AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Identification and fungicide sensitivity of *Phytophthora nicotianae* for controlling pineapple heart rot in Thailand

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Article Info

Article history:
Received 10 September 2024
Revised 10 December 2024
Accepted 2 January 2025
Available online 28 February 2025

Keywords:
Dimethomorph,
Disease management,
Fungicide sensitivity,
Phytophthora nicotianae,
Pineapple heart rot

Abstract

Importance of the work: Pineapple heart rot caused by *Phytophthora* spp. can lead to substantial yield losses and significant economic impacts for pineapple growers. This highlights the need for accurate identification of the causative agent and effective control measures.

Objectives: To identify and characterize *Phytophthora* isolates, evaluate fungicide sensitivity and assess fungicide effectiveness in pineapple field trials.

Materials and Methods: Pineapple heart rot samples were collected for pathogen isolation. *Phytophthora* isolates were identified using morphological and molecular methods. Fungicide sensitivity was evaluated based on *in vitro* assays, with field trials being used to assess the efficacy of four fungicides. Disease incidence and severity were monitored and analyzed using two-way analysis of variance.

Results: Four fungal isolates (PHY-01 to PHY-04) were identified, with PHY-02 matching *Phytophthora nicotianae* morphologically and phylogenetically. Pathogenicity assays confirmed PHY-02 as the causal agent of pineapple heart rot. In fungicide sensitivity tests, dimethomorph achieved 100% mycelial growth inhibition at concentrations of 100 mg/L and 1,000 mg/L, with a half maximal effective concentration (EC_{50}) value of 0.17 mg/L. Metalaxyl was effective at higher concentrations (EC_{50}: 5.3 mg/L). Fosetyl-Al and phosphonic acid showed no efficacy. The field trials demonstrated that dimethomorph (9.5 mL/L) was most effective, reducing disease severity to 1 after 45 d, compared to 3 in control plots. Metalaxyl and fosetyl-Al provided moderate control, while phosphonic acid was less effective.

Main finding: *Phytophthora nicotianae* was confirmed as the cause of pineapple heart rot. The results demonstrated dimethomorph's superior efficacy for disease control, advancing effective fungicide use and integrated management strategies for pineapple cultivation.

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https://doi.org/10.34044/j.anres.2025.59.1.10

Introduction

Pineapple (*Ananas comosus* L. Merr.) is a major tropical fruit of major global economic value; in 2023, ever-increasing global pineapple production reached approximately 29.36 million t, with further increases projected for many years (Food and Agriculture Organization of the United Nations, 2023a). Leading producers include Indonesia, the Philippines, Costa Rica, Brazil, China, India, Thailand, Nigeria, Mexico and Colombia (Hossain, 2016; Food and Agriculture Organization of the United Nations, 2023b). This widespread cultivation highlights the economic importance of pineapples but also underscores the impact of diseases, such as pineapple heart rot caused by *Phytophthora* spp., that can lead to substantial yield losses and adverse economic impacts for pineapple growers (Green and Nelson, 2015; Bosco et al., 2018; Jasper et al., 2019). Pineapple cultivation in Thailand is prominent in the western and eastern regions, with overall production of approximately 1.65 million t of 'Pattavia' variety pineapples, where the key production provinces are Prachuap Khiri Khan, Phetchaburi, Ratchaburi, Kanchanaburi, Chonburi and Rayong (Office of Agricultural Economics, 2023). In Thailand, pineapple heart rot reduces both the quantity and quality of yields, resulting in considerable financial losses for farmers and stakeholders, worth billions of dollars annually (Office of Agricultural Economics, 2023). Therefore, it is crucial to advance research into identifying the fungal pathogens responsible and to develop effective control methods.

Pineapple heart rot disease (also referred to as pineapple wilt or pineapple dieback) is a severe fungal infection impacting pineapple plants. Primarily, this disease manifests as the decay of the central portion (called the heart) of the pineapple plant, leading to symptoms such as wilting, stunting and ultimately the death of the plant (Bosco et al., 2018). Often, the infected fruits have water-soaked lesions, discoloration and softening, which render them unsuitable for commercial sale (Bosco et al., 2018; Serrato-Diaz et al., 2023). Pineapple heart rot, caused by pathogens including *Phytophthora nicotianae*, represents a major threat to pineapple cultivation on a global scale (Ocwa et al., 2018a, 2018b; Afandi et al., 2021). *P. nicotianae*, a soil-borne oomycete, is one of the primary causal agents of pineapple heart rot and various plant diseases as this pathogen thrives in warm, humid conditions (Serrato-Diaz et al., 2023), making tropical pineapple-growing regions particularly susceptible to its effects. In addition, the persistence of *P. nicotianae* in soil and plant debris exacerbates the challenge of disease management (Ocwa et al., 2018b). Effective management strategies are crucial to mitigate the impact of this disease on global production and trade (Sapak and Nusaibah, 2024). Effective control of pineapple heart rot relies on integrated disease management approaches, including cultural practices, sanitation and chemical control (Rohrbach, 1985). While fungicides are essential in mitigating disease spread and minimizing yield losses, the emergence of fungicide-resistant strains underscores the importance of continuous monitoring and evaluation of fungicide sensitivity in *P. nicotianae* populations.

