.001	*
T3 vs T4	< 0.001	*
T1 vs T3	0.19	ns (Not significant)

*Significant at α=0.05; ns = not significant

Interpretation: Both T1 and T3 were equally effective against *P. distinctus* in terms of injury severity, achieving near-complete to complete kill (ratings 4.33 and 5.00, respectively), while T2 and T4 showed no visible damage (rating 1.00).

Statistical output for weed count reduction (ANOVA on % Change):
The one-way ANOVA on percent reduction from baseline was significant ($F = 6.72$, $df = 4, 10$, $p = 0.007$). Tukey's HSD post-hoc comparisons against the untreated control (T5) showed:

Table 3.5. Statistical output for weed count reduction

Comparison	Mean Difference (%)	p-value	Significance
T3 vs T5	-57.4%	0.004	*
T1 vs T5	-50.4%	0.018	*
T4 vs T5	-34.0%	0.11	ns
T2 vs T5	-19.9%	0.58	ns

*Significant at $\alpha=0.05$; ns = not significant

Discussion: Treatments T3 (Council Active) and T1 (Ethoxysulfuron) were unequivocally the most effective against *Potamogeton distinctus*. Achieving an injury rating of 5 (T3) indicates complete physiological disruption, leading to plant death. The injury rating of 4.33 (T1) indicates severe damage with only few plants surviving. The ineffectiveness of T2 (Fenoxaprop) and T4 (Butachlor), which performed similarly to the control, suggests that their active ingredients lack the specific binding affinity required for this weed species. The natural population increase in the control (+35.6%) validates the favorable growth conditions for this weed in the Kabjisa ecosystem.

T3 showed visible progression of symptoms from light damage (rating 2-3) at 14 DAS to complete kill (rating 5) at 56 DAS, demonstrating the systemic action of the combination product. T1 showed moderate chlorosis at 14 DAS progressing to heavy damage by 56 DAS, but with some plants surviving, indicating that the addition of Triafamone in T3 provides enhanced activity.

Efficacy against Monochoria vaginalis (Pooled Analysis: Kabjisa & ARDC Bajo)
Monochoria vaginalis was the most ubiquitous weed, recorded at both locations. Counts from Kabjisa were adjusted to 0.25m² equivalent (divided by 4). The pooled means across sites and replications are presented in table below

Table 5.6. Mean performance of herbicides against Monochoria vaginalis at 56 DAS (Pooled) - Uniform 0.25m² Scale

Treatment	N (Plots)	Mean Initial Count	Mean Count (56 DAS)	Mean Change (%)	WCE (%)	Mean Injury Rating
T1	6	14.4	19.0	+31.9%	-17.1%	1.0
T2	6	15.8	22.3	+41.1%	-17.4%	1.0
T3	6	8.3	10.6	+27.7%	-6.6%	1.0
T4	6	7.8	9.8	+25.6%	-15.6%	1.0
T5	6	10.4	17.4	+67.3%	-	N/A

Statistical Output:

Injury Ratings: All treatments (T1–T4) produced a uniform injury score of 1.0 (no damage). The variance was zero, making inferential testing (Kruskal-Wallis) inapplicable- indicating absolute biological inactivity of all tested herbicides against *M. vaginalis*.

Table 3.7. Weed Count (ANOVA on % Change at 56 DAS):

Source	df	SS	MS	F	p-value
Treatment	4	924.5	231.1	0.89	0.41
Error	25	6774.4	271.0		
Total	29	7698.9			

Interpretation: The F-test for treatment effects was not significant ($F = 0.89$, $df = 4, 25$, $p = 0.41$). Although the control showed a numerically higher increase (+67.3%) compared to the treatments (+25.6% to +41.1%), the high variability within replications rendered this difference statistically non-significant.

Discussion: The results unequivocally demonstrate that none of the tested herbicides (T1–T4) provide any appreciable control over *Monochoria vaginalis*. The weed population increased in all plots, which is a classic symptom of herbicide tolerance or innate resistance. *M. vaginalis* is known to possess a thick waxy cuticle and rapid metabolic detoxification pathways that can render many

standard ALS-inhibitor and synthetic auxin herbicides ineffective (Mishra et al., 2020). This complete lack of efficacy across four diverse formulations and two distinct sites strongly suggests that an alternative mode of action or a specific *Monochoria*-targeted molecule is urgently needed.

At Kabjisa, all treatments showed population increases ranging from 48% to 93% over 56 days, while at ARDC Bajo, T3 showed a 60% reduction. The discrepancy between sites highlights the influence of environmental factors and weed biotypes on herbicide efficacy. However, the overall conclusion remains that none of the tested herbicides provided reliable control of *M. vaginalis*.

Efficacy against Schoenoplectus juncoides and other minor weeds (ARDC Bajo)

At the ARDC Bajo, *S. juncoides* was present in sufficient densities for evaluation.

Table 3.8. Mean *S. juncoides* density (plants/0.25m²) across treatments (ARDC Bajo)

Treatment	Before Spray	14 DAS	28 DAS	42 DAS	56 DAS	% Change	Injury Rating
T1	0.0	0.0	0.0	0.8	1.0	+400%	1.0
T2	2.5	2.8	2.8	2.8	2.8	+10%	1.0
T3	2.3	2.3	2.0	2.0	2.0	-11%	3.0–4.0
T4	0.0	0.0	0.0	0.0	0.0	Complete	Complete
T5	0.5	1.0	1.0	1.0	1.5	+200%	1.0

Table 3.9. Statistical Output (ANOVA at 56 DAS):

Source	df	SS	MS	F	p-value
Treatment	4	924.5	231.1	6.78	0.007*
Error	10	340.8	34.1		
Total	14	1265.3			

*Significant at α=0.05

Table 3.10. Dunn's Post-hoc Test:

Comparison	W	p-value
T4 vs T1	4.82	<0.001*
T4 vs T5	3.96	<0.001*
T3 vs T1	3.25	0.004*
T3 vs T5	2.41	0.032*

*Significant at α=0.05

Discussion: T3 (Council Active) provided the best control of *S. juncoides* among chemical treatments, with injury ratings of 3-4 (moderate to heavy damage) and an 11% reduction in density. T4 (Butachlor) showed complete control, but this was due to the absence of weeds in the plots prior to treatment, as noted in the BTOR: "Not a single weed species was recorded from the treatment 4." T1 (Ethoxysulfuron) showed emergence of *S. juncoides* at 42-56 DAS, suggesting that it has no activity against this sedge-like weed. T2 (Fenoxaprop) showed no effect (injury rating 1.0), confirming its lack of activity against sedges.

Efficacy against Echinochloa spp. (ARDC Bajo)

Echinochloa spp. (barnyard grass) was present at low densities at ARDC Bajo.

Table 3.11. Mean *Echinochloa* spp. Density (plants/0.25m²) Across Treatments (ARDC Bajo)

Treatment	Before Spray	14 DAS	28 DAS	42 DAS	56 DAS	Injury Rating
T2	0.3	0.3	0.5	0.5	0.5	2.0
T3	0.0	0.0	1.0	1.0	1.0	1.0
T5	0.3	0.3	0.5	1.0	1.8	1.0

Discussion: T2 (Fenoxaprop) provided the best control of *Echinochloa* spp., with a moderate injury rating of 2.0 (light symptoms), though densities remained relatively static. This is expected as Fenoxaprop is a grass-specific herbicide. T3 showed no effect on *Echinochloa* (injury rating 1.0), and the control showed a six-fold increase in density. However, due to the low initial densities, statistical analysis was not performed for this species.

Summary of injury ratings (56 DAS)

Table 3.12. Injury ratings by species and treatment at 56 DAS

Treatment	***Monochoria vaginalis***	***Potamogeton distinctus***	***Schoenoplectus juncoides***	***Echinochloa* spp.**
T1	1.0 (Tolerant)	4.33 (Susceptible)	1.0 (Tolerant)	-
T2	1.0 (Tolerant)	1.0 (Tolerant)	1.0 (Tolerant)	2.0 (Moderate)
T3	1.0 (Tolerant)	5.0(Complete kill)	3.0-4.0 (Susceptible)	1.0 (Tolerant)
T4	1.0 (Tolerant)	1.0 (Tolerant)	Complete control	-
T5	1.0 (Tolerant)	1.0 (Tolerant)	1.0 (Tolerant)	1.0 (Tolerant)

Site comparison (Kabjisa vs. ARDC Bajo)

A mixed-model interaction term (Site × Treatment) was specifically tested for *M. vaginalis* (the only species with sufficient replication at both sites, treated with T1). The analysis yielded:

- F-value: 0.03
- Degrees of freedom: 1, 8
- p-value: 0.86

This indicates no significant interaction between site and treatment. The efficacy (or lack thereof) of the herbicides was consistent across the two distinct geographical locations. This consistency strongly reinforces the conclusion that the observed inefficacy against *M. vaginalis* is due to the inherent weed biology rather than environmental or edaphic factors.

Overall Discussion

T3 (Triafamone + Ethoxysulfuron - Council Active) demonstrated the most consistent broad-spectrum efficacy across both sites and weed species:

- Best control of *P. distinctus*: 21.8% reduction with complete kill (Injury 5), significantly better than T5 ($p=0.004$) and T2 ($p=0.048$).
- Best control of *S. juncoides*: Injury rating 3–4 with 11% reduction, significantly better than T1 and T5.
- Moderate control of *Echinochloa*: Static population with injury rating 1.0.
- Complete failure against *M. vaginalis*: Population increased by 27.7%, injury rating 1.0.

T1 (Ethoxysulfuron - Sunrice) showed moderate to good efficacy against specific broadleaf weeds:

- Good against *P. distinctus*: 14.8% reduction with heavy damage (Injury 4), significantly better than T5 (p=0.018).
- Poor against *M. vaginalis*: Population increased by 31.9%, injury rating 1.0.
- Poor against *S. juncoides*: Population increased by 400%.
- Narrow spectrum - primarily broadleaf selective with limited activity.

T2 (Fenoxaprop - Ricestar) performed as expected for a grass-specific herbicide:

- Best against *Echinochloa*: Moderate control (Injury 2.0).
- No effect on broadleaf weeds: *M. vaginalis*, *P. distinctus*, and *S. juncoides* all showed injury rating 1.0.
- Not suitable for fields with broadleaf weed problems.

T4 (Butachlor) showed variable efficacy:

- Complete control at ARDC Bajo where no weeds were present in the plots.
- No effect on broadleaf weeds at Kabjisa: Injury rating 1.0 for both *M. vaginalis* and *P. distinctus*.
- Confirms previous reports of limited broadleaf activity; should not be relied upon for emerged broadleaf weed control.

T5 (Untreated Control) demonstrated the critical importance of weed management:

- Weed populations increased 35.6-67.3% over 56 days across species.
- Confirms the need for timely and effective weed management practices.

1. with different modes of action, including contact herbicides or specifically labeled broadleaf herbicides.

3.1.4 Conclusion

Table 3.13. Key findings by species

Species	Best Treatment	2nd Best	Least Effective
Monochoria vaginalis	**None** - All treatments failed	-	T2, T4
Potamogeton distinctus	T3	T1	T2, T4
Schoenoplectus juncoides	T3	-	T1, T2
Echinochloa spp.	T2	T3	T5

Table 3.14. Treatment performance summary

Treatment	Broadleaf Weeds	Grass Weeds	Sedges	Overall Rating
T1	Moderate-Good	Poor	Poor	Moderate
T2	Poor (No effect)	Good	Poor	Poor
T3	Good-Best	Moderate	Good	**Best**
T4	Variable	Good	Complete (Bajo only)	Variable
T5	Poor	Poor	Poor	Poor

Statistical Conclusions

1. **For *Potamogeton distinctus*:** T3 and T1 were highly effective and statistically superior to T2, T4, and the untreated control ($p < 0.05$). T3 achieved a perfect injury score (5.0) and a population reduction of 21.8%. T1 is a strong alternative (injury 4.33, reduction 14.8%). These two treatments are recommended for commercial use against this specific weed.
2. **For *Monochoria vaginalis*:** All treatments (T1-T4) failed to show effectiveness. Statistical tests ($F = 0.89$, $df = 4, 25$, $p = 0.41$) confirmed no significant difference between any herbicide treatment and the weedy check.
3. **For *Schoenoplectus juncoides*:** T3 provided the best control (injury 3-4), significantly better than T1 and T5 ($p < 0.05$). T1 provided zero control, indicating a broad spectrum of inefficacy against non-grass monocots.
4. **General Site Effect:** Treatment efficacy was consistent across Kabjisa and Bajo (Site × Treatment interaction $p = 0.86$), confirming the results are robust and widely applicable.

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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 widely cultivated cereal crops globally and serves as a staple food in many 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). In Bhutan, maize is the second most important cereal crop after rice. Due to its wide adaptability, maize is grown across diverse agroecological zones throughout the country, with extensive cultivation in the mid-altitude and low-altitude regions in the eastern and southern parts of Bhutan.

However, Maize production in Bhutan has shown a consistent declining trend in recent years, raising concerns about its implications for national food security. According to the National Statistics Bureau (NSB, 2024), maize production in 2023 was estimated at 25,118 metric tonnes (MT) from a harvested area of 17,140.71 acres. This shows a decrease of 863 MT (3%) compared to the 2022 production level and a decrease of 21,117 MT from the 46,235 MT produced in 2019. The decline in maize production is due to reduction in cultivated land and the number of maize growers, crop damage caused by insects, diseases, weeds, and wild animals, declining soil fertility, and other constraints affecting domestic agricultural production (NSB, 2024).

Among these challenges, weed infestation is the major constraint, primarily due to the high labour costs associated with weeding. Currently, manual weeding is the only available method for weed control in maize cultivation resulting in labour-intensive and expensive. Both annual and perennial weed species compete with maize plants for vital resources such as nutrients, water, and sunlight, thereby reducing crop growth and yield (Salceanu et al., 2024). Globally, weeds are estimated to account for nearly 37% of maize yield losses (Oerke & Dehne, 2004; Sharma & Rayamajhi, 2022). In addition, severe weed infestations can hinder root development, weakening the crop's ability to absorb water and nutrients efficiently.

A weed inventory conducted by the Weed Science Program across several regions of Bhutan, particularly in the eastern dzongkhags, showed that maize growers face difficulties in managing the weed in their fields. Farmers reported that labour shortages and the increasing cost of hiring workers have made weed control both challenging and expensive. As a result, weed management has become a major production constraint in maize cultivation. Currently, manual weeding is the only method of weed control, as there are no selective herbicides available for use in maize fields. Although Metribuzin is available in Bhutan, it is widely used for potato cultivation. The absence of suitable herbicide options for maize further complicates weed management for maize growers, highlighting the urgent need for alternative weed control solutions.

Considering the significance of maize as a major cereal crop in Bhutan and the increasing challenges associated with labour shortages and weed infestations, the development of efficient and sustainable weed management strategies has become important. One promising approach is the introduction of new-generation herbicides that can reduce labour requirements while improving weed control effectiveness. In this regard, the Weed Science Program has identified a potential herbicide candidate for use in maize cultivation. However, before recommending its adoption by farmers, it is important to evaluate its efficacy and suitability under local agroecological conditions. Such assessments are important to determine its effectiveness against prevalent weed species and crop safety.

Objectives

- Evaluate the efficacy of Tembotrione 34.5% SC as a post-emergence weedicide against complex weed flora in maize.
- Compare the weed control efficiency of manual weeding with the new weedicide under study.

3.2.2 Materials and Method

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 herbicide
- 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 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 before 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

The trial was conducted at the Agricultural Research and Development Centre (ARDC), Wengkhar; farmers' fields in Phosorong, Monggar Gewog; and the Agriculture Research and Development Sub-Centre (ARDSC), Tsirang. The trial was initiated in March and will go on until August 2026.

Trial set up at the on-station site of ARDC-Wengkhar: **March 19, 2026**

Trial set up in a farmer's field in Phosorong: **March 20, 2026**

Trial set up at the on-station site of ARDSC-Tsirang: **April 10, 2026**

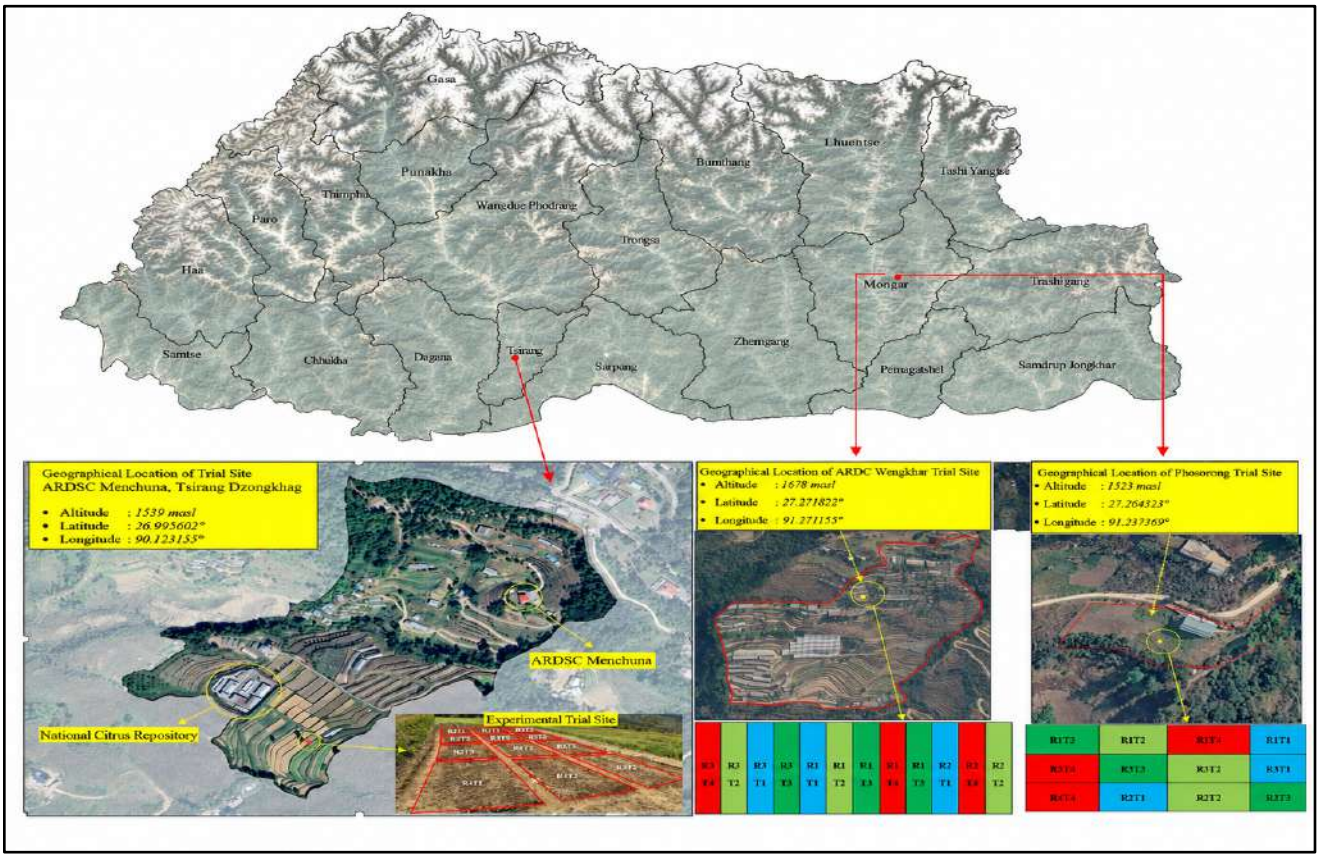

Figure 3.2. Google map representation of trial sites

Trial Design

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

  T1: Manual weeding

  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 m² per plot)

Field preparation and seed sowing at ARDC Wengkhar

The field chosen for the trial establishment at the Centre is a long, single terrace used for maize cultivation. It was ploughed using a power tiller. Then, the entire field was measured to ensure it was uniformly divided into 12 equally sized plots. Once the measurements were completed, the team prepared the beds, each measuring 3 m × 2 m, with a 50 cm gap between beds to allow for easy movement. The beds were arranged in a single long line due to the field's layout.