Therefore, the current study aimed to identify and characterize *Phytophthora* isolates from pineapple fields in Thailand, to evaluate their sensitivity to commonly used fungicides and to assess the effectiveness of selected fungicides in field trials. The results should provide insights into effective management strategies that can help to mitigate the impact of heart rot disease and support the sustainability of pineapple production.

Materials and Methods

Sample collection and isolation of pathogens

Two pineapple fields were surveyed in Sam Roi Yot district, Prachuap Khiri Khan Province, Thailand. Pineapple samples of the 'Pattavia' variety displaying symptoms of heart rot were collected from these plantations for pathogen isolation using the tissue transplanting method (Agrios, 2005). Excisions (each approximately 0.5 cm × 0.5 cm in size) were made of pineapple leaf tissues showing disease symptoms, as well as asymptomatic leaf areas. The plant tissue pieces were surface sterilized by immersing them in a 0.5% sodium hypochlorite solution for 3 min, followed by rinsing with sterilized distilled water three times to remove the bleach. Subsequently, the plant tissue pieces were placed on potato dextrose agar (PDA) and incubated at 25°C for 7 d. After incubation, the emerging fungal hyphae from the plant tissue were transferred to fresh PDA plates for observation of morphology.

Morphological identification

Fungal growth and sporulation were induced using the baiting technique. First, pineapple leaf pieces (each approximately 0.5 cm × 0.5 cm in size) were boiled in distilled

water for 1 min and then allowed to cool. A natural spring water-to-tap water ratio of 1:1 was prepared, boiled and then allowed to cool. A cork borer with a diameter of 0.5 cm was used to take agar plugs of fungal mycelia from the edges of fungal colonies grown on PDA for 7 d. Then, these agar plugs were transferred to Petri dishes each containing 10 boiled pineapple leaf pieces and the cooled water mixture. The cultures were incubated at 25°C for 24 hr before being examined under a stereomicroscope (Olympus; Tokyo, Japan) and a compound microscope (Carl Zeiss; Jena, Germany) to observe the structural characteristics of the *Phytophthora* species such as sporangia, oospores and chlamydospore production.

Molecular phylogenetic analysis

DNA extraction and species identification were conducted. DNA of *Phytophthora* (PHY-02) isolate was extracted, as described by Zelaya-Molina et al. (2011), using cultures at 7 d. The internal transcribed spacer (ITS) region was amplified using the ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers (White et al., 1990) and the cytochrome oxidase 1 and 2 spacer (cox 1 and 2 spacer) was amplified using FM35 (5'-CAGAACCTTGGCAATTAGG-3') and FMPhy-10b (5'-GCAAAAGCACTAAAAATTAAATATAA-3') (Martin, 2000; Martin et al., 2004). Amplification was performed using PCRBIO Taq Mix (PCR Biosystems; London, UK) and conditions as described by White et al. (1990) and Martin et al. (2004). Sequencing was carried out by Macrogen Inc. (Seoul, Republic of South Korea). Sequencing chromatograms were visualized and trimmed using the 4Peaks software (Nucleobytes B.V.; Aalsmeer, the Netherlands; nucleobytes.com), assembled using the CAP3 Sequence Assembly program (Huang and Madan, 1999) and then blasted against the sequences in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the Phytophthora Database (Park et al., 2013). The ITS and the cox 1 and 2 spacer sequences were deposited in the NCBI database under accession numbers OM846622 and OM869879, respectively.

For phylogenetic analysis, the retrieved sequences from the Phytophthora Database and GenBank (https://phytophthoradb.org) were used to compare with the sequences of the PHY-02 isolate. The MUSCLE alignment version 3.8.31 software (Edgar, 2004) was used in the Seaview version 5.0.4 multiplatform graphical user interface for sequence alignment and phylogenetic tree building (Gouy et al., 2010). Phylogenetic tree estimation was performed using PhyML (Guindon et al., 2010) and Parsimony in Seaview version 5.0.4 (Gouy et al., 2010) with 1,000 bootstrap replicates and using Bayesian evolutionary analysis in the BEAUti and Beast version 2.6.6 software (Drummond et al., 2012; Bouckaert et al., 2019) with a chain length of 10,000,000 and sampling every 1,000 generations. The phylogenetic tree was arranged and edited in the FigTree version 1.4.4 software (http://tree.bio.ed.ac.uk/software/figtree/).

Pathogenicity tests

First, healthy pineapple leaves of the 'Pattavia' variety were cleaned using tap water and then sterilized by immersion in a 0.5% sodium hypochlorite solution for 1 min. After sterilization, the leaves were rinsed twice with sterile water and allowed to air dry. Each sterilized leaf was inoculated with the fungal pathogen, with a single inoculation point marked on each sample. Mycelial discs from cultures of the pathogen age 7 d were placed at the inoculation point, while agar discs without fungal isolates served as controls. There were 10 replicates. The inoculated leaves were placed in plastic boxes and incubated in the dark at 25°C for 24 hr. Disease progression was monitored daily based on visual assessment. Pathogenicity tests were conducted on 10 pineapple plants in a greenhouse to confirm the pathogen's role. After inoculation, infected tissues were sampled and the pathogen was re-isolated. Then, this re-isolated pathogen was compared with the original to confirm its identity and to validate its role as the causal agent of the disease (Darapanit et al., 2021).

In vitro assay for fungicide sensitivity

Four fungicides (dimethomorph 50% SC, metalaxyl 25% WP, fosetyl-Al 80% WG and phosphonic acid 40% SL), were tested. The selection of these four fungicides was based on their frequent use in managing diseases caused by oomycetes such as *Phytophthora* spp., as has been documented in pineapple cultivation practices in Thailand, where these fungicides are commonly applied due to their mode of action targeting oomycete pathogens and their availability in the region (Fungicide Resistance Action Committee, 2024). However, frequent application of these chemicals raises concerns about potential resistance development, which was why the current study evaluated the sensitivity of *P. nicotianae* to these fungicides. The sensitivity of the mycelial growth

of the *Phytophthora* isolates to these fungicides was evaluated using an agar dilution method, as described by Piasai et al. (2021). Each fungicide was dissolved in sterilized distilled water and added to PDA to achieve final concentrations of 0 mg/L, 0.01 mg/L, 0.1 mg/L, 1 mg/L, 10 mg/L, 100 mg/L, 1,000 mg/L and 10,000 mg/L. The *Phytophthora* isolates were cultured on PDA and incubated for 7 d. Mycelial discs (5 mm in diameter) were excised from the PDA colonies and placed in the center of PDA plates amended with each fungicide at varying concentrations. Five replicates were conducted for each treatment. The plates were incubated at 25°C under a cycle of 12 hr light and 12 hr darkness. The colony growth was measured in millimeters on the underside of the colonies after 7 d and 12 d of incubation period. The percentage of mycelial growth inhibition (MGI) was calculated using the average diameters of mycelial colonies at 7 d and 12 d according to the formula: MGI = [(C - T) / C] × 100, where *C* is the average diameter of the mycelial colony on the control plate and *T* is the average diameter of the mycelial colony on the treated plate, with all measurements in millimeters. The half maximal effective concentration (EC_{50}) values for each treatment on PDA were calculated at average radial growth using Prism 8 software (GraphPad Software, Inc.; Boston, MA, USA) and the EC_{50} Calculator (AAT Bioquest Inc.; Pleasanton, CA, USA).

Field trial experiments

A randomized complete block design experiment was conducted in the field. The experiment consisted of six treatments with three replications each. The planting distances were set at 30 cm between plants and 50 cm between rows. After planting the pineapple seedlings in the designated plots, the preparation of the inoculum commenced the following day. The fungal inoculum was prepared by diluting the suspension of fungal spores cultured on potato carrot agar medium. Then, the cultures were incubated at room temperature for 10 d. Subsequently, fungal cultures from 25 plates were combined with 1 L of water and homogenized using a blender to prepare a suspension. Next, this suspension was diluted to a concentration of 1/30 L of water/plot. The resulting suspension was applied at a rate of 300 mL per plant around the base of the pineapple seedlings at age about 30 d. When symptoms of disease became apparent on the pineapple plants, the infected plants were uprooted and any diseased plant residues remaining in the plot were removed to prepare the area for subsequent planting. This was done as part of the natural soil inoculum preparation to evaluate the efficacy of the fungicides for disease control. The fungicide treatments were prepared: 1.2 mL/L and 9.5 mL/L of dimethomorph 50% SC; 2.5 g/L of metalaxyl 25% WP; 2.5 g/L of fosetyl-Al 80% WG; and 2.5 mL/L of phosphonic acid 40% SL. The concentrations of each fungicide were based on the Department of Agriculture recommendations for effective control of oomycete pathogens and adjusted according to their labeled application rates in agricultural practices (https://esc.doae.go.th/). These concentrations represented realistic field application rates that growers used for pineapple crops, ensuring practical relevance in sensitivity testing. Pineapple seedlings of the 'Pattavia' variety were dip-treated in one of the fungicide solutions for 5 min. Pineapple seedlings of control plots were dip-treated in sterile distilled water. Subsequently, these treated seedlings were transplanted into the experimental plots where the fungal inoculum had been applied previously. The location of the plots was in Mueang district, Kanchanaburi province, Thailand, which an isolated area away from general pineapple cultivation. In total, 20 pineapple plants/plot were systematically sampled, selected diagonally across each plot, to assess disease incidence and severity at 7 d, 15 d, 30 d, 45 d and 60 d post-planting. The assessment of disease severity and the calculation of the disease severity index (DSI) were conducted using a scale ranging from 0 to 4, where 0 = the absence of observable disease symptoms in pineapple plants; 1 = the presence of slight symptoms, affecting 1–15% of the total plant area; 2 = the manifestation of mild symptoms, impacting 16–40% of the overall plant surface; 3 = moderate symptoms, spanning 41–75% of the entire plant area; and 4 = severe symptoms, affecting more than 75% of the total plant area. The DSI was assessed using the formula: DSI = [Σ (A × B) / N × M] × 100, where A is the class frequency, B is the score of the rating class, N is the total number of plants and M is the maximum disease index (Jasper et al., 2020; Serrato-Diaz et al., 2023).