Yangtsepa maize variety was used for the trial. A total of 27 planting points were created per plot, with a 50 cm distance between plant to plant. Wooden dribblers were used to create holes at the designated points, with two seeds planted in each hole/point. The final step was the installation of tag poles in each plot, with the specific treatment and replication details indicated on the tags. Once the set up was completed, the field was thoroughly irrigated.

Figure 3.3 Bed preparation for the trial

Field preparation and seed sowing at Phosorong, Monggar Gewog

The team, along with the PP focal, visited the farmer's field in Phosorong to establish the trial. The field is located at an elevation of 1823 masl. The team identified the field, which was then ploughed using a mini tiller. Then the field was measured to ensure it could uniformly accommodate all 12 plots. During the ploughing, large stones and plant debris were removed from the site.

Similar to the setup at the on-station field, the team prepared the beds, each measuring 3 m × 2 m, with a 50 cm gap between plots to facilitate easy movement. The beds were arranged in three rows, each containing four beds. Each row was taken as one replication, and the placement of replications and treatments was randomised.

Field preparation and seed sowing at ARDSC Tsirang

The Weed Science Team, in collaboration with the Field Crop Sector focal person of the Centre, established the trial at ARDSC Tsirang on 10 April 2026. The experimental site is situated at an elevation of 1,539 masl. The selected field was identified and prepared using a power tiller. A

similar procedure was followed for the preparation of the raised beds to ensure uniform field conditions for the trial establishment.

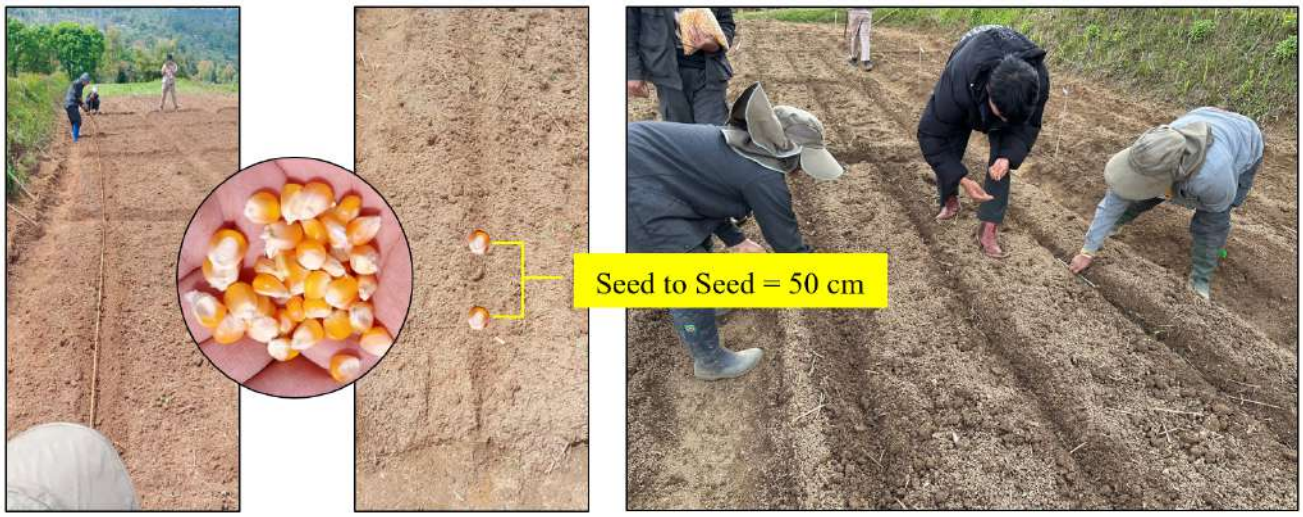

Figure 3.4. Maize seed sowing (Yangtsepa variety)

Application of treatments (weedicides)

Following the collection of pre-spray data, the treatments were prepared and applied according to the experimental protocol. Plots assigned to Treatment 1 across all three replications were maintained under manual weeding. In contrast, Treatments 2 and 3 were subjected to herbicide application using Laudis.

For Treatment 2 (T2), a spray solution was prepared by mixing 0.3 ml of herbicide with 300 ml of water and 0.74 ml of surfactant per plot. For Treatment 3 (T3), the mixture consisted of 0.6 ml of herbicide, 300 ml of water, and 4.44 ml of surfactant per plot. The solution was sprayed using a hand sprayer.

Table 3.14. Dilution rate of treatments

Treatment	**Description**	**Dosage per plot**		
		Tembotrione 34.5% SC	**Water**	**Surfactant**
T1	Manual Weeding			
T2	Low dose	0.3 ml	300 ml	0.74 ml
T3	High dose	0.6 ml	300 ml	4.44 ml
T4	Control	No treatment		

Figure 3.5. Solution preparation

Data Collection

Data collection was conducted both prior to and following herbicide application to assess treatment effects over time. A 1 m × 1 m quadrat (square method) was used, and sampling points were randomly selected within the designated experimental area. The quadrat locations were permanently marked to ensure that all subsequent observations were taken from the same fixed positions throughout the study period.

To date, two rounds of post-treatment data have been recorded, while additional data collection is scheduled for the next financial year to capture longer-term effects. All collected observations have been systematically compiled and organised using Microsoft Excel to facilitate subsequent data analysis.

Pre-spray data collection

Pre-spray data collection was focused on identifying weed species present within the sampled quadrats. Within each marked 1 m × 1 m quadrat, all weed species were identified and the number of individual plants per species was counted and recorded. This baseline information was collected before herbicide application to enable comparison with post-treatment responses. Before sampling, 1 m² quadrats were randomly demarcated within each treatment plot using jute rope and four wooden stakes placed at the corners to ensure consistent sampling points. All weed observations from each quadrat were recorded in an online Excel spreadsheet.

Table 3.15. List of common weed species recorded in trial sites

Sl. No.	Name of Weeds	Presence of weed in Trial sites

1	*Galinsoga quadriradiata*	✓
2	*Galinsoga parviflora*	✓
3	*Bidens pilosa*	✓
4	*Persicaria nepalensis*	✓
5	*Spergula arvensis*	Present in ARDSC Tsirang only
6	*Oxalis latifolia*	✓

Post-spray data collection

Post-spray data collection was conducted every 14 days after herbicide application and continued at 14-day (biweekly) intervals until 56 days after sowing (DAS). During each assessment, the number of individual weed species present within the sampled quadrant was recorded. In addition, any newly emerged weed species that were not observed during the pre-spray assessment were identified and their numbers were documented. Overall, a reduction in weed density was observed across all herbicide-treated plots, whereas the control plots showed no reduction.

Data Analysis

Data was compiled and managed using Microsoft Excel. The analysis was focused on the four most dominant weed species in all sites, *Galinsoga quadriradiata*, *Galinsoga parviflora*, *Oxalis latifolia* and *Persicaria nepalensis*. At the ARDSC Tsirang site, *Spergula arvensis* was also included in the analysis. Herbicide efficacy was assessed using the mean weed count per treatment, calculated by averaging observations across all replications.

3.2.3 Results and Discussion

The study showed that *Galinsoga quadriradiata*, *Galinsoga parviflora*, *Oxalis latifolia* and *Persicaria nepalensis* were the dominant weed species in all study sites. In addition, the ARDSC Tsirang site recorded the presence of *Spergula arvensis* as an additional weed species in the study area. All recorded weed species exhibited a strong response to herbicide application, except *Bidens Pilosa*. *Bidens pilosa* showed a comparatively weaker response to herbicide application, while most species were effectively suppressed. The application of herbicides effectively suppressed weed growth, resulting in a reduction in population density in treated plots compared to the control plots.

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4 Pest Surveillance Program

4.1 Strengthening the Capacity of Agricultural Officials on Wheat Diseases

Historically, wheat has been considered a minor crop in Bhutan, as it is cultivated on a relatively small scale compared to other major cereal crops such as rice (paddy) and maize. Consequently, wheat diseases were not perceived as a significant concern among farmers or agricultural authorities in the past. Due to its limited importance within the national cereal production system, agricultural officials had minimal exposure to training on wheat pest and disease management. As a result, many agriculture extension agents currently possess limited knowledge and practical experience in identifying wheat diseases and implementing appropriate management practices.

To address this knowledge gap, the National Plant Protection Centre in collaboration with the Wheat Commodity Program of the Agriculture Research and Development Centre, Bajo with financial support from the WISER AP Phase I and WISER AP Phase II initiatives under the International Maize and Wheat Improvement Centre (CIMMYT) project, has been actively conducting capacity-building programs. Over the past two consecutive years, training sessions have been organised for agriculture extension officials from Punakha Dzongkhag, Wangdue Phodrang, Trongsa, Zhemgang, Bumthang, Haa, Paro, Thimphu, Samtse and Chhukha Dzongkhag, as well as plant protection officers and field crop officials from the Agriculture Research and Development Centres of four different regions of the country. With Wiser 3rd Phase's fund support, the primary focus of this year's training was to enhance participants' skills in conducting systematic field surveillance of wheat diseases.

The training program was held at six different locations for the extension agents of 10 dzongkhags (Figure 4.1). At Wangdue Phodrang for the extension agents of Wangdue Phodrang and Punakha dzongkhags, at Zhemgang for the extension agents of Bumthang and Zhemgang dzongkhags, at Samtse for the extension agents of Samtse, at Phuntsholing for the extension agents of Chhukha and Thimphu dzongkhags, at Trashigang for the extension agents of Trashigang dzongkhag and at Paro for the extension agents of Paro and Haa dzongkhags. A total of 82 participants (56 males and 26 females) attended the training, including resource persons from the NPPC. The training was conducted over one day and consisted of both theoretical and practical components.

During the first half of the day, participants attended theoretical sessions covering key concepts related to wheat disease surveillance. The sessions included guidance on assessing disease incidence, measuring disease severity, and determining host plant reactions. Participants were also introduced to standardised protocols for collecting both live and dead samples of wheat plants affected by various diseases (Figure 4.2).

The second half of the training focused on practical field applications. Participants were trained on how to conduct field surveillance using the Open Data Kit (ODK) digital data collection system. In addition, awareness was raised about Wheat Blast, a serious disease currently reported in neighboring countries. The training covered the identification of its symptoms, potential risks, and recommended management practices in case the disease is detected in Bhutan in the future.

Participants were also updated on the status of wheat diseases in the country based on findings from field surveys conducted during the previous and current cropping seasons. Furthermore, the NPPC shared information regarding its ongoing collaboration with CIMMYT, including details on project activities, funding support, implementation progress, and the key achievements.

In conclusion, participants were encouraged to conduct regular wheat disease surveillance within their respective gewogs. Extension agents were also advised to seek technical guidance from NPPC officials if they encounter any challenges while conducting field surveillance activities. This initiative is expected to strengthen early disease detection, improve reporting systems, and enhance the overall management of wheat diseases in Bhutan.

Figure 4.1. Geographic coverage of the extension agent training program

Figure 4.2. Images from the training conducted at different locations

4.2 Awareness Raising on Key Wheat Diseases and Their Management Practices for Wheat Growing Farmers

A one-day awareness workshop on wheat diseases and their management practices was conducted at 13 locations (Langthil & Nubi block under Trongsa dzongkhag, Chhoekhor & Ura under Bumthang, Barp & Dzomi under Punakha, Ge-nyen and Maedwang under Thimphu, Dokar under Paro, and Uesu, Samar, Bji and Kar-tshog under Haa dzongkhag (Figure 4.3 & 4.4). The program aimed to enhance farmers' understanding of major wheat diseases, their impacts on crop productivity, and the appropriate management practices to minimize potential losses.

A total of 459 participants attended the workshop, out of which 324 female and 135 male farmers. The session began with a presentation highlighting the importance of rust diseases in wheat and the need for timely management to prevent severe yield losses. Particular emphasis was placed on how effective disease management practices at the national level contribute to the broader understanding of global wheat disease epidemiology.

Farmers were introduced to the major wheat diseases affecting crop production, including the different types of rust diseases, namely Stripe Rust (yellow rust), Leaf Rust (brown rust), and Stem Rust, as well as Fusarium Head Blight. The training focused on helping participants recognize the characteristic symptoms of these diseases and understand their potential impacts on wheat yield and quality.

In addition, farmers were trained in appropriate disease management strategies based on Integrated Pest Management principles. They were advised to adopt preventive and cultural management practices as the primary control measures, while the use of chemical fungicides was recommended only as a last resort when disease outbreaks become severe.

The workshop also included an awareness session on Wheat Blast, a highly destructive disease that has already been reported in neighboring countries. Farmers were informed about the symptoms associated with wheat blast and were encouraged to remain vigilant in monitoring their fields. They were further advised to promptly report any suspected cases to their respective extension agents for immediate investigation and response. To reinforce the importance of early detection and management, demonstrations were provided to illustrate the potential severity and destructive nature of wheat blast. Farmers were also guided on appropriate response measures in the event that the disease is detected in their fields in the future.

Overall, the awareness campaign helped improve farmers' knowledge of major wheat diseases, strengthened their capacity to identify symptoms in the field, and promoted the adoption of sustainable disease management practices to safeguard wheat production.

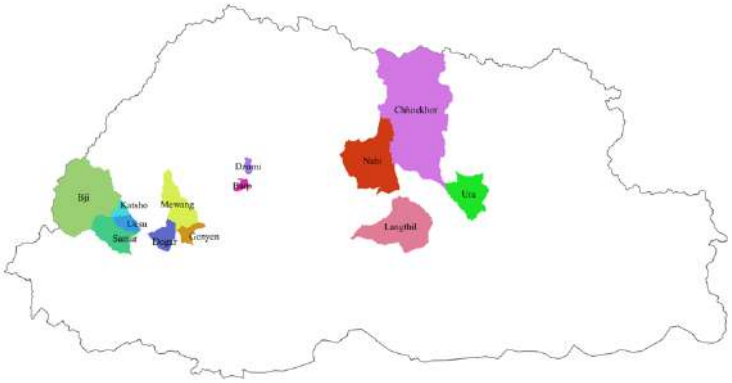

Figure 4.3. Map showing the geographic coverage of the awareness raising

Figure 4.4. Images from the awareness raised at different locations

4.3 Capacity building of Plant Protection & Research officials on barberry survey & laboratory work by the wheat disease experts

Wheat rust is a highly destructive fungal disease that relies on both its primary host (wheat) and alternate hosts, like barberry, to complete its sexual life cycle**.** The Himalayan region is a major hotspot for *Berberis* species. In this environment, these alternate hosts facilitate genetic recombination, driving the emergence of aggressive pathogen strains that pose a serious threat to global cereal production. Although Bhutan grows wheat on only a limited scale, its immense barberry diversity has a disproportionately large impact on regional disease epidemiology. Consequently, pinning down which specific barberry species act as reservoirs for particular rust pathogens is essential for developing effective management strategies.

To address this challenge, CIMMYT wheat disease experts facilitated a six-day capacity-building training program from April 8 to 13, 2026. Attended by 18 plant protection officials (8 women, 10 mwn) from Bhutan's National Plant Protection Centre, the training was led by specialists from CIMMYT, the University of Minnesota, USDA, and Cornell University. The program aimed to equip local researchers with the technical skills needed to survey barberry populations, assess host susceptibility, and detect the presence of yellow and stem rust pathogens.

The training integrated theoretical lectures with practical field and laboratory sessions (Figure 4.5). Participants learned to conduct field surveys using the Open Data Kit platform, identify relevant barberry and grass species, and prepare herbarium specimens for future reference. Laboratory sessions focused on pathogen detection techniques and evaluating the susceptibility of different barberry species to wheat rust strains.

This capacity-building initiative successfully established a stronger technical foundation for Bhutan's national wheat rust surveillance and early detection systems. By mastering advanced field data collection and laboratory diagnostics, the National Plant Protection Centre is now better equipped to generate reliable data. These enhanced competencies will support evidence-based agricultural policies and improve Bhutan's ability to mitigate the impacts of wheat rust both locally and regionally.

Figure 4.5. Training on identification and isolation of cereal rust from alternative host (Barberry)

4.4 Targeted surveys of barberry plants

4.4.1 Introduction

Following the completion of the technical training, systematic surveillance and research initiatives were initiated to investigate the epidemiological role of barberry (*Berberis*) species in wheat rust diseases. This investigation was primarily prompted by wheat rust surveillance reports from the preceding cropping season, which had detected a novel wheat rust pathotype-previously unrecorded in the country- specifically within the Thimphu, Paro, and Haa valleys. Because barberry species can serve as alternate hosts that facilitate the hybridization and survival of rust pathogens, these three valleys were designated as critical target zones to determine if local barberry populations are driving the emergence of new rust races.

Following the technical training, systematic surveillance and research initiatives were launched to investigate how barberry (*Berberis*) species affect the epidemiology of wheat rust diseases. This investigation was driven by rust surveillance reports from the previous cropping season, which had detected a novel wheat rust pathotype never before recorded in Bhutan. This new strain was found specifically within the Thimphu, Paro, and Haa valleys. Because barberry species act as alternate hosts for the disease and allow the rust pathogens to hybridize and survive, these three valleys were designated as critical target zones. This classification will help researchers determine whether local barberry populations are actively driving the emergence of new rust races.

4.4.2 Materials and Method

Field surveys were conducted year-round across the Thimphu, Paro, and Haa valleys to locate, document, and geo-reference barberry bushes. Field teams collected comprehensive botanical and spatial data using the ODK platform, and each identified bush was systematically tagged to ensure precise identification during subsequent visits. These tagged bushes were monitored monthly throughout the active growing season until they entered dormancy. During these regular inspections, researchers examined the foliage for the presence of aecia, collecting infected leaf samples containing live aeciospores whenever symptoms were observed.

Laboratory work-1: To test the presence of rust pathogen on the barberry plants

To determine whether the rust pathogens harboured by wild barberry plants have the capacity to infect wheat crops, controlled laboratory inoculation studies were established. Seedlings of locally available wheat lines, along with "Morocco" (a highly susceptible reference line sourced from the CIMMYT nursery in Kenya), were pre-germinated and cultivated in tubes containing coco peat. The seedlings were fertilised with Suphala at sowing to support early development, and upon coleoptile emergence, they were treated with maleic hydrazide to suppress excessive vertical elongation. This treatment ensured the development of short, robust, and structurally uniform plants optimised for inoculation.