Statistical analysis

Statistical analysis was performed using two-way analysis of variance with the GraphPad Prism 7.0 software (GraphPad Software, Inc.; Boston, MA, USA). The subsequent mean comparisons were conducted using Duncan's New Multiple Range Test (DMRT) with statistical significance set at $p < 0.05$.

Results and Discussion

Morphological and molecular identification

The isolation of fungi from the rotting pineapple plants resulted in four fungal isolates, designated as PHY-01, PHY-02, PHY-03 and PHY-04. The stimulation of reproductive structures was achieved using baiting techniques. Subsequently, slides were prepared for microscopic examination of the fungal structures, with PHY-02 used as the representative isolate for fungal identification. The morphological characteristics of isolate PHY-02, consistent with the genus *Phytophthora*, included white, fluffy mycelial colonies, with ovoid sporangia 35–40 µm in length and 30–40 µm in width. Papilla formation was observed at the apex of the sporangia, serving as the exit point for zoospores. Chlamydospores were infrequently observed and were spherical in shape with an average diameter of 35–40 µm (Fig. 1). Molecular identification matched PHY-02 to *P. nicotianae*. The ITS and the *cox1* and *2* sequences of PHY-02 isolate had more than 99.5% identity with the *P. nicotianae* sequences in the NCBI database and 100% identity with the *P. nicotianae* sequences in the Phytophthora Database. The ITS sequence of PHY-02 was 831 nucleotides and the *cox1* and *2* spacer was 951 nucleotides. The combined alignment of the both regions was 2,077 characters, including gaps (ITS: 1–990; *cox1* and *2* spacer: 991–2,077). Phylogenetic analyses were performed using PhyML, Parsimony and Bayesian methods; the tree in Fig. 2 was generated from PhyML. PHY-02 was in the same clade as other *P. nicotianae* isolates, with 100% bootstrap values from PhyML, Parsimony and Bayesian posterior probability analyses.

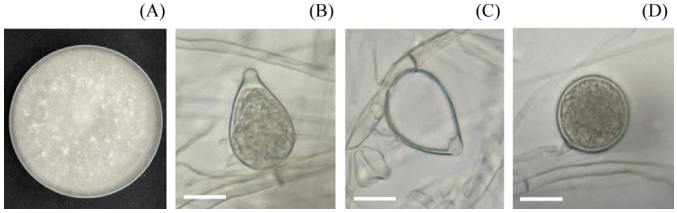

Fig. 1 Morphology of *Phytophthora nicotianae* isolate PHY-02: (A) colony on PDA at 7 d; (B) sporangium containing zoospores; (C) sporangium after zoospore release; (D) terminal chlamydospore; scale bar = 20 µm