The prepared wheat seedlings were subsequently inoculated with the freshly collected field aeciospores. To facilitate infection under highly controlled conditions, the inoculated plants were maintained in a laminar airflow cabinet for 48 hours before being transferred to an incubation chamber for regular disease monitoring (Figure 4.6).

4.4.3. Results and Discussion

While multiple inoculation experiments have been conducted during the current wheat growing season, no visible symptoms of infection have developed on any of the evaluated wheat lines, including the susceptible Morocco control.

Because successful infection has not yet been achieved, the inoculation experiments are ongoing, and subsequent trials will continue to evaluate fresh field samples under varying experimental parameters. If future inoculation studies successfully produce rust symptoms on the wheat seedlings, infected tissue samples will be harvested and submitted to the Global Rust Reference Centre (GRRC) in Denmark for advanced pathotype analysis to determine their exact race identity and potential impact on cereal production.

Figure 4.6. Laboratory work to test the presence of rust pathogen on the barberry

Laboratory Work-2: Assessing the Susceptibility of Diverse Barberry Species to the Wheat Rust Pathogen

To evaluate the susceptibility of various barberry species (*Berberis* spp.) to wheat rust pathogens, wild specimens were collected from multiple field sites and established in pots within a controlled greenhouse environment. Concurrently, additional barberry seedlings of different species were germinated from seed under laboratory conditions (Figure 4.7).

Inoculation was performed on young shoots, newly emerged sprouts, and laboratory-raised seedlings using teliospores of stripe (yellow) rust and stem rust sourced from various field locations. To trigger germination, the teliospores were pre-soaked in water for 72 hours. Following this soaking period, the germinating teliospores were transferred onto filter paper and secured within Petri dishes. Both the host plant tissues and the teliospores were thoroughly moistened immediately before inoculation to optimise infection conditions. The Petri dishes containing the hydrated teliospores were then inverted over the target plants within transparent containers, and the entire set-up was transferred to an incubation chamber to promote pathogenesis.

To provide essential aeration and prevent stagnation, the inoculum-bearing Petri dishes were removed for 1–2 hours daily before being replaced to resume incubation. Despite conducting multiple experimental runs using this protocol, no macroscopic symptoms of infection have yet been observed on any of the inoculated barberry hosts. Consequently, these evaluations remain active, and further experimental trials are underway to secure definitive results.

Figure 4.7. Laboratory work to test the susceptibility of different species of barberry towards wheat rust

4.5 Wheat Rust Sentinel plot establishment and monitoring

4.5.1 Introduction

The National Plant Protection Centre, in collaboration with the CIMMYT office (under the DEWAS & WISER Phase III Subgrant), has established wheat rust sentinel plots at six different locations (Figure 4.8). Two of these plots are situated at the lowest elevations: one at ARDC-Samtenling (261 masl) and the other at Serzhong gewog (234 masl), both in Sarpang dzongkhag. Two plots are located at mid-elevations: one at ARDC-Bajo (1191 masl) in the west-central region, and the other at ARDC-Wengkhar (1626 masl) in the eastern region. The remaining two plots are positioned at the highest elevations: one at Susuna (2363 masl) in Nagya gewog, Paro dzongkhag, and the other at Dorikha (2923 masl) in Haa dzongkhag, both in the western region. The sentinel plots were established with the following objectives: 1)To trap rust spores, which will be collected and sent to the Global Rust Reference Centre in Denmark for rust race analysis; and 2) To assess the resistance of different wheat varieties against rust diseases under various environmental conditions.

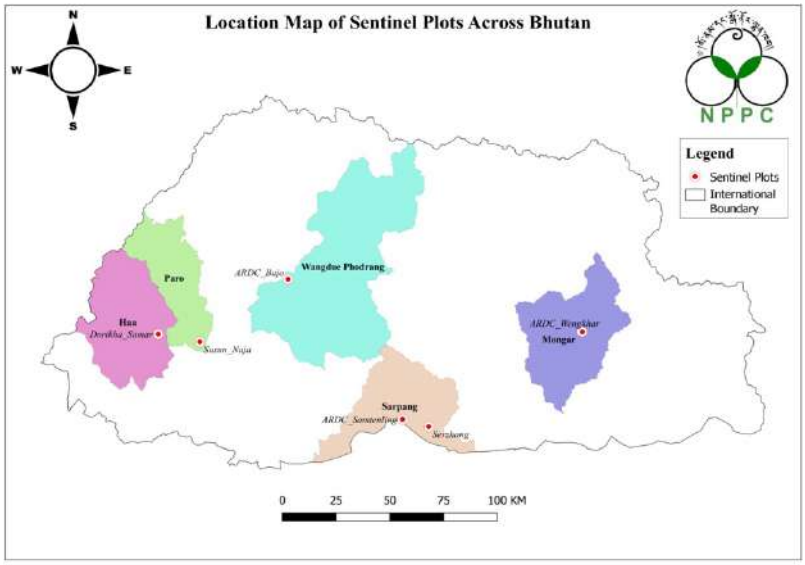

Figure 4.8. Locations where the plots were established

4.5.2 Materials and Method

A total of 33 lines of seeds were sown in each sentinel plot. These included 24 different lines of wheat seeds imported from the CIMMYT office in Mexico, along with nine locally released or local varieties (Table 4.1). Seeds were sown in two plots, each measuring 70 cm x 600 cm. For each line, 3.5 grams of seeds were sown per row in the bed, maintaining an approximate line-to-line distance of 20 cm. Data from the sentinel plots were collected monthly using ODK

applications, starting from the tillering stage until maturity. Whenever rust diseases were observed during data collection, samples were collected and shipped to the GRRC in Denmark for rust race analysis.

Table 4.1. List of wheat lines included in the sentinel plots

Sl. No	Lines	Sl. No	Lines
1	YR5/6*AOC **(CX86.6.1.20-OMXI**)	18	SR31/6*LMPG (**SR31**)
2	SUPER KAUZ **(CM67458-4Y-1M-3Y-OB)**	19	W2691 SR36TT1 (**W2691 SR36TT1**)
3	YR10/6*AOC **(CX93.53.3.1-OMXI)**	20	TETRA CANTHATCH/AE. SQ-SR33, SR5 (**RL5405**)
4	YR15/6*AOC (**CX89.1.1.27-OMXI**)	21	W3763=SR35 (W3763-SR35)
5	YR26/3*AOC (**CX96.17.1-OMXI**)	22	SR50/4/3*KACHU*2/3ND643/2*PRL/2*PASTOR (**CMSS16BO1884T-099MABY-099M-099Y-47M-OWGY)**
6	OPATA/PASTOR (**C05607-A020**)	23	KASUKO (**CMSS11BOO123S-099M-099NJ-1RGY-0B-4M-ORGY**)
7	AOC-YR3*/PASTOR (**CGSS00Y00207T-099M-1Y**)	24	CNSSRTMP (**SRTMP)**
8	YR33 **(###513182-OMXI**)	25	Bumthangka Drukchu
9	Yr35 98M71 **(###734610-OMXI**)	26	Gumasokha
10	Yr37 **(###734609-OMXI**)	27	Bajosokha
11	Yr51 **(###734607-OMXI**)	28	Bajoka 3
12	YRSP/6*AOC (**CX94.14.1.15-OMXI**)	29	Drubeka
13	W2691Sr13 **(###23300)**	30	Bhoeka
14	SWSR22T.B. (**II.70.565.11-?)**	31	Yueka
15	LC-SR25-ARS **(###250760**)	32	Susuna local
16	EAGLE-SR26, SR9G (**EAGLE-SR26, SR9G**)	33	Jho
17	COORONG TRITICALE-SR27 (**COORONG TRITICALE-SR27**)		

4.5.3 Results and Discussion

The overall disease pressure across all sites was comparatively lower than in the previous season (Figure 4.9). At ARDC-Wengkhar, traces of leaf rust, powdery mildew, and Fusarium head blight were observed. Similarly, at Susuna in Paro dzongkhag, minor incidences of yellow rust and low levels of leaf rust were recorded. In addition, nearly all spikes of CIMMYT line number 17 were found to be infected with loose smut. A low incidence of leaf rust was also noted in the plot established at Dorikha in Haa dzongkhag. Conversely, both plots located in the southern region of the country remained free from any detectable diseases. These disease-free plots were located at ARDC-Samtenling and Serzhong in Sarpang dzongkhag. Regarding crop stages, plots in Haa and Paro dzongkhags had reached flowering to maturing stages, depending on the varieties cultivated and their harvest stage, while the crops at the remaining sites had already been harvested.

Figure 4.9. Diseases observed in the sentinel plots at different locations

4.6 General wheat disease surveillance

4.6.1 Introduction

Wheat rust survey is a regular activity conducted annually by the NPPC in collaboration with the ARDCs and Extension agents, with funding support from the CIMMYT project (DEWAS and WISER AP Phase I, II, and III). This year, with financial support from the WISER AP Phase III, the National Plant Protection Centre conducted comprehensive wheat rust surveillance and monitoring across major wheat-growing areas in Punakha, Wangdue Phodrang, Trongsa, Samtse, Haa, and Bumthang dzongkhags. This activity is also part of the global wheat rust monitoring system, wherein rust outbreaks and the possible emergence of new rust races are monitored. The wheat rust surveillance for this growing season began in the third week of February 2026 and will continue until the end of August 2026.

Objectives:

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

4.6.2 Materials and Method

The rust monitoring survey was conducted in selected gewogs of major wheat-growing dzongkhags. The survey was carried out using the ODK application by scanning the QR code of the project, which was customised by the CIMMYT office for conducting wheat rust surveillance in specific countries. In each gewog, major wheat fields were randomly selected. Within a field, a diagonal pattern was followed, observing plants at every 10-15 steps. Information on location, variety name, crop stage, rust type, incidence, severity, and reaction type was recorded. Live rust samples, including those found on grass and barberry plants growing adjacent to wheat fields, were also collected for analysis of the rust races present in the country. Similarly, samples of diseases that could not be identified in the field were collected and brought to the office for confirmation through microscopic observation of spores, which were cultured using wet tissue culture.

4.6.3 Results and Discussion

At this time of the year, crops cultivated in low- and mid-elevation regions have already been harvested. In contrast, fields in higher elevation areas such as Bumthang, Thimphu, Paro, Haa, and Gasa still have standing crops, which are at various growth stages ranging from flowering to maturing, depending on the location and varieties planted.

Unlike previous years, the overall disease pressure this season has been comparatively low (Figure 4.10). In the past, wheat cultivated in the Wangdue and Punakha valleys was often heavily infected with yellow rust. This year, however, crops across nearly all surveyed sites appear relatively clean. The incidence of Fusarium head blight has also been lower compared to the previous season. Nevertheless, yellow rust and leaf rust have begun to emerge on the standing crops, and their severity is gradually increasing.

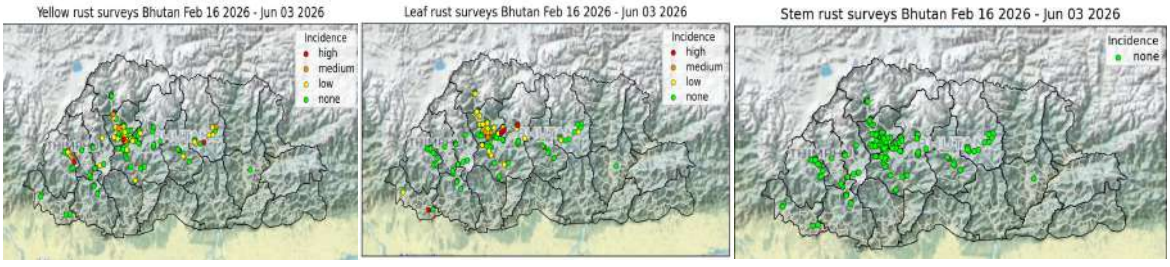

Figure 4.10. Incidence maps of three types of rust in the country

4.6.4 Conclusion

This season's wheat crops exhibited comparatively low disease pressure across most surveyed sites, marking a notable improvement from previous years when yellow rust was widespread, particularly in the Wangdue and Punakha valleys. Although Fusarium head blight incidence was minimal, emerging cases of yellow rust and leaf rust on standing crops suggest a potential rise in severity as the season progresses. Continuous monitoring will therefore be essential to manage these late-season infections effectively.

Figure 4.11. Types of diseases recorded from the field during surveillance

4.7 Development and distribution of Wheat Diseases Awareness Materials

A "Pictorial Guide for Field Identification of Wheat Diseases - Version II" (Figure 4.12) was distributed to extension agents and training participants during wheat disease training sessions conducted at various locations. The guide was used as a key reference tool to support field-level identification and diagnosis of major wheat diseases. In addition, 100 copies each of pictorial posters illustrating the symptoms and signs of the three wheat rust diseases, as well as Fusarium Head Blight (FHB), wheat blast, and their respective management practices, were printed and disseminated. Complementary posters depicting alternate and accessory hosts, including barberry (*Berberis* spp.), *Mahonia* spp., and various grass species, were also produced (Figure 4.12). These materials were utilised during farmer awareness activities to strengthen understanding of disease epidemiology and the role of alternate hosts in disease cycles. The printed materials were distributed to extension agents, who were encouraged to display them in their respective offices and use them as visual aids during farmer outreach and advisory services.

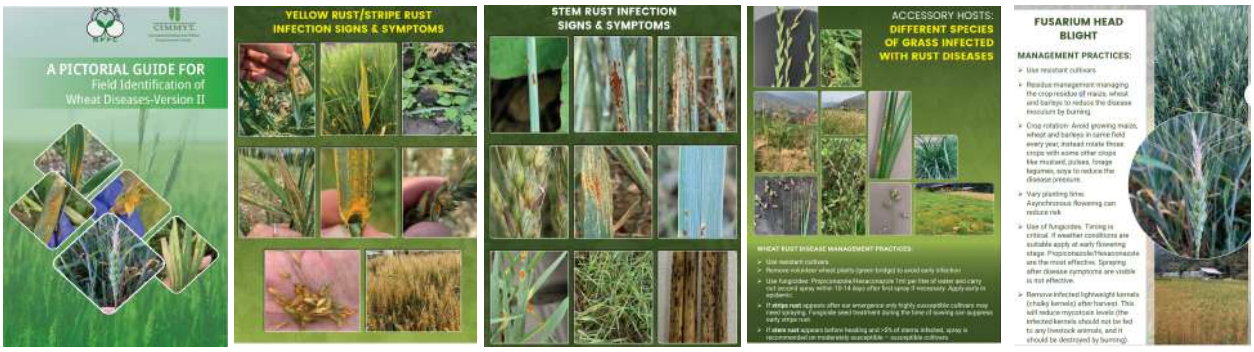

Figure 4.12.Posters and the booklets distributed to the field officials

4.8 Dissemination of Weekly Wheat Disease Early Warning Advisories

The Pest Surveillance program continued the dissemination of weekly Wheat Disease Early Warning Advisories to support timely disease management and safeguard wheat production. The advisory service forms an integral component of the national wheat disease surveillance and forecasting program, providing stakeholders with evidence-based information to facilitate informed decision-making.

Field surveillance data were systematically collected from wheat-growing areas through routine monitoring of disease incidence and severity. The surveillance data were analyzed and used to forecast the potential occurrence and spread of major wheat diseases. Based on these analyses, weekly early warning advisories were developed and disseminated to Regional Plant Protection Officers, Dzongkhag Agriculture Officers, and Extension Agents through emails and NPPC officials web portal (Figure 4.13).

The advisory information was subsequently communicated to wheat-growing farmers through the agricultural extension system, enabling the timely implementation of appropriate disease management interventions. The dissemination of regular advisories enhanced the preparedness of extension personnel and farmers by providing up-to-date information on disease risks, recommended control measures, and emerging disease trends.

The continued implementation of the early warning advisory system strengthened the national capacity for disease surveillance and forecasting, promoted timely response to disease outbreaks, and contributed to improved wheat health management and sustainable crop production through informed, science-based decision-making.

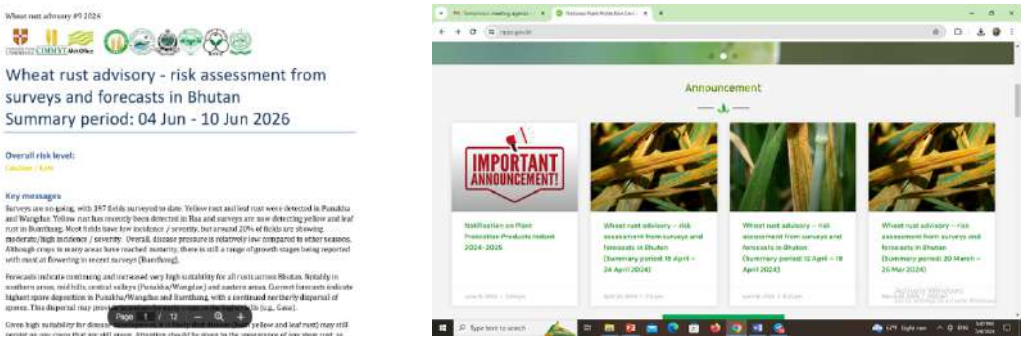

Figure 4.13. Information dissemination through different channels

4.9 Capacity Building on Wheat Genome Sequencing and Bioinformatics

The Centre organized a three-day capacity-building program on Wheat Genome Sequencing and Bioinformatics, facilitated by three experts from the John Innes Centre and The Sainsbury Laboratory. The program aimed to enhance national capacity in the application of genomic and bioinformatics tools for wheat research, disease resistance studies, and plant protection.

A total of 25 participants (13 female and 12 male) attended the training, including plant protection officials from the National Plant Protection Centre and Regional Plant Protection Offices, as well as laboratory officers from the ARDCs representing the four regions (Figure 4.14).

The training combined lectures (Figure 4.14) and hands-on practical sessions covering recent advances in genome sequencing technologies, utilisation of genetic diversity in wheat landraces for resistance breeding, high-performance computing, command-line computing, and bioinformatics pipelines for genomic data analysis. Participants gained practical experience in sequence quality control, sequence alignment, SNP calling and annotation, phylogenetic analysis, k-mer clustering, haplotype analysis using IBSpy, principal component analysis (PCA), and scientific writing, including the preparation of publication-ready figures.

The program strengthened participants' technical competencies in genomic data analysis and bioinformatics, equipping them with the knowledge and practical skills required to support wheat improvement, disease surveillance, and evidence-based plant protection. The training also fostered collaboration among researchers, laboratory personnel, and plant protection professionals, contributing to the development of national expertise in the application of genomics for sustainable agricultural research and crop improvement.

Figure 4.14. Training on Wheat Genome Sequencing and Bioinformatics

4.10 Application of MARPLE Diagnostics for Wheat Rust Surveillance in Bhutan

4.10.1 Introduction

The Surveillance program, in collaboration with pathology and the laboratory officials, applied MARPLE (Mobile and Real-time PLant disEase) diagnostics for molecular characterisation of wheat rust pathogens as part of ongoing national disease surveillance activities.