The comprehensive characterization and molecular identification of isolate PHY-02 confirmed its taxonomic affiliation as *P. nicotianae*. These observations of the *P. nicotianae* characteristics aligned with the descriptions provided in the literature (Waterhouse, 1963; Santos, 2016). The fungus has wide mycelial growth, with elongated sporangiophores branching sympodial. The sporangia are papillated, persistent and predominantly ovoid or spherical, measuring 56.0 µm × 35.0 µm–33.3 µm × 24.5 µm with a length-to-breadth ratio of 1.4:1 (Santos, 2016). Typically, sporangia develop at the tips, although intercalary pedicel formations are occasionally observed, measuring around 2 µm in length (Waterhouse, 1963). Chlamydospores are terminal or intercalary in the mycelium and develop thick walls measuring 3–4 µm when aged 1–2 wk. *P. nicotianae* is heterothallic and isolates from both the A1 and A2 compatibility groups were recovered. Oospores measured 23–38 µm in diam. The antheridia are amphygynous (Santos, 2016). Phylogenetic analysis confirmed the morphological results and classified *P. nicotianae* into Clade 1, which was consistent with the report of Kroon et al. (2012). *Phytophthora* Clade 1 comprises 13 species: *P. andina*, *P. cactorum*, *P. clandestina*, *P. hedraiandra*, *P. idaei*, *P. infestans*, *P. ipomoeae* nom. inval., *P. iranica, P. mirabilis*, *P. nicotianae*, *P. phaseoli*, *P. pseudotsugae* and *P. tentaculata* (Kroon et al., 2012).

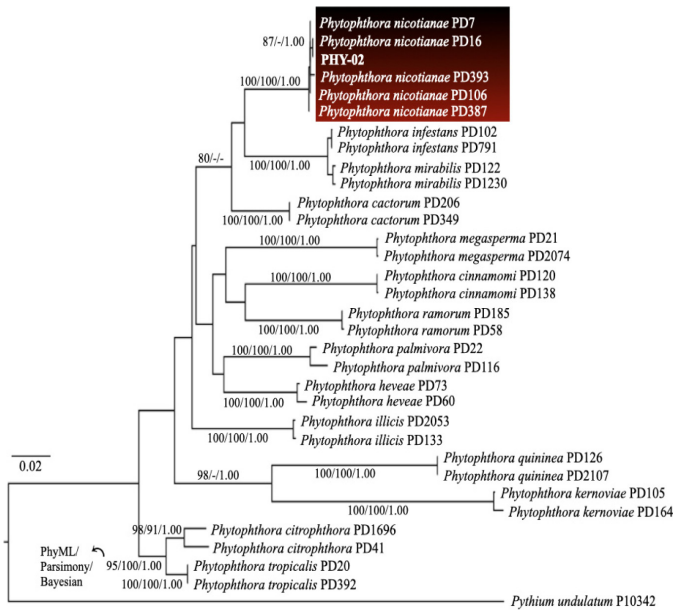

Fig. 2 Phylogenetic tree based on concatenated sequences of internal transcribed spacers and the cox 1 and 2 spacer from PHY-02 isolate, sequences from the Phytophthora Databases, with outgroup of *Pythium undulatum* P10342 from the NCBI database, the tree is based on PhyML analysis, numbers (x/y/z) at nodes are bootstrap values of more than 80% from PhyML, Parsimony and Bayesian posterior probability, respectively

Pathogenicity assays

Following incubation for 7 d in a moist chamber, pineapple leaves in proximity to the inoculated area exhibited characteristic symptoms of fungal infection (dark brown tissue, smooth edges and enlarged lesions), indicative of fungal dissemination through the plant's vascular system. Subsequent decay of the lesions led to their extension across the entire leaf (Fig. 3). In contrast, there were no signs of disease in the control group, where PDA plugs had been applied to the lesions. These findings were consistent with the disease symptoms observed on pineapple plants in greenhouse conditions (Fig. 3). *P. nicotianae* was successfully recovered upon re-isolation of the fungus from the diseased pineapple leaves. Based on these observations, it was inferred that *P. nicotianae* isolate PHY-02 was the causal agent of pineapple heart rot disease. *P. nicotianae* Breda de Haan (syn. *P. parasitica* Dastur) has long been recognized as a major plant pathogen with a broad host range (Erwin and Ribeiro, 1996; Sonavane and Sriram, 2021; Liu et al., 2022). The pathogenicity tests aligned with other findings that reported damage from *P. nicotianae* to pineapples in countries such as the USA (Rohrbach, 1985), Uganda (Ocwa et al., 2018b), and Puerto Rico (Serrato-Diaz et al., 2023). The morphological and molecular data presented in the current study provided insights into the genetic diversity and pathogenicity of *P. nicotianae*, which should facilitate the development of more effective management strategies for pineapple heart rot. Consequently, research into controlling pineapple heart rot disease through fungicides and bioagents is crucial for advancing effective disease management strategies.

Sensitivity of P. nicotianae to fungicides

This experiment aimed to evaluate the efficacy of dimethomorph, metalaxyl, fosetyl-Al and phosphonic acid in inhibiting the mycelial growth of *P. nicotianae* isolate PHY-02 after 7 and 12 d of incubation at various concentrations (Fig. 4). Dimethomorph had the highest efficacy across all concentrations tested, achieving 100% inhibition of fungal growth at concentrations of 100 mg/L, 1,000 mg/L and 10,000 mg/L. Metalaxyl had good efficacy at higher concentrations but was less effective than dimethomorph at lower concentrations. Fosetyl-Al and phosphonic acid had zero efficacy even at the highest concentration tested (10,000 mg/L). The evaluation was extended to 12 d of incubation and the results were compared to those obtained after 7 d (Fig. 5). Dimethomorph continued to have high efficacy, achieving 100% inhibition of fungal growth at all concentrations tested. Metalaxyl had moderate efficacy, achieving 100% inhibition at the highest concentration (10,000 mg/L) but was less effective than dimethomorph (Fig. 5).