4.10.2 Materials and Method

Field sampling targeted yellow rust (*Puccinia striiformis* f. sp. *tritici*) from late-season wheat crops in high-altitude regions and stem rust (*Puccinia graminis* f. sp. *tritici*) from off-season and volunteer wheat crops in Haa Dzongkhag (Figure 4.18). In addition, approximately 30 rust-infected samples were collected from farmers' fields during the current season surveillance. These samples are currently undergoing laboratory processing for DNA extraction, sequencing, and subsequent molecular analysis.

DNA was extracted from collected samples (Figure 4.18) and subjected to sequencing for pathogen genotyping and comparative analysis. The resulting genomic data were used to assess the genetic diversity and population structure of wheat rust pathogens across different agro-ecological zones.

4.10.3 Results and Discussion

Preliminary results indicate a high level of genetic diversity within national rust populations (Figure 4.18). Yellow rust samples collected from Laya in Gasa Dzongkhag showed pronounced genetic differentiation, with isolates obtained from very small geographic areas exhibiting distinct genotypic profiles. Similarly, stem rust isolates collected from the same field plots demonstrated subtle but consistent genetic variation among samples.

These findings suggest the presence of a heterogeneous and evolving rust population within the country, with multiple pathogen lineages co-existing within localised environments (Figure 4.18). This highlights the dynamic epidemiology of wheat rust pathogens in Bhutan and underscores the importance of continuous molecular surveillance for early detection of emerging variants.

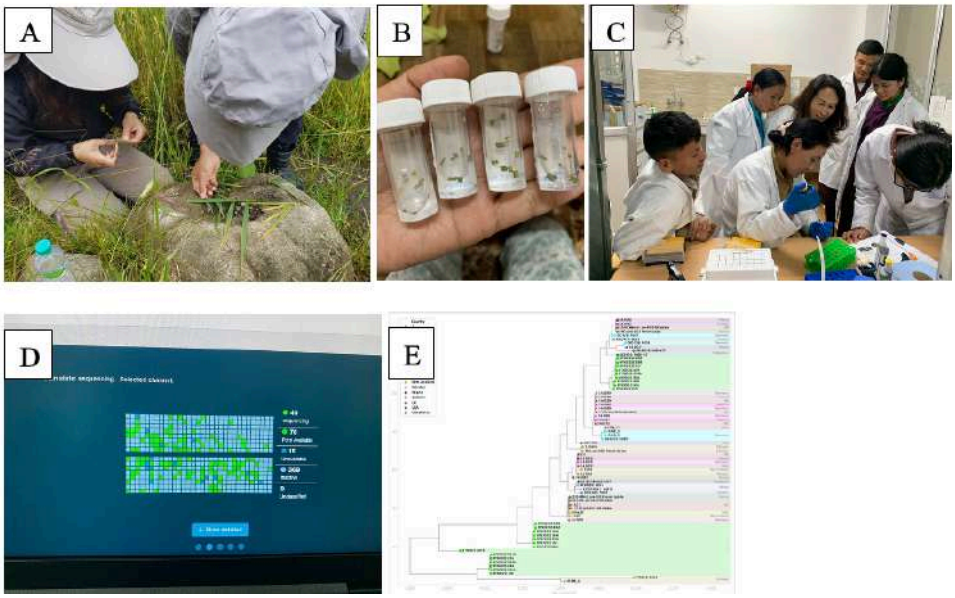

Figure 4.15. MARPLE Diagnosis procedure; A-Field sample collection, B-Sample preservation in RNA later, C-Sample DNA extraction, D-Sample sequencing, E- Phylogenetic tree of yellow & stem rust

The application of MARPLE diagnostics has significantly strengthened national capacity for rapid pathogen genotyping and provided valuable insights into the population biology of wheat rusts. The ongoing analysis of additional field samples will further enhance understanding of pathogen diversity and support the development of targeted, evidence-based disease management strategies for sustainable wheat production.

4.11 Development of ePest Surveillance System under GEF Project Support

With funding support from the Global Environment Facility (GEF) project initiatives, the Surveillance program initiated the development of a prototype ePest surveillance system aimed at strengthening national pest monitoring, reporting, and decision-support mechanisms.

To ensure that the system incorporates inclusive and functional design components, a multi-stakeholder consultation process was undertaken during the prototype development phase. Key stakeholders included representatives from all technical programs of the Centre, Dzongkhag Agriculture Officers (DAOs) and the field extension agents from selected Dzongkhags and Gewogs, and Regional Plant Protection Officers of four regions (Figure 4.16). This participatory approach ensured that user requirements from different administrative and operational levels were adequately reflected in the system design.

In addition, the GEF project focal person was engaged during the consultation process to provide an overview of the project's goals and objectives and to ensure alignment of the prototype with the project's priority areas. The focal person also provided technical feedback on the prototype development, particularly with respect to its relevance, scope, and sustainability within the framework of the funded project. The consultative development process facilitated the integration of practical field-level requirements with national surveillance priorities, thereby enhancing the relevance and usability of the ePest system. The prototype is expected to serve as a foundation for a more robust, real-time pest surveillance platform to support evidence-based decision-making in plant protection and sustainable agricultural management.

Figure 4.16. Consultation meeting on development of ePest Surveillance System

5 Plant Protection Products Program

5.1 Plant Protection Products Indents and Procurement

During FY 2025–26, the NPPC received consolidated demands from farmers and extension agencies across the country for 7,707.88 kg/L of insecticides, 14,531.95 kg/L of fungicides, 467,367.45 kg of herbicides, 6,215.25 kg/L of bio-pesticides, and 9,909 traps and lures.

Based on these indents and the availability of funds, the NPPC procured a total of 9,019 kg/L of fungicides, 3,872 kg/L of insecticides, 146,431 kg of herbicides, 4,772 kg/L of bio-pesticides, and 3,930 traps and lures. In addition, under the RNR-JOBS Project, an additional 1,710 kg of fungicides, 250 kg of herbicides, 200 kg of bio-pesticides, and 200 traps were procured for distribution under the project interventions.

Including the additional procurements and carry-over stocks from previous years, the total stocks available during the financial year amounted to 18,502.75 kg/L of fungicides, 10,550.4 kg/L of insecticides, 460,242.5 kg of herbicides, 9,503.5 kg/L of bio-pesticides, and 15,585 traps and lures. The available stocks ensured an adequate supply of plant protection inputs to support pest and disease management interventions across the country.

5.2 PP Products Distribution

5.2.1 Overall Distribution

During FY 2025–26, a total of 480177.39 kg/L of pesticides were distributed to farmers across the country based on submitted demands. The distribution comprised herbicides, fungicides, insecticides, and bio-pesticides to support the management of weeds, diseases, and insect pests in different cropping systems.

Pesticide distribution varied considerably among dzongkhags according to cropping intensity, cultivated area, and crop protection requirements. The highest quantities were distributed in Paro (164,296.11 kg/L), Punakha (85,836.9 kg/L), Wangdue Phodrang (48,078.78 kg/L), Trashi Yangtse (39,963.98 kg/L), Thimphu (35,148.4 kg/L), and Trashigang (32,071 kg/L). These six dzongkhags together accounted for the majority of the total volume distributed during the fiscal year. Moderate quantities were supplied to Lhuentse, Trongsa, Tsirang, Chukha, Dagana, and Mongar, while relatively small quantities were distributed to Bumthang, Haa, Pema Gatshel, Samtse, Samdrup Jongkhar, and Sarpang. No pesticide distribution was recorded in Gasa and Zhemgang during the reporting period.

The distribution pattern indicates continued reliance on plant protection products to address diverse crop protection challenges across the country. The high demand observed in major agricultural dzongkhags reflects the importance of timely interventions for managing weeds, diseases, and insect pests and safeguarding crop productivity and quality. The availability and distribution of these products supported farmers in implementing appropriate pest management measures and contributed to sustaining agricultural production during the reporting period.

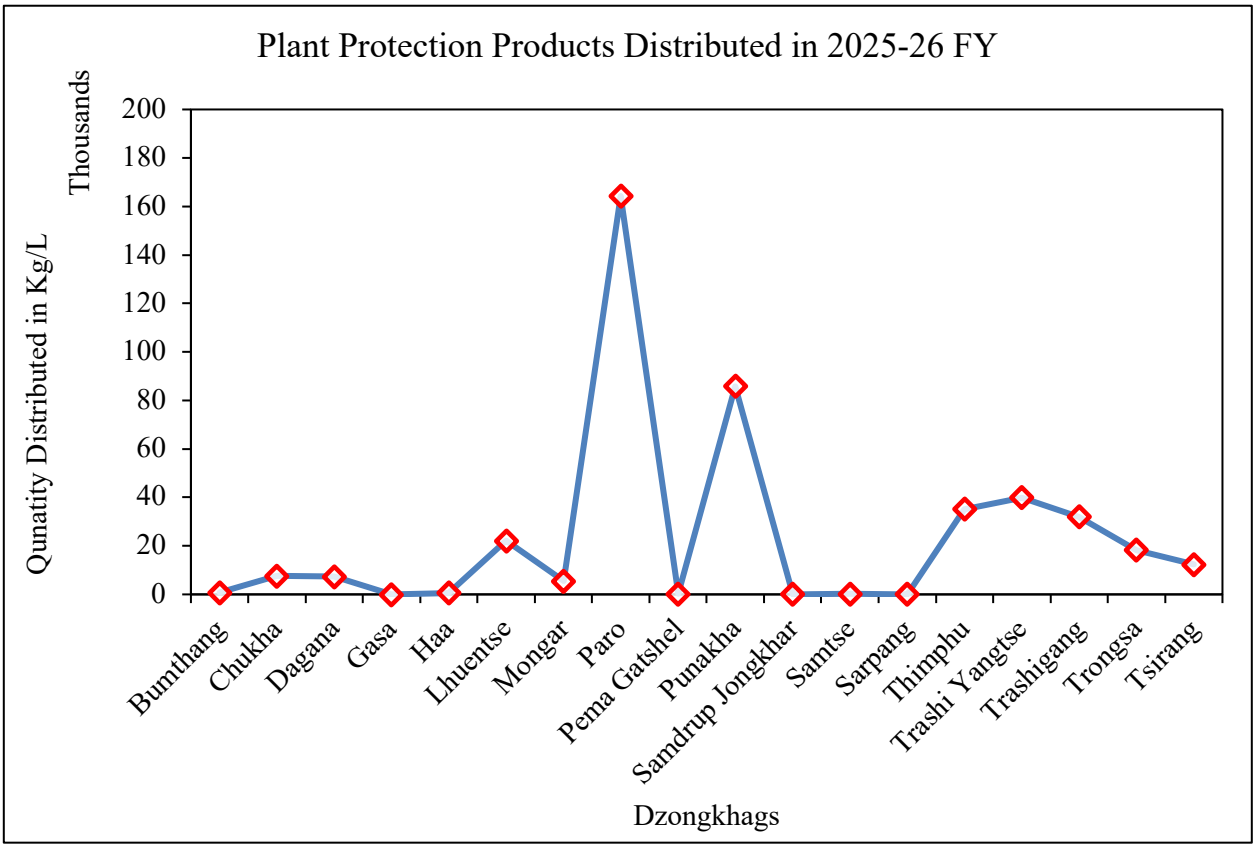

Figure 5.1. Overall distribution of plant protection products to dzongkhags and central agencies during FY 2025–2026

5.2.2 PPP distribution by types

Insecticides

During FY 2025–26, a total of 6,804.54 kg/L of insecticides were distributed to farmers across the country to support the management of insect pests affecting agricultural and horticultural crops. Insect pests continue to pose a significant threat to crop production by causing direct damage to plant tissues and reducing both yield and quality. Timely access to insecticides enabled farmers to respond effectively to pest outbreaks and minimize economic losses.

The distribution of insecticides varied among dzongkhags depending on crop types, pest incidence, and seasonal requirements. The highest quantities were distributed in Paro (2,468.16 kg/L), followed by Wangdue Phodrang (1,134.73 kg/L), Thimphu (840.1 kg/L), and Trashi Yangtse (568 kg/L). Moderate quantities were supplied to Punakha, Chukha, Haa, Trongsa, Sarpang, and Trashigang, while relatively small quantities were distributed to Bumthang, Dagana, Lhuentse, Mongar, Pema Gatshel, Samtse, Samdrup Jongkhar, and Tsirang. No insecticide distribution was recorded in Gasa and Zhemgang during the reporting period.

The distribution pattern reflects the continued need for effective insect pest management interventions, particularly in major agricultural production areas. While insecticides remain an important component of crop protection, their use is increasingly being integrated with pest monitoring, biological control, and cultural practices to promote more sustainable and environmentally sound pest management approaches. The availability of insecticides during the year supported timely interventions against insect pests and contributed to safeguarding agricultural productivity and food security.

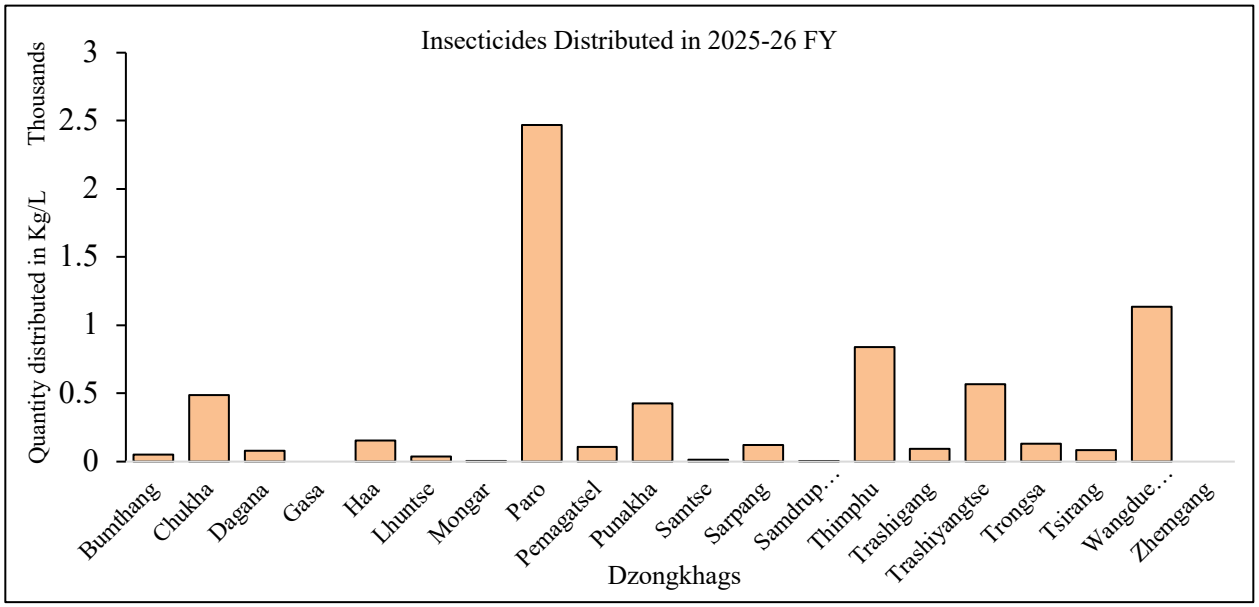

Figure 5.2. Dzongkhag-wise distribution of insecticides during FY 2025–26

Fungicides

During FY 2025–26, a total of 12,821.6 kg/L of fungicides were distributed to farmers across the country to support the management of fungal diseases affecting field, fruit, and vegetable crops. Bhutan's diverse agroecological conditions, coupled with periods of high humidity and rainfall,

create favorable environments for the development and spread of fungal pathogens, necessitating timely disease management interventions.

The distribution of fungicides varied considerably among dzongkhags depending on cropping patterns, disease prevalence, and crop protection requirements. The highest quantity was distributed in Wangdue Phodrang (8,175.3 kg/L)**,** accounting for nearly two-thirds of the total fungicide distribution. This was followed by Thimphu (1,419.05 kg/L) **and** Paro (1,378.05 kg/L). Moderate quantities were supplied to Trashi Yangtse, Trashigang, Mongar, Bumthang, Tsirang, Punakha, and Dagana, while relatively small quantities were distributed to Haa, Chukha, Pema Gatshel, Sarpang, Samtse, Lhuentse, Trongsa, and Samdrup Jongkhar. No fungicides were distributed in Gasa and Zhemgang during the reporting period.

Fungicides remain an essential component of crop protection programs, helping farmers safeguard crop health, maintain yield potential, and improve produce quality. The distribution pattern indicates the continued occurrence of fungal disease challenges across agricultural production systems and highlights the importance of timely disease management interventions. The availability of fungicides during the reporting period supported farmers in managing disease outbreaks, reducing crop losses, and sustaining agricultural productivity.

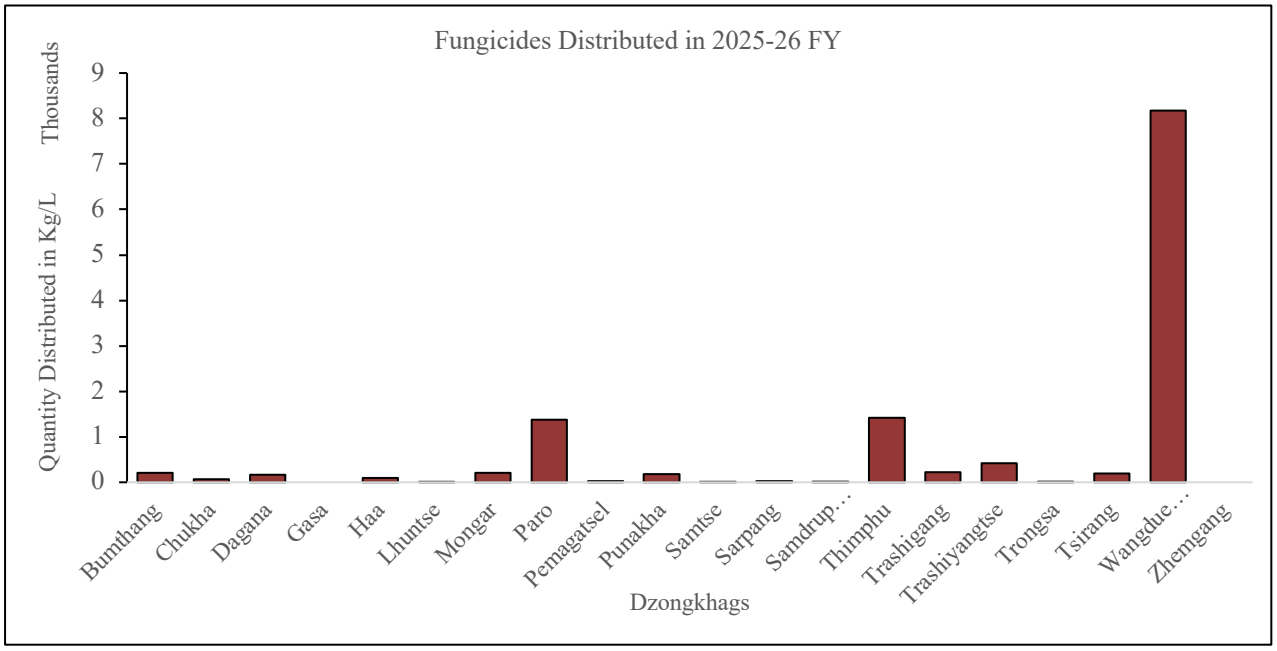

Figure *5.3.* Dzongkhag-wise distribution of fungicides during FY 2025–26

Herbicides

During FY 2025–26, a total of 456,181.65 kg of herbicides were distributed to farmers across the country, making herbicides the most widely distributed category of plant protection products. The predominance of herbicide distribution reflects the persistent challenge of weed management in Bhutan's agricultural production systems and the increasing labour constraints faced by farming communities.

Herbicide distribution varied considerably among dzongkhags according to cultivated area, cropping intensity, and weed management requirements. The highest quantity was distributed in Paro (158,125.4 kg), followed by Punakha (85,211 kg)**,** Trashigang (39,644.15 kg)**,** Wangdue Phodrang (38,427.9 kg)**,** Thimphu (31,322.75 kg)**,** and Trashi Yangtse (31,088 kg)**.** Substantial quantities were also supplied to Lhuentse (22,035.35 kg)**,** Trongsa (18,198.15 kg)**,** and Tsirang (12,000 kg). Moderate quantities were distributed in Chhukha, Dagana, and Monggar, while relatively small amounts were supplied to Bumthang and Haa. No herbicides were distributed to Gasa, Pema Gatshel, Samtse, Sarpang, Samdrup Jongkhar, and Zhemgang during the reporting period.

The distribution pattern indicates that weed management continues to be a major priority for farmers across the country. The availability of herbicides supported timely weed control interventions, reduced labour requirements for manual weeding, and contributed to improved crop establishment, productivity, and the efficient management of agricultural production systems during the reporting period.

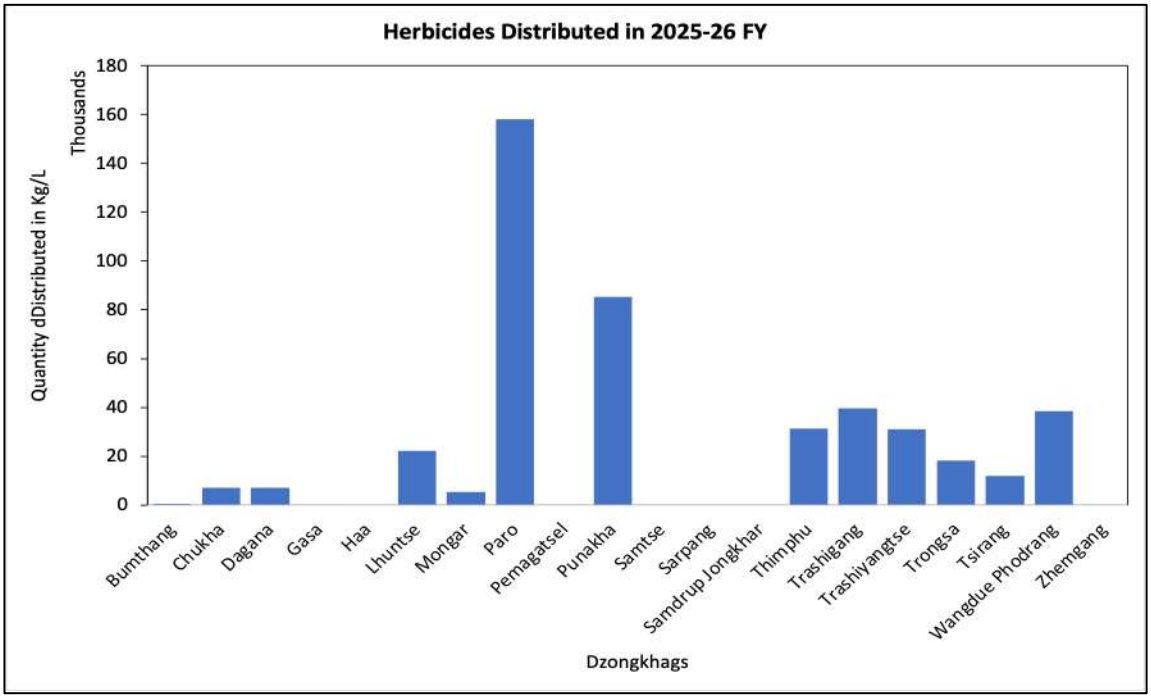

Figure 5.4. Dzongkhag-wise distribution of Herbicides during FY 2025–26

Bio-pesticides, Traps and Lures

During FY 2025–26, a total of 4,369.6 kg/L of bio-pesticides and 2,586.25 traps and lures were distributed to farmers across the country to support environmentally sustainable and integrated approaches to pest management. Bio-pesticides and traps and lures are important components of integrated pest management (IPM), helping to reduce reliance on conventional pesticides through pest monitoring, early detection, and the use of environmentally friendly control measures.

The distribution of bio-pesticides was concentrated primarily in Paro (2,324.5 kg/L) and Thimphu (1,566.5 kg/L)**,** which together accounted for the majority of the total quantity distributed during the reporting period. Moderate quantities were supplied to Wangdue Phodrang (340.85 kg/L), while relatively smaller amounts were distributed to Dagana, Pemagatshel, Punakha, Bumthang, Haa, Tsirang, Chhukha, Monggar, and Sarpang. No bio-pesticides were distributed in Gasa, Lhuentse, Samdrup Jongkhar, Trashigang, Trashi Yangtse, Trongsa, Samtse, and Zhemgang.

The highest numbers of traps and lures were distributed in Pemagatshel (539), followed by Trongsa (337)**,** Dagana (319)**,** Samtse (300)**,** Mongar (218)**,** Paro (208)**,** Tsirang (180)**,** and Wangdue Phodrang (174)**.** Smaller quantities were distributed in Punakha, Thimphu, Trashi Yangtse, and Sarpang, while no traps and lures were distributed in Bumthang, Chukha, Gasa, Haa, Lhuentse, Samdrup Jongkhar, Trashigang, and Zhemgang.

The distribution pattern indicates increasing efforts to promote integrated and environmentally sound pest management practices across the country. The availability of bio-pesticides, traps, and lures supported pest surveillance, facilitated timely interventions, and encouraged the adoption of sustainable crop protection approaches, thereby contributing to improved agricultural productivity and reduced dependence on conventional pesticides.

Table 5.1. Dzongkhag-wise distribution of bio-pesticides and traps and lures during FY 2025–26

Dzongkhag	**Traps and Lures** (Numbers)	**Bio-pesticides** (Kg/L)
Bumthang	0	7
Chhukha	0	1.5
Dagana	319	53
Gasa	0	0
Haa	0	6.6
Lhuntse	0	0
Monggar	218	0.2
Paro	208	2324.5
Pemagatsel	539	43.5
Punakha	71.25	16

Samtse	300	0
Sarpang	25	4.2
Samdrup Jongkhar	0	0
Thimphu	75	1566.5
Trashigang	0	0
Trashiyangtse	140	0
Trongsa	337	0
Tsirang	180	5.75
Wangdue Phodrang	174	340.85
Zhemgang	0	0
Total	**2586.25**	**4369.6**

5.3 Conclusion

During FY 2025-2026, the Plant Protection Products Program ensured the timely procurement and distribution of plant protection inputs to meet the diverse crop protection needs of farmers across the country. A total of 9,019 kg/L of fungicides, 3,872 kg/L of insecticides, 146,431 kg of herbicides, 4,772 kg/L of bio-pesticides, and 3,930 traps and lures were procured, while total available stocks, including carry-over stocks, remained adequate to support pest and disease management interventions nationwide. Overall, 480,177.39 kg/L of pesticides and 2,586.25 traps and lures were distributed to farmers and agencies based on demand.

The distribution pattern highlighted the continued importance of crop protection products in addressing weeds, diseases, and insect pests across Bhutan's agricultural production systems. Herbicides accounted for the largest share of distribution, reflecting the persistent challenge of weed management and increasing labour constraints, while the distribution of fungicides, insecticides, bio-pesticides, and traps and lures supported integrated and environmentally sound pest management practices. The program contributed to timely pest management interventions, improved crop health and productivity, and strengthened the capacity of farmers to manage biotic stresses and sustain agricultural production.

6 Biocontrol Program

6.1 Diversity and Composition of Natural Enemies Associated with Fall Armyworm (*Spodoptera frugiperda*) in Maize Ecosystems of Bhutan

6.1.1 Introduction

Fall armyworm (*Spodoptera frugiperda* J.E. Smith) (Lepidoptera: Noctuidae) is a highly destructive invasive pest that has rapidly expanded its distribution beyond its native range in the Americas, causing significant yield losses in maize and other crops worldwide. Since its first detection in Bhutan in 2019, the pest has become established in major maize-growing areas, posing a serious challenge to sustainable maize production. Management of FAW has largely relied on chemical insecticides; however, increasing concerns regarding insecticide resistance, environmental impacts, and disruption of beneficial organisms highlight the need for integrated pest management (IPM) approaches.

Natural enemies, including parasitoids, predators, and entomopathogenic microorganisms, play an important role in regulating FAW populations in agricultural ecosystems. Among these, parasitoids such as *Cotesia* spp., *Chelonus* spp., and *Telenomus* spp., along with microbial agents including entomopathogenic fungi, nematodes, and nucleopolyhedrovirus, have been reported as important biological control agents against FAW in different regions. However, information on naturally occurring FAW-associated natural enemies in Bhutan remains limited.

Understanding the diversity and abundance of native natural enemies is essential for developing conservation-based biological control strategies and identifying potential candidates for incorporation into FAW management programs. Therefore, this study aimed to document the composition and relative abundance of parasitoids and microbial natural enemies associated with FAW larvae collected from maize fields in Bhutan.

6.1.2 Materials and Method

Field Collection of Fall Armyworm Larvae

Field surveys were conducted in 2026 in maize fields across different locations in Bhutan. Maize plants showing symptoms of fall armyworm infestation were inspected, with particular attention given to leaf whorls, leaves, and tassels where larvae are commonly observed. Larvae were identified in the field based on characteristic morphological features, including the inverted “Y”-shaped marking on the head capsule and longitudinal body stripes.

Individual larvae were collected using soft forceps or a camel-hair brush and placed separately in labelled plastic containers to prevent cannibalism. Collection information, including date, location, GPS coordinates, and host plant, was recorded for each sample. The collected larvae were transported to the laboratory in ventilated containers placed inside a cooler box and protected from

direct sunlight during transportation. The collection sites and number of larvae collected from each location are presented in Table 1.

Table 6.1. Collection sites and number of fall armyworm larvae collected from maize fields in Bhutan

Sl. No	Place	Dzongkhag	No. of FAW larvae collected
1	Yonphula, Kanglung	Trashigang	19
2	Maangthong, Kanglung	Trashigang	9
3	Ritsangdoong, Kanglung	Trashigang	17
4	Rongthoong Kanglung	Trashigang	18
5	Phoshing, Samkhar	Trashigang	19
6	ARDSC, Tsirang	Tsirang	16
7	Phosorong	Mongar	14
8	ARDC Wengkhar	Mongar	15
9	Basnatey, Dorokha	Samtse	9
10	Dorokha, Dophuchen	Samtse	26
11	Jogijaon, Lhamoi Dzingkha	Dagana	12
12	Daragai, Lhamoizingkha	Dagana	2
13	Karmaling	Dagana	14
14	Jemathang, Karmaling	Dagana	34
15	Pangna, Drukjeygang	Dagana	9
16	Tsendagang	Dagana	12
17	Tashiding	Dagana	18
18	Karmaling, Samphelling	Chukha	65
19	Khenpaithang, Samphelling	Chukha	23
20	Rilangthang, Serghithang	Tsirang	172
21	Bajo town, Thedtsho	Wangdue Phodrang	9
22	Wongjokha, Thedtsho	Wangdue Phodrang	18
23	Khilikhar, Dzomi	Punakha	55
24	Dzomlingthang, Guma	Punakha	19

Figure 6.1. Collection of fall armyworm larvae from infested maize fields (left) and damage symptoms caused by fall armyworm larvae (right)

Laboratory Rearing and Parasitoid Observation

Collected larvae were individually reared in plastic cups under controlled conditions of 27 ± 1°C and 70 ± 5% relative humidity. Initially, larvae were provided with fresh untreated maize leaves, and after six months, an artificial diet was used for colony maintenance and parasitoid recovery. The artificial diet was prepared using chickpea flour, maize leaf powder, brewer's yeast, vitamins, preservatives, agar, and water. Larvae were monitored daily for feeding activity, development, mortality, pupation, and parasitoid emergence. Emerged parasitoids were collected, while non-parasitized larvae were allowed to complete development.

Figure 6.2. Laboratory rearing of fall armyworm larvae; Artificial diet (left) and Maize (right)

Parasitoid Identification and Preservation

Emerging parasitoids were collected after emergence and identified based on morphological characteristics observed under a stereomicroscope. Diagnostic features, including body size, colouration, wing venation, antennal structure, and abdominal morphology, were examined for identification. Specimens were preserved in 99.9% ethanol, assigned unique identification codes, labeled with collection details, and stored at 4°C for further examination.

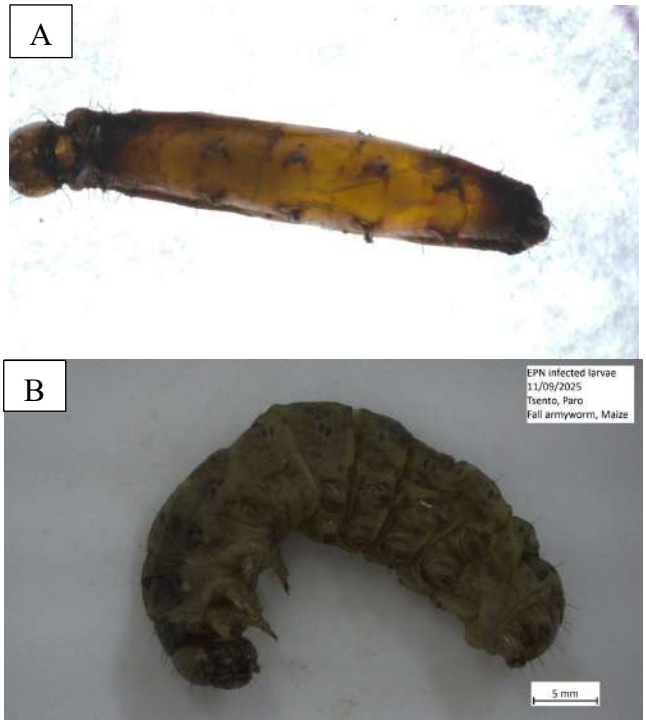

Figure 6.3. Aleiodes-infected (A) and entomopathogenic nematode-infected (B) fall armyworm larvae

6.1.3 Results and Discussion

Natural Enemy Composition Associated with Fall Armyworm

Among the 624 fall armyworm larvae collected from maize fields, 107 larvae were found to be associated with natural enemies. Four groups of natural enemies were recorded: parasitoids, entomopathogenic fungi (EPF), entomopathogenic nematodes (EPN), and nucleopolyhedrovirus (NPV).

Parasitoids were the predominant natural enemies, accounting for 88.79% of all recovered natural enemies (95 occurrences). Entomopathogenic fungi represented 5.61% (6 occurrences), followed by entomopathogenic nematodes (4.67%; 5 occurrences) and nucleopolyhedrovirus (0.93%; 1 occurrence).

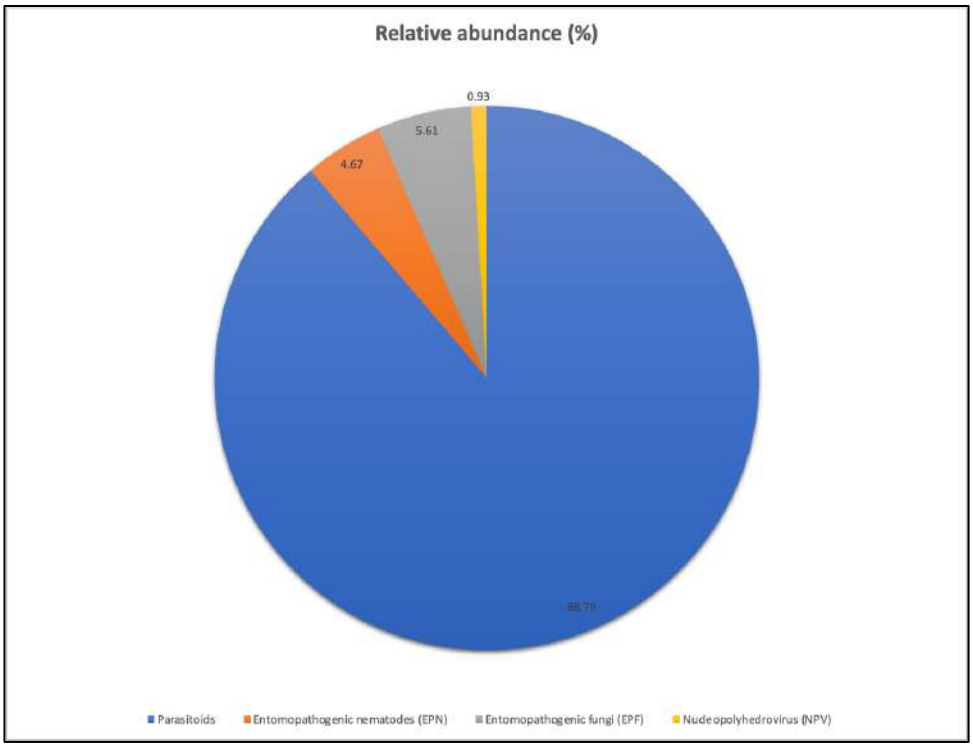

Figure 6.4. Relative abundance of natural enemy groups recovered from fall armyworm larvae collected in maize fields of Bhutan. Parasitoids represented the dominant natural enemy group, followed by entomopathogenic fungi (EPF), entomopathogenic nematodes (EPN), and nucleopolyhedrovirus (NPV).