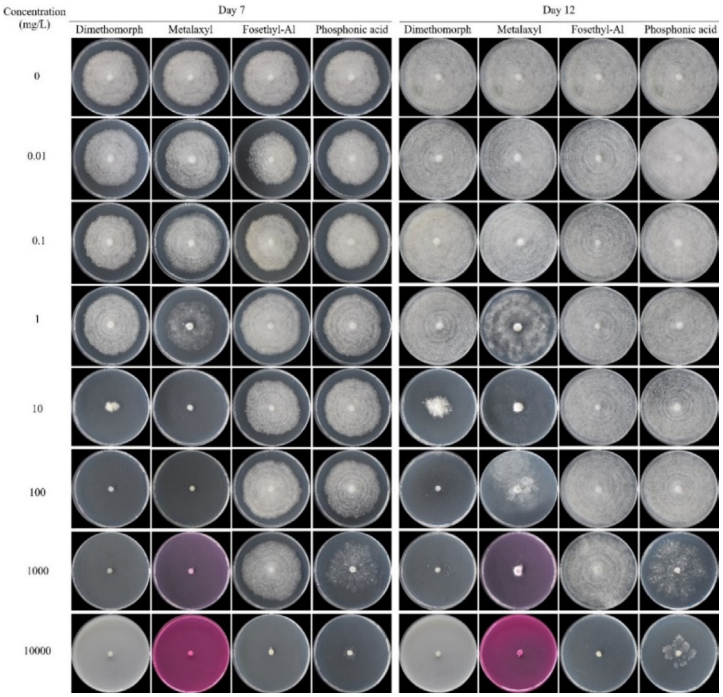

Fig. 4 Mycelial growth of *Phytophthora nicotianae* isolate PHY-02 on PDA medium containing different concentrations of fungicides and incubated at 25 ± 2°C for 7 d and 12 d

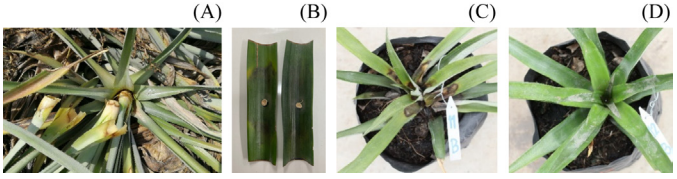

Fig. 3 Pathogenicity tests: (A) sample of pineapple heart rot disease observed in field; (B) symptoms observed on detached pineapple leaves, displaying dark brown tissue, smooth edges and enlarged lesions; (C) pineapple plant in greenhouse after being inoculated with *Phytophthora nicotianae* isolate PHY-02 for 3 wk; (D) control pineapple plant

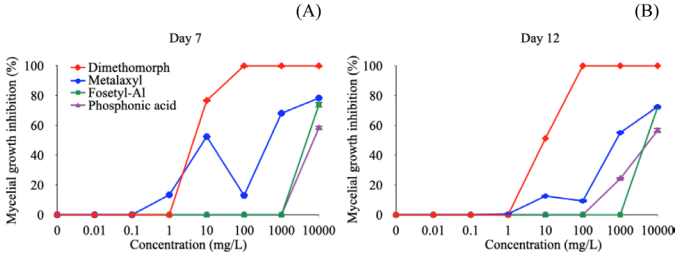

Fig. 5 Sensitivity of fungicide concentrations on inhibition of mycelial growth in *Phytophthora nicotianae* after incubation for (A) 7 d; (B) 12 d

Analysis of the EC_{50} values and hill coefficients on both day 7 and day 12 revealed consistent trends (Table 1). Dimethomorph consistently had the lowest EC_{50} values (7.5783–9.8848 mg/L), indicating its superior efficacy in inhibiting the mycelial growth of *P. nicotianae* isolate PHY-02. Metalaxyl had moderate efficacy (1,692.549–470.1751 mg/L), while fosetyl-Al consistently had the least efficacy, with EC_{50} values consistently greater than 10,000 mg/L. Phosphonic acid showed moderate efficacy, with EC_{50} values lower than for fosetyl-Al but higher than for dimethomorph and metalaxyl (Table 1).