Figure 6.5. Entomopathogenic fungi infected fall armyworm larvae: *Beauveria bassiana* (left) and *Metarhizium anisopliae* (right)

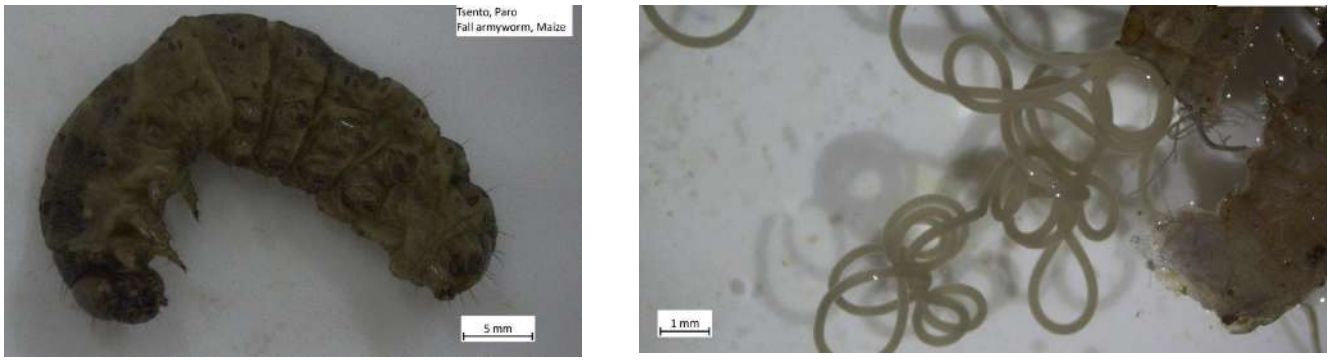

Figure 6.6. Entomopathogenic nematode-infected fall armyworm larvae

Table 6.2. Natural enemy composition associated with fall armyworm larvae collected from maize fields in Bhutan.

Natural Enemy Type	**Frequency of occurrence (n)**	**Relative abundance (%)**
Parasitoids	95	88.79
Entomopathogenic fungi (EPF)	6	5.61
Entomopathogenic nematodes (EPN)	5	4.67
Nucleopolyhedrovirus (NPV)	1	0.93

Parasitoid Composition Associated with Fall Armyworm

A total of 95 parasitoid individuals were recovered from fall armyworm larvae collected from maize fields. The parasitoid community consisted of eight groups, with *Cotesia* spp. being the most abundant parasitoid, accounting for 64.21% (61 occurrences) of all recovered parasitoids. This was followed by *Aleiodes* spp. with 14.74% (14 occurrences) and *Charops* spp. with 9.47% (9 occurrences).

Other parasitoid groups were recovered at lower frequencies, including *Bothrophora* spp. (4.21%; 4 occurrences), Tachinid flies (3.16%; 3 occurrences), *Chelonus* spp. (2.11%; 2 occurrences), and *Coccygidium* spp. and Ichneumonidae spp. (1.05%; 1 occurrence each).

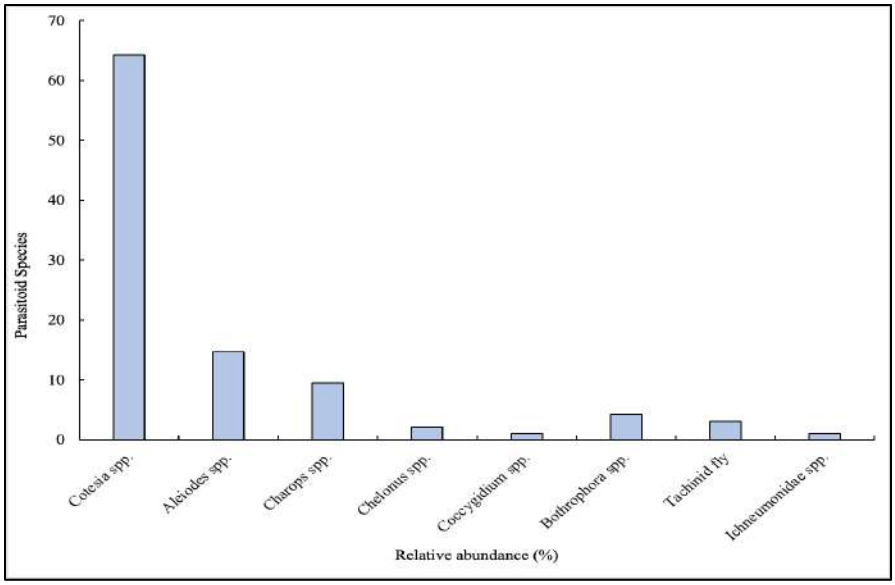

Figure 6.7. Relative composition of parasitoid groups recovered from fall armyworm larvae collected from maize fields in Bhutan

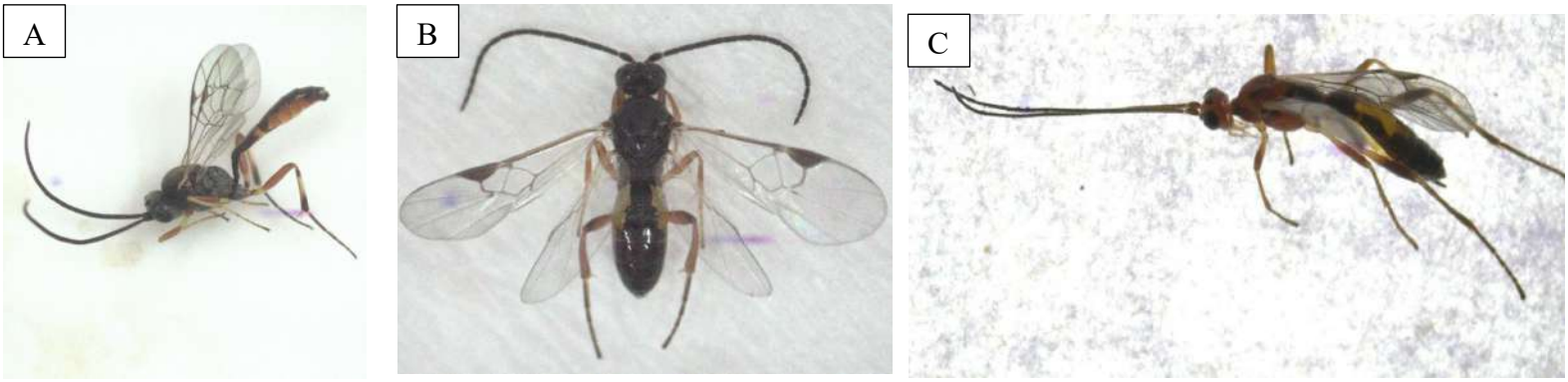

Figure 6.8. Representative parasitoids recovered from fall armyworm larvae: *Charops* spp. (A), *Cotesia* spp. (B), *Aleiodes* spp. (C),

The present study showed a diverse natural enemy complex associated with fall armyworm in maize fields of Bhutan, with parasitoids representing the dominant group among the recovered natural enemies. The predominance of parasitoids (88.79%) suggests that naturally occurring parasitoid communities may play an important role in regulating FAW populations under field conditions. Similar to observations from other FAW invasion regions, parasitoids have been reported as one of the major components of the natural enemy complex contributing to the suppression of FAW populations.

Among the recovered parasitoids, *Cotesia* spp. was the most abundant group, accounting for 64.21% of all parasitoid recoveries. Species within the genus *Cotesia* are widely recognised as

important larval parasitoids of FAW and other *Spodoptera* species due to their high parasitism capacity, wide distribution, and potential for biological control. The high occurrence of *Cotesia* spp. in maize fields in Bhutan indicates their potential contribution to natural FAW suppression and highlights the need for further studies on species identification, parasitism rates, and seasonal population dynamics.

Other parasitoids, including *Aleiodes*, *Charops*, *Chelonus*, *Coccygidium*, *Bothrophora*, tachinid flies, and Ichneumonidae, were recorded at lower frequencies. Although their abundance was comparatively low, the presence of multiple parasitoid taxa indicates a diverse parasitoid community associated with FAW in Bhutan. Such diversity may enhance ecosystem resilience by providing complementary biological control services across different FAW developmental stages and environmental conditions.

In addition to parasitoids, entomopathogenic fungi, entomopathogenic nematodes, and nucleopolyhedrovirus were detected, although at relatively low frequencies. The occurrence of these microbial natural enemies demonstrates the presence of additional biological control agents within maize ecosystems. Further investigation of native EPF and EPN isolates, including their pathogenicity and potential for development as microbial biopesticides, could support the integration of locally adapted biological control options into FAW integrated pest management programs in Bhutan.

Overall, the findings provide the first evidence of a diverse natural enemy complex associated with FAW in Bhutan and establish a baseline for future studies on conservation biological control and sustainable FAW management strategies.

6.1.4 Conclusion

This study represents the first comprehensive survey of parasitoids emerging from field-collected fall armyworm larvae in Bhutan and provides important baseline information on the diversity and distribution of indigenous natural enemies associated with the pest. A total of 202 positive records were obtained from 846 field-collected larvae, demonstrating that naturally occurring parasitoids and pathogens are widely present in Bhutanese maize ecosystems. The predominance of *Cotesia* spp., together with the occurrence of *Aleiodes*, *Charops*, *Chelonus*, *Coccygidium*, tachinid flies, and other parasitoids, indicates a diverse natural enemy complex with potential to contribute to the biological suppression of fall armyworm populations. The detection of entomopathogenic fungi, entomopathogenic nematodes, and NPV further suggests that microbial agents also play a role in the natural regulation of the pest.

The results highlight the importance of conserving indigenous natural enemies as part of sustainable integrated pest management strategies for fall armyworm in Bhutan. Although parasitoid identification was primarily based on morphological characteristics and some specimens could not be identified to species level, the study establishes a valuable foundation for future research. Further investigations using molecular identification, long-term monitoring, and

evaluations of parasitism rates and biological control effectiveness will improve understanding of these natural enemies and support the development of environmentally sustainable management programmes for fall armyworm in Bhutan.

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6.2 Survey, Collection, and Identification of Predators Associated with Fall Armyworm (*Spodoptera frugiperda*) in Major Maize-Growing Areas of Bhutan

6.2.1 Introduction

Fall Armyworm (*Spodoptera frugiperda*) is a highly destructive invasive pest and one of the most important insect pests of maize worldwide(Montezano et al., 2018). Since its spread across Africa and Asia, the pest has become a major threat to maize production due to its high reproductive capacity, rapid dispersal, and ability to cause severe yield losses (Day et al., 2017).

In Bhutan, Fall Armyworm was first detected in maize fields in Punakha in September 2019 and has since spread to most maize-growing dzongkhags of the country. The pest has become a major constraint to maize production, increasing crop damage and management costs (Mahat et al., 2021)

Although chemical insecticides remain an important management option, overreliance on pesticides can lead to insecticide resistance, environmental contamination, and adverse effects on beneficial organisms. Therefore, conservation and utilisation of natural enemies are essential components of sustainable Fall Armyworm management and Integrated Pest Management (IPM) strategies (Abbas et al., 2022).

Among the natural enemies of Fall Armyworm, predators play an important role by feeding (Abbas et al., 2022) eggs, larvae, and other developmental stages, thereby helping to suppress pest populations. However, information on predator species associated with Fall Armyworm in Bhutan is still limited, despite preliminary surveys documenting a range of native natural enemies(Clarkson et al., 2022)

Therefore, this study was conducted to collect, identify, and document predator species associated with Fall Armyworm in selected maize-growing areas of Bhutan. The findings provide baseline information to support future biological control research and strengthen Integrated Pest Management (IPM) programs.

6.2.2 Materials and Method

Study Sites

Field surveys were conducted from 1st January to 30th May 2026 in selected maize-growing areas of Bhutan where Fall Armyworm (FAW), *Spodoptera frugiperda*, infestations had been reported. The survey sites included Dagalapang, Drugyel, Rilangthang, Dzomi Gewog, Chhali Gewog, ARDC Bajo, Phobjikha, and Karmaling (Samphelling). These locations represent diverse agroecological zones and maize production systems across the country.

Figure 6.9. Collection site

Collection of Predator Samples

Predator surveys were conducted in maize fields during the vegetative and reproductive growth stages of the crop. Predatory arthropods associated with FAW were collected through direct visual observations and hand collection. Each field was systematically inspected by walking along transects covering representative sections of the maize crop. Predators observed feeding on FAW eggs, larvae, or other maize-associated insects were recorded and collected whenever possible.

In addition, pheromone traps installed for FAW monitoring were examined for the incidental capture of beneficial insects. Captured predator specimens were carefully collected and preserved for future reference.

The following information was recorded during each survey:

- Date of collection
- Survey location
- Predator group
- Number of individuals observed
- Life stage (adult, larva, or nymph)

- Collection method
- Habitat and field conditions
- Presence of FAW infestation

Collected specimens were preserved in vials, labelled appropriately, and transported to the laboratory for identification.

Figure 6.10. Spider (left) and Lacewing (right)

Laboratory Identification

Predator specimens were identified in the laboratory using morphological characteristics and available taxonomic keys and reference materials. Identification was based on diagnostic features including body shape, colouration, wing morphology, antennae structure, mouthparts, leg characteristics, and other distinguishing traits.

Figure 6.11. Larva of Ladybug (left) and Earwig(right)

Specimens were identified to the lowest possible taxonomic level (family, genus, or species) depending on the availability and condition of the specimens. Representative specimens were photographed and documented for future reference.

Figure 6.12. Identification of Predators under Stereo Microscope

Data Analysis

Survey data were compiled and summarised using Microsoft Excel. Predator abundance was determined by calculating the total number of individuals recorded for each predator group across all survey sites. The occurrence and distribution of predator species were assessed based on the number of locations where each predator group was observed. Descriptive statistics were used to present the diversity and abundance of predators associated with Fall Armyworm in the surveyed maize-growing areas.

6.2.3 Results and Discussion

Table 6.3. Predator Species Recorded

Predator Group	Total Observed
Earwig	40
Lady Beetle (*Coccinellidae*)	26
Green Lacewing (*Chrysopidae*)	1
Lynx Spider	2
Ground Beetle	3
Crab Spider	1
Spider	2
Rove Beetle	2
Monolepta signata	1

Total Predators Recorded: 78 Individuals

Table 6.4. Site-wise Observation

Site	Place	Predators group	Number	Total Numbers
1	Dagalapang,Kawang	Earwig	4	11
		Lady Beetle (Coccinellidae)	7	
2	Drugyel,Paro	Earwig	7	7
3	Rilangthang,Sergithang	Green Lacewing	1	
		Lady Beetle	3	5
		Monolepta signata	1	
4	Dzomi,Punakha	Lady Beetle	3	4
		Earwig (Nymph)	1	
5	Chhaling,Monggar	Earwig	5	5
6	Drukjeygang	Lady beetles	2	
		Ground beetles	1	5
		Earwig	2	
7	Tashiding	Lyrinx Spider	1	
		Earwig	1	
		Lady beetle	3	7
		Earwig	2	
8	Tsendagang	Lady beetle	3	3
9	Tsangkha	Earwig	1	
		Ground beetle	1	2
10	Guma	Lady beetle	4	
		Earwig	1	5
11	Thoedtsho	Lady beetle	1	
		Earwig	1	4
		Spider	2	
12	ARDC Bajo	Earwig	1	1
13	Phobjikha	Earwig	12	12
14	Kabjisa	Earwig	2	2
15	Karmaling, Samphelling	Lynx spider	1	
		Ground Beetle	1	
		Crab Spider	1	5
		Rove Beetle	2	

The survey documented a diversity of predator species associated with Fall Armyworm habitats across different agroecological zones of Bhutan. Earwigs were the most abundant predators observed, accounting for approximately 51.28% of all recorded individuals. Their frequent occurrence in maize fields suggests they may play an important role in suppressing Fall Armyworm populations.

Lady beetles were the second most abundant predator group and were widely distributed across survey sites. Other beneficial predators including green lacewings, lynx spiders, crab spiders,

ground beetles, and rove beetles were recorded in lower numbers but are recognised as important biological control agents in agricultural ecosystems.

The presence of multiple predator groups indicates that maize fields support a diverse community of natural enemies that may contribute to natural regulation of Fall Armyworm populations.

6.2.4 Conclusion

The survey successfully documented predator species associated with Fall Armyworm in selected maize-growing areas of Bhutan. A total of 50 predator individuals representing eight predator groups were recorded. Earwigs and lady beetles were the dominant predators observed during the survey. The findings provide valuable baseline information for future studies on biological control and the conservation of natural enemies within Integrated Pest Management (IPM) programs. The diversity of predator groups observed indicates the presence of a natural enemy complex that may contribute to the suppression of Fall Armyworm populations. Continued monitoring and further studies on the abundance, seasonal dynamics, and predation potential of these predators are recommended to support the development of sustainable and ecologically based Fall Armyworm management strategies in Bhutan.

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6.3 Mass Rearing of *Corcyra cephalonica* and *Sitotroga cerealella* (Lepidoptera: Pyralidae and Gelechiidae) as Factitious Hosts for Egg Parasitoid Production

6.3.1 Introduction

Biological control is an environmentally friendly and sustainable method of managing pests by using natural enemies such as parasitoids, predators, and pathogens. It helps reduce the use of chemical pesticides and supports long-term pest suppression (van Lenteren, 2012).Compared with chemical control, biological control has fewer negative effects on non-target organisms, reduces environmental pollution, and is safer for farmers and consumers (van Lenteren, 2012). With the growing importance of Integrated Pest Management (IPM) and sustainable agriculture, the use of natural enemies has become an important component of crop protection programs worldwide (Calatayud et al., n.d.).

Parasitoids are important biological control agents because they attack specific pests and can effectively reduce pest populations. To use parasitoids in biological control programs, large numbers of healthy and effective individuals must be produced at a reasonable cost (Hassan, 1993). Therefore, mass rearing is an essential step. Successful rearing systems should provide a continuous supply of suitable host insects while ensuring that the parasitoids remain healthy, fertile, and capable of locating and parasitizing pests after release (Castellanos et al., 2019)

To meet this need, many programs rely on factitious hosts, non-pest species that are easy to rear in the laboratory to produce parasitoids at scale. Factitious hosts reduce cost and biosecurity risk relative to rearing large populations of the target pest, and they often perform well on simple, inexpensive diets such as cereal grains (Greenberg et al., 1998). Key criteria for effective factitious hosts include high reproductive rate, short generation time, ease of handling, and suitability for the development of the parasitoid species of interest.

Among the commonly used factitious hosts, *Corcyra cephalonica* (rice moth) and *Sitotroga cerealella* (Angoumois grain moth) are widely used for rearing parasitoids because they are easy and inexpensive to culture. Both species can be reared on common cereal grains, reproduce rapidly, and produce large numbers of eggs and larvae suitable for parasitoid development (Vargas et al., 2001). As a result, these moths are widely used in biological control programs around the world to support the mass production of parasitoids for agricultural and stored-product pest management.

Although rice moth and Angoumois grain moth are widely used in many countries, there is limited information on their mass rearing under Bhutanese conditions. Factors such as temperature, humidity, grain type, and laboratory facilities can affect their survival, reproduction, and suitability as hosts for parasitoids (Rajasekhar, 2016). Therefore, establishing reliable and locally adapted rearing methods is important to ensure a continuous supply of host insects for biological control programs in Bhutan.

The present study was conducted to establish and maintain mass cultures of rice moth and Angoumois grain moth using locally available grains to provide a continuous supply of host insects for parasitoid rearing and biological control programs in Bhutan. The study aimed to develop practical and cost-effective rearing techniques that can be easily adopted under laboratory conditions. Establishing reliable cultures of these factitious hosts will support the mass production of parasitoids and strengthen biological control efforts as part of integrated pest management programs in the country.