The results of the fungicide sensitivity study provided valuable insights into the efficacy of dimethomorph, metalaxyl, fosetyl-Al and phosphonic acid in inhibiting the growth of *P. nicotianae* isolate PHY-02 over 7 d and 12 d of incubation periods. Dimethomorph was consistently the most effective fungicide, achieving complete inhibition of fungal growth at concentrations as low as 100 mg/L. Although metalaxyl had good efficacy at higher concentrations, its effectiveness was notably inferior to that of dimethomorph, particularly at lower concentrations. Furthermore, the analysis of EC_{50} values confirmed dimethomorph's superior efficacy, as it consistently had the lowest values among the tested fungicides. Metalaxyl showed moderate efficacy, while fosetyl-Al demonstrated the least efficacy, with EC_{50} values consistently exceeding 10,000 mg/L. The superior performance of dimethomorph highlighted its recognized potential as a potent agent for controlling *Phytophthora* species (Elansky et al., 2007; Zhu et al., 2008; Jackson et al., 2012).

Field trial experiments

The efficacy was evaluated of various fungicidal treatments in managing disease incidence and severity, based on the severity index (DSI) during 60 d (Fig. 6, Table 2). Among the treatments, dimethomorph at 9.5 mL/L was the most effective with a DSI of 36.6 at 60 d after application (DAA), significantly reducing all disease parameters compared to other treatments and the control group which had a DSI of 65.0. This represented a 1.77-fold reduction in disease severity. This higher concentration of dimethomorph consistently lowered disease incidence and severity starting from day 30. Metalaxyl at 2.5 g/L and fosetyl-Al at 2.5 g/L were moderately effective, significantly controlling disease progression. Phosphonic acid at 2.5 mL/L was less effective but still reduced disease parameters compared to the control group, which exhibited a continuous increase in disease metrics. As shown in Table 2, dimethomorph at 9.5 mL/L maintained a disease level of 1 from 7–45 DAA, rising slightly to level of 2 at 60 DAA. In contrast, dimethomorph at 1.2 mL/L, metalaxyl at 2.5 g/L, fosetyl-Al at 2.5 g/L and phosphonic acid at 2.5 mL/L sustained a disease level of 2 from 30 DAA onwards. However, the control group had a progressive increase in disease level, reaching level 3 at 60 DAA, underscoring the lack of effective disease management without fungicidal intervention. These results underscored the superior efficacy of dimethomorph at 9.5 mL/L in controlling heart rot in pineapples. While fosetyl-Al, metalaxyl at 2.5 g/L and phosphonic acid at 2.5 mL/L offered alternative treatment options, they were less effective than dimethomorph. The worsening disease metrics in the control group further emphasized the need for effective fungicidal treatments in managing heart rot.

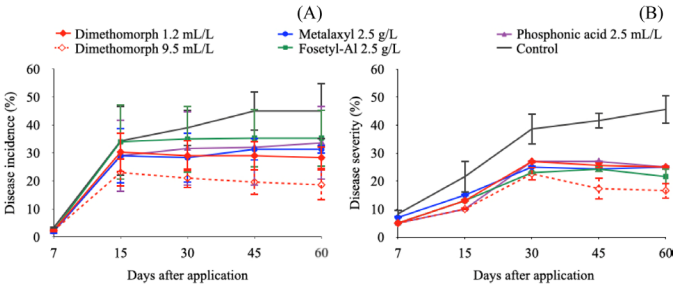

Fig. 6 Field trial experiments of pineapple heart rot disease assessed at different times post application: (A) disease incidence; (B) disease severity; error bar = ± SD

Table 1 EC_{50} values of fungicides for inhibition of mycelial growth of *Phytophthora nicotianae*

Fungicide	FRAC code*	Target site	Day 7		Day 12	
			EC_{50} (mg/L)	Hill coefficient	EC_{50} (mg/L)	Hill coefficient
Dimethomorph	40	Cellulose synthase	7.5783	-4.2969	9.8848	-4.4539
Metalaxyl	4	RNA polymerase I	1692.549	-0.2563	470.1751	-1.3289
Fosetyl-Al	P07	Host plant defense induction	>10,000	-3.0539	>10,000	-3.1204
Phosphonic acid	P07	Host plant defense induction	>10,000	-3.2526	1,084.7366	-3.5595

FRAC = Fungicide Resistance Action Committee (Fungicide Resistance Action Committee, 2024); EC_{50} = half maximal effective concentration.