6.3.2 Materials and Method

Collection sites

Infested stored-grain samples were collected from five locations in Bhutan: Doteng, Paro Dzongkhag (27.465643° N, 89.421487° E; 2336 m asl), Wokuna under Kabjisa Gewog, Punakha Dzongkhag (27°39'12.97" N, 89°46'27.37" E; 1338 m asl), Tashiding, Dagana Dzongkhag (26°56′11″ N, 90°00′16″ E; 850.6 m asl), Upper Karmaling, Dagana Dzongkhag (26°46'03" N, 89°55'17" E; 120 m asl), and Jemathang, Dagana Dzongkhag (26°44'41" N, 89°52'36" E; 129 m asl). Samples were collected from stored grains exhibiting visible insect infestation and transported to the laboratory for further study. Rice moth cultures were established from larvae collected from infested stored rice, whereas Angoumois grain moth cultures were established from moths collected from infested stored paddy.

Rearing of Rice moth and Angoumois grain moth

Rearing conditions

All rearing experiments were conducted under controlled laboratory conditions at 27 ± 2 °C, 70 ± 5% relative humidity (RH).

Preparation of culture media

Rice moth

A total of 2.5 kg of rice was oven-dried at 100 °C for 30 minutes to eliminate contaminants and incidental insect stages. After cooling to room temperature, the kernels were coarsely broken into approximately 3–4 pieces per grain. The broken grains were treated with 0.1% formalin to reduce microbial contamination and allowed to air-dry briefly before being transferred into clean rearing basins as culture substrate.

Angoumois grain moth

2.5 kg of locally available cereal grains (maize, wheat, or rice) was oven-dried at 100 °C for 30 minutes and used directly as whole kernels without grinding or further processing.

Figure 6.13. Oven drying of Medium

Inoculation and culture maintenance

Larvae of each species were introduced separately into their respective rearing media. Rearing containers were covered with muslin cloth to ensure aeration while preventing escape and external contamination. All containers were clearly labelled with species name, date of inoculation, and batch number, and maintained under the controlled laboratory conditions described above until adult emergence.

Figure 6.14. Egg-inoculated container (left) and Incubation Setup (right)

Cultures were inspected every 2-3 days to monitor development and to remove any mould-infested or deteriorated grains to maintain colony health.

Adult emergence and feeding

Emerging adults were collected daily using aspirators and soft nets and transferred into oviposition cages. Adult emergence was recorded for each batch.

Adults were provided with a solution consisting of 50 ml honey, 50 ml distilled water, and five vitamin E capsules. The solution was supplied via sterile cotton wicks and replaced every 48 hours to maintain freshness and prevent microbial growth.

Figure 6.15. Adult rearing cage with feeder

Egg collection and culture continuation

Oviposition drums were placed inside adult cages for egg deposition. Eggs were collected at 24-hour intervals using fine soft brushes and sieves to minimize physical damage.

After collection, eggs were cleaned to remove any adhering debris, weighed using an analytical balance to determine egg yield, and immediately introduced into fresh culture media for colony propagation, ensuring a continuous supply of overlapping generations of both moth species.

Figure 6.16. Oviposition funnel (left), Eggs of rice moth (middle) and Eggs of Angoumois grain moth (right)

6.3.3 Results and Discussion

A total of 0.339 g of rice moth eggs and 0.047 g of Angoumois grain moth eggs were produced under laboratory conditions. Based on the standard laboratory estimate that 1 cc (approximately 1 g) of moth eggs contains approximately 16,000 eggs, these quantities corresponded to approximately 5,424 rice moth eggs and 752 Angoumois grain moth eggs. A total of 1,025 adult moths emerged from the cultures, comprising 648 Angoumois grain moths and 377 rice moths. Both species completed their life cycles and produced sufficient adults for colony maintenance and continued egg production. Higher larval mortality was observed in rice moth cultures during

the rearing period. Cultures are still being maintained, and further data on egg production, survival, and adult emergence will be collected in subsequent generations.

The successful emergence of 1,025 adult moths demonstrates that both the Angoumois grain moth and rice moth can be reared under laboratory conditions using locally available grain substrates. Angoumois grain moth produced slightly more adults than Rice moth. Relatively high larval mortality was observed in rice moth cultures, which may have been associated with the quality and suitability of the rearing medium. The use of packaged rice could have influenced larval development due to processing, polishing, or storage conditions that may affect its nutritional value.

Despite this limitation, both species produced sufficient adults to sustain laboratory cultures and support egg production. Mass rearing is ongoing, and continued culture maintenance is expected to provide a reliable supply of host eggs for future parasitoid multiplication and biological control programs.

6.3.4 Conclusion

Mass cultures of the rice moth and Angoumois grain moth were successfully established and maintained under laboratory conditions. Although higher larval mortality was observed in rice moths, both species completed their life cycles and produced adults for colony maintenance and egg production. Rearing activities are ongoing, and further optimisation of the rearing medium and environmental conditions is expected to enhance production efficiency and ensure a reliable supply of host material for future parasitoid rearing and biological control programs.

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6.4 Isolation and Characterisation of Native Entomopathogenic Fungi Associated with Fall Armyworm (*Spodoptera frugiperda*) in Bhutan

6.4.1 Introduction

The fall armyworm (*Spodoptera frugiperda* J.E. Smith) is an invasive, highly polyphagous lepidopteran that has rapidly expanded its range across Africa and Asia since its detection outside the Americas. Its high reproductive capacity, mobility, and capacity to feed on multiple host plants allow rapid establishment and severe damage. Larval feeding in maize whorls and ears leads to defoliation, tassel damage, and poor grain fill, with reported yield losses that vary widely; severe outbreaks have produced estimated reductions of 20-50% or more under unmanaged conditions (Day et al., 2017; Early et al., 2018).

In Bhutan, FAW was first observed damaging maize in Punakha in September 2019 and has since been detected in 15 dzongkhags, becoming a recurrent threat (Mahat et al., 2021; National Plant Protection Centre [NPPC], 2023). Farmers have predominantly relied on chemical insecticides as the primary control option; however, field-level effectiveness is often inconsistent due to challenges in correct application timing and the pest's rapid reinfestation potential. Continued reliance on synthetic insecticides also raises concerns about environmental contamination, impacts on non-target organisms, and the evolution of pesticide resistance, underlining the need for sustainable, locally adapted integrated pest management (IPM) strategies (Day et al., 2017; Food and Agriculture Organization [FAO] & Department of Plant Protection and Quality Assurance, 2021).

Entomopathogenic fungi (EPF), especially *Beauveria bassiana* and *Metarhizium anisopliae*, are among the most studied fungal biocontrol agents for lepidopteran pests. These fungi infect insect hosts through the cuticle, germinate, and proliferate internally, ultimately killing the host via nutrient depletion, mechanical damage, and toxin production. Entomopathogenic fungi present several advantages, including broad host applicability, relative safety to non-target organisms, and persistence in agroecosystems (Zimmermann, 2007; McGuire & Northfield, 2020).

Locally adapted or native EPF isolates frequently perform better under local environmental conditions than exotic strains because of their acclimation to local temperature, humidity, and ecosystem interactions. Naturally occurring EPF populations in cropping systems can contribute to suppression of pest populations, particularly where microclimatic conditions (e.g., humidity) favour infection and sporulation. Despite their promise, documentation of the diversity, prevalence, and pathogenicity of native EPF associated with FAW larvae in Bhutanese maize fields remains limited, representing a knowledge gap for developing locally tailored biocontrol measures.

This study aimed to isolate and identify native entomopathogenic fungi associated with naturally infected FAW larvae collected from maize fields in Bhutan and establish baseline information for future biocontrol applications.

6.4.2 Materials and Method

Collection of Entomopathogenic Fungi-Infected Larvae

Field surveys were conducted in maize-growing areas of Khempaithang (Tading Gewog, Samtse Dzongkhag; 26°54′07″N, 89°16′16″E; 1,046 m asl) and Karmaling (Samphelling Gewog, Chukha Dzongkhag; 26°51′29″N, 89°26′26″E; 315 m asl), Bhutan. These sites were selected based on reported fall armyworm infestations in maize fields.

Naturally infected fall armyworm larvae were collected based on visible fungal infection symptoms, particularly white or green mycelial growth on the larval body. Infected larvae were carefully picked using sterile forceps, placed in sterile labeled containers, and transported to the laboratory for further processing

Figure 6.16. Fall armyworm larva (L3) infected with *Beauveria* sp. (left) and *Metarhizium* sp. (right)

Surface Sterilization of Larvae

Surface sterilization was conducted to remove external contaminants while allowing internally colonizing entomopathogenic fungi to be isolated from infected tissues. Infected larvae were surface sterilized prior to fungal isolation. Larvae were immersed in 70% ethanol for 10 seconds

and rinsed 2–3 times with sterile distilled water to remove residual disinfectant. Sterilized larvae were dried on sterile filter paper under aseptic conditions in a laminar airflow cabinet.

Isolation of Entomopathogenic Fungi

Sterilized larvae were aseptically cut into small sections under a laminar airflow cabinet to expose internal tissues. Tissue fragments showing fungal growth were transferred onto Potato Dextrose Agar (PDA) plates using a sterile inoculating loop. Plates were incubated at 28 °C in complete darkness until fungal growth was observed.

Purification and Preservation of Fungal Isolates

Emerging fungal colonies with typical entomopathogenic morphology were sub-cultured onto fresh PDA plates to obtain pure cultures. Sub-culturing was repeated until contamination-free and morphologically uniform isolates were obtained.

Coding of Fungal Isolates

Each purified fungal isolate was assigned a unique identification code based on its genus, geographical origin (Country, Dzongkhag, Gewog), host crop, and host insect to ensure traceability and proper documentation throughout the study. *Beauveria* sp. isolate BEA-CHU-KAR-BH-MZ-FAW-001 was obtained from Karmalling, Chukha Dzongkhag, while *Metarhizium* sp. isolate MET-SAM-TAD-BH-MZ-FAW-001 was obtained from Tading, Samtse Dzongkhag.

Identification of Entomopathogenic Fungi

Fungal isolates were identified based on macroscopic and microscopic characteristics. Colony morphology, including color, and pigmentation, was recorded from 7–10-day-old cultures grown on Potato Dextrose Agar (PDA). Microscopic observations were conducted using a compound light microscope at 40× and 100× magnifications following Lactophenol cotton blue staining, and fungal structures such as hyphae, conidiophores, and conidia (shape and size) were examined. Identification was performed using standard taxonomic keys for entomopathogenic fungi. Isolates with white, cottony colonies and globose to sub globose conidia were tentatively identified as *Beauveria* sp., while isolates with green, powdery colonies and cylindrical to oval conidia were tentatively identified as *Metarhizium* sp. Conidial size measurements supported morphological identification. Molecular characterization (ITS sequencing) was not conducted due to resource constraints; therefore, identification was based solely on morphological criteria, which may limit species-level resolution.

Figure 6.18. Colony morphology of *Metarhizium* sp. (left) and *Beauveria* sp. cultured on PDA after 7 days of incubation (Image source: Biocontrol program, NPPC)

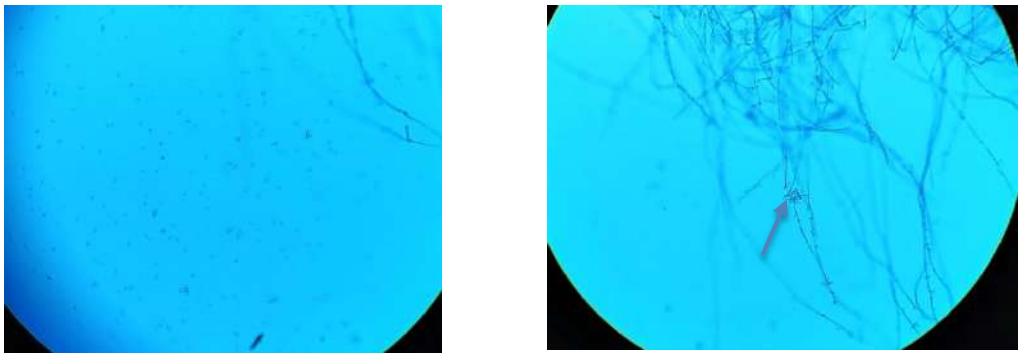

Figure 6.19. Microscopic morphology of *Beauveria* sp. showing globose to sub-globose conidia observed under 40× magnification (Image source: Biocontrol program, NPPC)

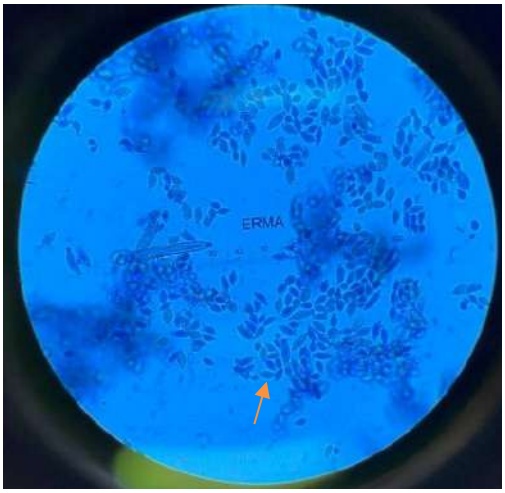

Figure 6.20. Microscopic morphology of *Metarhizium* sp. showing cylindrical to oval conidia observed under 100× magnification (Image source: Biocontrol program, NPPC)

Preparation of Conidial Suspension

Well-sporulated fungal cultures (8-10 days old) were used for conidial harvesting. Sterile distilled water was added onto the surface of the fungal cultures, and conidia were dislodged by gentle scraping using a sterile loop. The resulting suspension was filtered through sterile muslin cloth to remove mycelial debris and obtain a uniform conidial suspension. A 3 mL aliquot of the prepared conidial suspension was used as inoculum for solid-state mass multiplication.

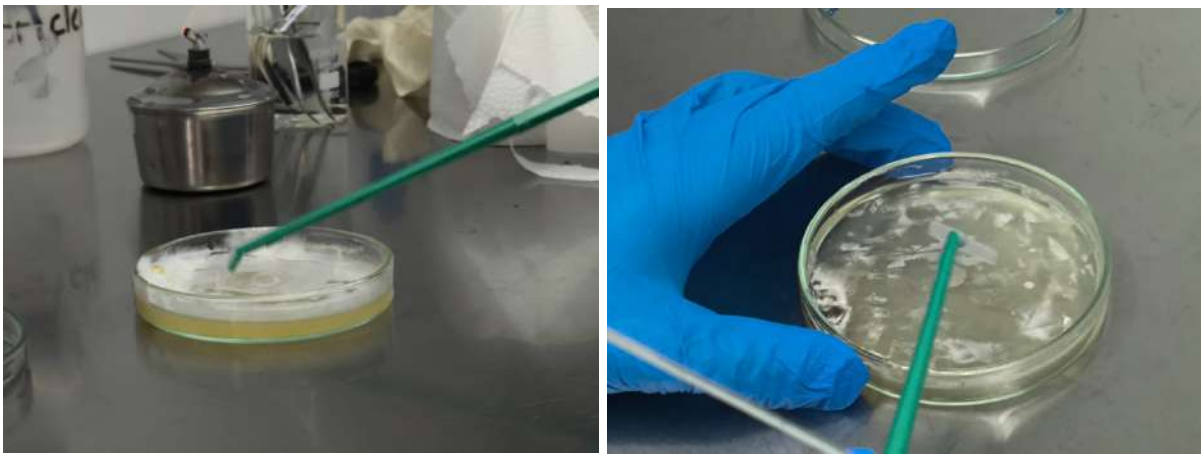

Figure 6.21. Preparation of conidial suspension by addition of sterile distilled water and gentle scraping of fungal cultures using a sterile loop (Image source: Biocontrol program, NPPC)

Liquid multiplication of Beauveria spp.

Beauveria spp. was initially multiplied in Potato Dextrose Broth (PDB) by inoculating 75 mL of sterilised broth in conical flasks with 8-day-old mother cultures. The flasks were sealed with cotton plugs and incubated on an orbital shaker at 120 rpm for three days to promote fungal growth. However, the liquid culture became contaminated and was subsequently discontinued.

Figure 6.22. Liquid multiplication of *Beauveria* spp. in sterilised Potato Dextrose Broth (PDB)

Solid-state multiplication

Following contamination in liquid culture, solid-state multiplication was adopted. Sterilised broken rice (~250 g per autoclavable bag) was used as substrate for both *Beauveria* spp. and *Metarhizium* spp. The substrate was sterilised at 121 °C (15 psi) for 30 minutes and allowed to cool. Each bag was inoculated with 3 mL of conidial suspension under aseptic conditions and gently mixed to ensure uniform distribution. The bags were fitted with cotton plugs for aeration and incubated at 28 °C under dark conditions for 14 days. The substrate was periodically mixed to ensure uniform colonisation. Complete fungal growth and sporulation were observed, characterised by white powdery growth in *Beauveria* spp. and green sporulation in *Metarhizium* spp.

Figure 6.23. Solid-state multiplication of Metarhizium sp. and Beauveria sp. on sterilised cooked rice showing fungal colonisation and sporulation.

Harvesting and Storage of Fungal Cultures

Following complete sporulation and air-drying of the colonized rice substrates, fungal biomass was harvested from both *Beauveria* sp. and *Metarhizium* sp. cultures. A total of 191.4 g of dried biomass was obtained from *Beauveria* sp**.**, while 151.7 g of dried biomass was obtained from *Metarhizium* sp.

Figure 6.24. Harvested fungal biomass of *Metarhizium sp*. and *Beauveria* sp.

6.4.3 Results and Discussion

The present study successfully isolated and characterised native entomopathogenic fungi associated with naturally infected fall armyworm larvae collected from maize fields. Two fungal genera, *Beauveria* sp. and *Metarhizium* sp., were recovered from infected larvae collected in Chukha and Samtse Dzongkhags, respectively. The occurrence of these fungi on field-collected larvae indicates their natural role in regulating insect pest populations in maize agroecosystems (Idrees et al. 2022).

The predominance of *Beauveria* and *Metarhizium* sp. in this study is consistent with previous reports identifying these genera as the most widespread entomopathogenic fungi associated with lepidopteran pests, including fall armyworm (Acharya et al. 2022; Palacio and Navasero 2021). Their success as natural pathogens can be attributed to their broad host range, ability to persist in soil and plant environments, and efficient infection through direct penetration of the insect cuticle (Idrees et al. 2022). These characteristics make them particularly effective under humid tropical and subtropical conditions, which are favorable for fungal germination, infection, and sporulation (Park et al. 2024).

Environmental conditions in the study areas may have contributed to the presence of these fungi. Both Samtse and Chukha Dzongkhags experience relatively warm temperatures and high humidity during the maize growing season, conditions known to enhance entomopathogenic fungal activity (Idrees et al. 2022). The detection of *Beauveria* sp. in Chukha and *Metarhizium* sp. in Samtse suggests that these fungi are well adapted to local ecological conditions and may already contribute to natural suppression of fall armyworm populations in Bhutanese maize fields.

From a biocontrol perspective, the isolation of native strains is particularly significant. Locally adapted entomopathogenic fungi are often more effective than exotic commercial strains because they are better suited to local climatic conditions and agroecosystems (Idrees et al. 2022; Hernandez et al. 2019). This suggests that the isolates obtained in this study may have strong

potential for development as biological control agents in integrated pest management (IPM) programs targeting fall armyworm in Bhutan (Shrestha et al. 2024).

However, the study is limited by the absence of molecular characterization (ITS sequencing), which restricts identification to the genus level and may overlook species-level diversity within isolates (Acharya et al. 2022; Carneiro et al. 2008). In addition, pathogenicity assays were not conducted, meaning the virulence of the isolates against fall armyworm could not be quantified (Idrees et al. 2022). Future studies should therefore incorporate molecular identification and bioassays to confirm species identity and evaluate insecticidal efficacy under laboratory and field conditions.

6.4.4 Conclusion

This study provides baseline information on the presence of native entomopathogenic fungi associated with fall armyworm larvae in maize fields of Bhutan. *Beauveria* sp. and *Metarhizium* sp. were successfully isolated and characterized based on morphological features, confirming their natural occurrence in FAW-infected populations across selected study sites.

The findings highlight the potential of native entomopathogenic fungi as environmentally sustainable biological control agents for fall armyworm management. Their presence in maize agroecosystems supports the possibility of integrating these fungi into future IPM strategies in Bhutan. However, further molecular identification and pathogenicity evaluation are required to fully validate their taxonomic status and biocontrol potential.

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6.5 Detection and Incidence of *Lecanicillium lecanii* infecting Fall Armyworm (*Spodoptera frugiperda*) in maize

6.5.1 Introduction

Fall Armyworm (*Spodoptera frugiperda*) is an invasive lepidopteran pest that has become widely established in maize-growing areas of Bhutan (Mahat et al., 2021; NPPC, 2023). Since its introduction in September 2019, it has caused substantial economic losses through direct foliar feeding, particularly during the vegetative and early reproductive stages of maize (NPPC, 2023; Day et al., 2017). The pest's high reproductive capacity (average fecundity of 1,000 eggs per female), wide host range (>350 plant species), and ability to spread rapidly (flying >100 km per night) makes it a persistent threat to maize production systems (Montezano et al., 2018; NPPC, 2023).

In agroecosystems, pest populations are naturally regulated by a range of biological control agents, including parasitoids, predators, and entomopathogenic microorganisms (Degaga & Degaga, 2024; Koffi et al., 2020). Among these, entomopathogenic fungi play a critical role in suppressing insect pest populations under favourable environmental conditions (Ard & Dubovskiy, 2024; Kumela et al., 2018). These fungi infect insects through cuticular penetration, leading to host mortality and contributing to natural population decline, especially in humid environments conducive to fungal growth (Ortiz-Urquiza & Keyhani, 2013; St. Leger et al., 2010).

During routine field surveillance in maize ecosystems, observations of FAW larvae exhibiting possible fungal associations were recorded and investigated. Such occurrences are important for understanding the presence and role of indigenous natural enemies in FAW management (Degaga & Degaga, 2024; NPPC, 2023). The present study documents and examines this fungal association to identify potential natural mortality factors contributing to FAW population dynamics in the field.

6.5.2 Materials and Method

Study Site and Period

Field monitoring was conducted in a maize field at Karmaling (Samphelling Gewog, Chhukha Dzongkhag; 26°51′29″N, 89°26′26″E; 315 masl) on 15 April 2026 to collect FAW and associated natural enemies.

Field Observation and Sample Collection

During routine field inspection, an unusual third instar (L3) FAW larva was observed on maize plants and collected due to suspected fungal infection. Although no clear external symptoms of disease were initially evident in the field, the typical condition of the larva suggested possible association with entomopathogenic fungi. The specimen was therefore transported to the laboratory for detailed examination and confirmation.

Figure 6.25. Field collection of FAW larva from maize plant

Laboratory Rearing

The field-collected FAW larva was maintained in the laboratory and fed fresh maize leaves under controlled conditions (27 °C, 70% RH). At the time of collection, no visible fungal growth was observed on the larval body. However, after approximately five days of incubation, the larva showed progressive development of white fungal growth, initially appearing in circular patches and later expanding to completely cover the entire body. This pattern of fungal emergence and rapid proliferation under humid laboratory conditions is consistent with infection by an entomopathogenic fungus, likely *Lecanicillium lecanii*. The observation suggests that the fungus was present in an early or later stage at the time of field collection and subsequently developed fully under favorable environmental conditions during incubation in the laboratory.

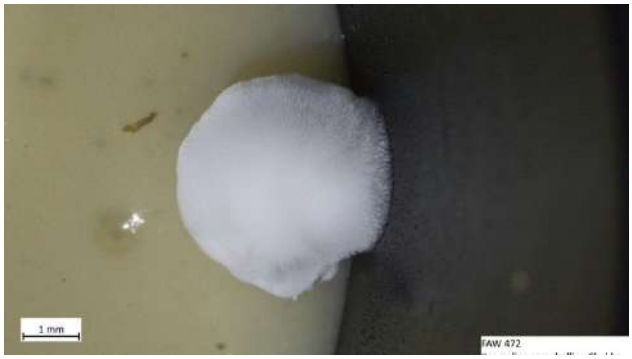

Figure 6.26. FAW larva completely covered with fungal growth, indicating infection by *Verticillium lecanii*.

Fungal Isolation and Culture

The collected FAW larva was surface-sterilized prior to fungal isolation to minimize external contamination. The specimen was first rinsed with sterile distilled water to remove debris,

followed by immersion in 70% ethanol for 10 seconds. It was then rinsed 2-3 times with sterile distilled water and blotted dry under a laminar flow cabinet.

Small sections of the larval body showing fungal growth were aseptically transferred onto Potato Dextrose Agar (PDA) plates. The medium was prepared and sterilized by autoclaving at 121°C for 30 minutes at 15 psi.

The inoculated plates were incubated at 28°C in darkness for 7 days and monitored regularly for fungal growth and contamination. Emerging fungal colonies were sub-cultured onto fresh PDA plates to obtain pure cultures using hyphal tip transfer.

Fungal growth was allowed to develop sufficiently for morphological characterization. Colony characteristics, including color, texture, growth rate, and sporulation, were recorded, and representative cultures were used for microscopic examination.

Figure 6.27. Colony morphology of *Lecanicillium lecanii* grown on Potato Dextrose Agar (PDA) showing characteristic fungal growth.

Microscopic Examination and Identification

Microscopic observations were carried out using a compound light microscope at 40× and 100× magnification. Fungal structures were examined for hyphal and conidial morphology. Identification was based on characteristic features of entomopathogenic fungi, particularly *Lecanicillium lecanii*, including hyaline, septate, branched hyphae and small, single-celled, oval to cylindrical conidia produced in clusters.

6.5.3 Results and Discussion

Fungal growth obtained on PDA from the sample showed characteristics of *Lecanicillium lecanii*. Microscopic examination revealed hyaline, septate, and branched hyphae forming a dense mycelial network. Conidia were small, single-celled, hyaline, and oval to cylindrical in shape, produced in clusters from conidiogenous cells and observed in slimy aggregations typical of *Lecanicillium lecanii.*

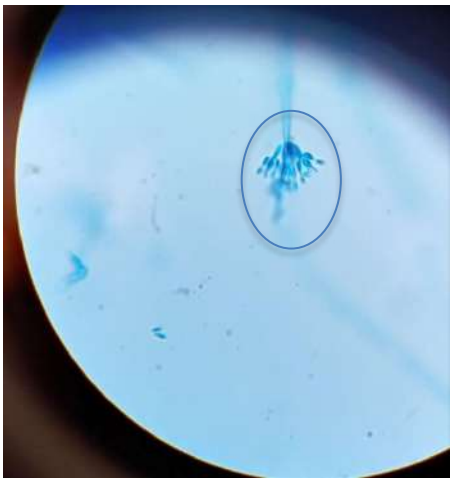

Figure 6.28. Microscopic view of conidia of *Lecanicillium lecanii* showing small, single-celled, hyaline, oval to cylindrical structures produced in clusters.

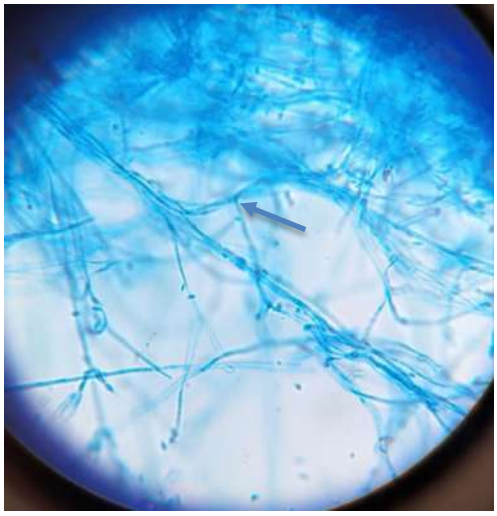

Figure 6.29. Microscopic image of *Lecanicillium lecanii* showing hyaline, septate, and branched hyphae forming a dense mycelial network.

The observation indicates the natural occurrence of *Lecanicillium lecanii* in maize agroecosystems of Samphelling Gewog and its possible role in infecting FAW under field conditions. This suggests the presence of indigenous entomopathogenic fungi contributing to natural pest suppression. However, the identification is based solely on morphological characteristics and lacks molecular confirmation. In addition, the record is based on a single larva, limiting inference on prevalence or epidemiological significance. Despite these limitations, the finding highlights the potential of naturally occurring fungal pathogens in FAW management systems in Bhutan.

6.5.4 Conclusion

This study reports a preliminary observation of *Lecanicillium lecanii* associated with FAW in maize at Karmalling, Chukha Dzongkhag. Although limited to a single specimen and morphological identification, the finding suggests the presence of natural fungal pathogens in the FAW system. Further systematic surveys and pathogenicity studies are recommended to assess distribution, prevalence, and biocontrol potential.

6.5.5 References

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7 Vertebrate Program

7.1 Technical Cooperation Project (TCP) with JICA - Project for Community-Based Human–Wildlife Conflict Management (HWCM)

7.1.1 Background and Rationale

Human-wildlife conflict (HWC) remains one of the most pressing challenges for Bhutan's rural farming communities. Declining rural populations, land abandonment, and the proximity of farmlands to forest habitats have intensified wildlife incursions, particularly from wild boar, monkeys, and deer. These incursions cause significant crop losses, undermining food security and rural livelihoods, and accelerating migration to urban centres.

Building on the foundation established in 2024–2025, the second year of the TCP focused on full-scale implementation of Universal Hybrid Fence (UHF) prototypes at selected pilot sites. This year's activities validated the feasibility of hybrid fencing systems, strengthened community management structures, and demonstrated replicable models for nationwide dissemination.

7.1.2 Project Objectives

The TCP continues to pursue its four core objectives:

1. National HWCM structure - Establish and refine guidelines for adoption across dzongkhags.
2. Government capacity building - Strengthen institutional ability to promote and implement HWCM.
3. Technology optimisation - Advance fencing systems suitable for Bhutan's diverse terrains and species challenges.
4. Community-based pilots - Validate models through participatory implementation and monitoring.

7.1.3 Governance and Implementation Structure

The project remains led by NPPC, with the Program Director as Project Director and the Vertebrate Pest Management Program Head as the Project Manager. The JICA expert team continues to provide technical and community engagement support, comprising:

- Chief Advisor on HWC management
- Community Development Specialist
- Training Coordinator

Oversight is provided by the Joint Coordination Committee (JCC), chaired by the Director, Department of Agriculture, Ministry of Agriculture and Livestock, with representation from NPPC, ARDC Bajo, relevant dzongkhags, the Nature Conservation Division, and JICA.

7.1.4 Pilot Site Implementation (2025–2026)

Three pilot sites were completed this year, with full UHF installation and community engagement activities:

- **Dawakha, Toedwang Gewog, Punakha Dzongkhag**
  - Fence length: 5 km
  - Area covered: 177 acres.
  - Wildlife threats: Predominantly wild boar, with monkey incursions
  - Activities: Site finalisation meeting, planning meeting, installation training, fence maintenance training
  - Outcome: Entire chiwog protected, reducing crop losses, and strengthening community confidence in HWCM interventions.
- **Nyalakha, Ruebisa Gewog, Wangdue Phodrang Dzongkhag**
  - Fence length: 2.5 km
  - Area covered: 47 acres.
  - Wildlife threats: Monkeys, wild boar, and deer
  - Activities: Community consultations, installation training, vegetation control awareness
  - Outcome: Farmers reported immediate reduction in crop damage, with strong local ownership of fence maintenance.
- **Pangserpo, Drujeygang Gewog, Dagana Dzongkhag**
  - Fence length: 700 m
  - Area covered: 6 acres (orange orchards)
  - Wildlife threats: Severe monkey damage
  - Activities: Planning meeting, installation training, fence maintenance training
  - Outcome: Orange orchards secured, with gewog administration pledging Nu. 1 million for future expansion.

Figure 7.1. Installation of Universal Hybrid Fence

7.1.5 Technology Development Focus

The Universal Hybrid Fence (UHF) was deployed at all three sites, incorporating:

- 2.5 m post height, 2 m net height
- Integrated electric wiring with voltage monitoring (>5,000V)
- 40 cm underground skirt to deter digging.
- Ring insulators

Community training emphasized:

- Fence installation and repair techniques
- Vegetation control along fence lines
- Voltage monitoring and maintenance routines
- Monkey deterrence measures

Figure 7.2. Installation of Universal Hybrid Fence at Three Sites

7.1.6 Capacity Building and Community Engagement

Community participation remained central to the HWCM approach. Key activities included:

- Site finalisation meetings: Ensuring consensus on fence routes and boundaries.
- Planning meetings: Assigning roles for operation and maintenance.
- Training sessions: Covering installation, repair, voltage monitoring, and vegetation management.
- Maintenance workshops: Focused on long-term sustainability and local ownership.

Strong local buy-in was evident, with households contributing labor and gewog administrations pledging financial or in-kind support. Farmers expressed confidence in the fencing system and willingness to expand coverage in future phases.

Figure 7.3. Capacity building and community engagement

7.1.7 Next Steps for 2026–2027

- Expand UHF installations in Tsirang, Tashi Yangjong chiwog.
- Develop and test primate-specific deterrence technologies.
- Strengthen baseline monitoring systems for crop damage and wildlife incursions.
- Draft and circulate HWCM guidelines for stakeholder review.
- Institutionalize training modules for gewog-level extension officers.

7.1.8 Conclusion

The second year of the JICA-NPPC Technical Cooperation Project has marked a decisive transition from planning to tangible implementation, with the successful installation of Universal Hybrid Fences (UHFs) at Dawakha, Nyalakha, and Pangserpo. These interventions have provided immediate protection to over 230 acres of farmland**,** safeguarding staple crops and high-value orchards from persistent wildlife incursions.

Beyond physical infrastructure, the project has demonstrated the strength of community-driven management models. Farmers actively participated in site finalisation, fence route planning, installation, and maintenance training, ensuring that the interventions were not only technically sound but also socially sustainable. Gewog administrations further reinforced this ownership by pledging financial and in-kind support, signalling strong local commitment to long-term HWCM solutions.

The deployment of UHFs has also validated the technical adaptability of hybrid fencing systems in Bhutan's diverse terrains. By integrating electric wiring, underground skirts, and locally sourced materials, the fences proved effective against multiple species—including wild boar and monkeys—while remaining affordable and maintainable for rural communities. Training modules on voltage monitoring, vegetation control, and repair techniques have equipped farmers with the skills necessary to sustain these systems independently.

Importantly, the project has begun to establish a replicable model for nationwide dissemination. The lessons learned from Dawakha, Nyalakha, and Pangserpo provide a blueprint for scaling interventions to other dzongkhags, while ongoing development of primate-specific deterrence measures and baseline monitoring systems will further refine the national HWCM framework.

In essence, the year's achievements underscore the project's dual success: delivering immediate livelihood protection for vulnerable farming households and laying the groundwork for a sustainable, scalable national strategy. By combining Japanese technical expertise with Bhutanese field experience and community ownership, the TCP is steadily advancing toward its vision of balancing conservation priorities with the resilience and prosperity of rural farmers.

8 Annexure: Publications and Academic Contributions from NPPC

8.1 Undergraduate and Postgraduate Thesis of NPPC staffs

Sl. No	Name	UG/PG/ Course	Thesis topic	University Details	Year of completion
1	Yeshey Dema	M.Phil	Nitrogen mineralisation from cattle FYM, a case study from Bhutan.	University of Reading, UK	2000
2	Ugyen Tshering	B.Sc Agriculture	Comparative Economic Analysis of Early and Regular season chilli production in, Mongar dzongkhag.	College of Natural Resources, RUB	2010
3	Yeshi Lhamo	B.Sc (Agriculture)	Efficacy of Garlic (*Allium sativum*) Intercropping for Management of Red Ant (*Dorylus orientalis*) Infestation in Potato	College of Natural Resources, RUB	2014
4	Tshomo	B.Sc (Agriculture)	Effect of 'Rebearth'- an organic bio-stimulant, on soil fertility and cabbage Production	College of Natural Resources, RUB	2017
5	Tshelthrim Zangpo	Master of Plant Sciences (Specialization: Entomology)	Crop diversification enhances the parasitism rate of *Cotesia glomerata* & Tertiary hyperparasitism in a Generalist pupal hyperparasitoid, *Gelis agilis* (Hymenoptera: Ichneumonidae)	Wageningen University & Research, The Netherlands	2022
6	Ugyen Tshering	M.Sc Ag. Mj Agronomy	Response of barley (*Hordeum vulgare* L.) to integrated nutrient management under Punjab conditions	Lovely Professional University, Punjab, India	2023
7	Dawa Dem Tamang	B.Sc (Agriculture)	Assessing farmers' knowledge on insect pest and disease management of areca nut in Karmaling gewog, Dagana	College of Natural Resources, RUB	2023
8	Chokey Lhamo	M.Sc	Exploring the role of Nickel dependent glyoxylase I in *Arabidopsis thaliana* plant.	South Asian University, New Delhi	2024
9	Tshencho Dem	B.Sc (Agriculture)	Documentation and Economic Analysis of Potato Production at Chang Gewog, Thimphu	College of Natural Resources, RUB	2024
10	Pema Tamang	B.Sc (Organic Agriculture)	Effects of Buckwheat and Mung Bean Cover Crops and their Mixture on Soil Nutrients and Weed Suppression under the Dry Sub-Tropical Condition	College of Natural Resources, RUB	2024

8.2. List of Publications in FY2025-2026

SI.No	Authors/Co-authors	Research Title	Publishing institute (s)	Year of Publication	Reference/DOI
1	Yeshey Dema, Yeshi Lhamo and Tshomo	Genetic analysis of the wheat yellow rust pathogen *Puccinia striiformis f.* sp. tritici in Bhutan reveals high levels of diversity	NPPC	2025	10.5281/zenodo.16743538
2	Yeshey Dema, Yeshi Lhamo and Tshomo	Genetic analysis of *Puccinia graminis f.* sp. tritici in Bhutan highlights the need for increased surveillance	NPPC	2026	10.5281/zenodo.18181952