Table 2 Level of disease and disease severity index of heart rot in pineapple treated with fungicides compared to control

Fungicide	Rate	Level of disease at day after application					Disease severity index at day after application				
		7	15	30	45	60	7	15	30	45	60
Dimethomorph	1.2 mL/L	1	1	2	2	2	24.58 ± 0.72	26.67 ± 0.72^{a}	49.17 ± 1.91^{bcd}	48.75 ± 1.25^{b}	49.58 ± 0.72^{b}
Dimethomorph	9.5 mL/L	1	1	2	2	1	23.75 ± 1.25	25.00 ± 0.00^{a}	42.92 ± 3.82^{b}	41.67 ± 6.88^{a}	36.67 ± 5.20^{a}
Fosetyl-Al	2.5 g/L	1	1	2	2	2	23.75 ± 2.17	29.17 ± 1.44^{a}	44.58 ± 2.89^{bc}	46.67 ± 4.73^{b}	45.83 ± 4.02^{b}
Metalaxyl	2.5 g/L	1	1	2	2	2	25.00 ± 0.00	35.42 ± 1.44^{b}	41.67 ± 1.44^{a}	42.08 ± 1.91^{a}	45.42 ± 0.72^{b}
Phosphonic acid	2.5 mL/L	1	1	2	2	2	23.75 ± 2.17	26.67 ± 0.72^{a}	50.00 ± 0.00^{cd}	50.00 ± 0.00^{bc}	47.50 ± 2.17^{b}
Control		1	2	2	2	3	25.83 ± 0.72	42.92 ± 7.64^{c}	56.67 ± 6.17^{d}	60.00 ± 2.50^{c}	65.00 ± 5.00^{c}
						%CV	5.35	10.19	7.52	8.04	7.41

CV = coefficient of variation.

Values (mean ± SD) in the same column with different lowercase superscripts are significantly ($p < 0.05$) different.

The data from the field trial experiment revealed significant variation in the efficacy of fungicides for controlling disease incidence and severity, depending on the type and concentration used. Notably, dimethomorph at a higher concentration (9.5 mL/L) had superior effectiveness, with the lowest disease incidence and severity during 60 d compared to the control, with the latter producing the highest disease metrics. Based on these results, higher concentrations of dimethomorph provided more sustained control, likely due to better coverage and prolonged fungicidal activity. In contrast, the lower concentration of dimethomorph (1.2 mL/L) was initially effective but failed to maintain control over time, indicating that while dimethomorph reduced disease initially, the lower concentration did not seem to offer long-lasting protection. Dimethomorph is a targeted fungicide primarily effective against oomycetes, such as *Phytophthora* species, with its mode of action involving the inhibition of the synthesis of crucial cell wall components in fungi, which leads to weakened cell walls, ultimately causing cell death (Fungicide Resistance Action Committee, 2024). Dimethomorph effectively suppresses fungal growth and reproduction by disrupting the production of these cell wall components and preventing the formation and spread of new spores (Kuhn et al., 1991). Its selectivity for oomycetes reduces the risk of resistance development in non-target fungi, making it a specialized tool in disease management. Generally, dimethomorph acts on the fungus through direct contact of the treated surface, making it more effective as a preventive measure rather than a cure.

The lack of antifungal activity *in vitro* against *P. nicotianae* isolate PHY-02 by phosphonic acid was unexpected, although it provided moderate control in the field trials. This discrepancy may have arisen because phosphonic acid enhances plant defense mechanisms rather than directly inhibiting fungal growth. Similarly, metalaxyl and fosetyl-Al did not affect *P. nicotianae* isolate PHY-02 growth but had moderate control in the field trials, which could be attributed to their systemic activity, targeting *P. nicotianae* within plant tissues rather than inhibiting mycelial growth directly.

Fosetyl-Al, metalaxyl and phosphonic acid are fungicides with distinct modes of action targeting various aspects of fungal physiology (Fungicide Resistance Action Committee, 2024). Fosetyl-Al functions as a systemic fungicide by converting into phosphonic acid within plant tissues. Then, the phosphonic acid inhibits the synthesis of critical phospholipids needed for fungal cell membranes, thereby compromising membrane integrity and hindering fungal growth. Metalaxyl is also systemic but specifically targets oomycetes by blocking RNA synthesis through interference with RNA polymerase, which disrupts fungal cell development and reproduction, while phosphonic acid, in its active form, similarly affects cell wall and membrane component synthesis (Fungicide Resistance Action Committee, 2024). The moderate effectiveness of these fungicides can be attributed to their specific modes of action (Cohen and Coffey, 1986) and the inherent resistance mechanisms of fungal species (Ocwa et al., 2018a; Sonavane and Sriram, 2021).

In conclusion, the current study underscored the importance of selecting appropriate fungicides and concentrations for effective disease management. The results supported the use of dimethomorph as a primary treatment option and highlighted the need for integrated disease management strategies that include fungicide rotation and application to mitigate resistance and optimize control. Continued research is essential into fungicide efficacy and the development of novel management approaches to address the impact of pineapple heart rot and to reduce crop losses.

Acknowledgements

The authors gratefully acknowledge the financial support provided by ADAMA (Thailand) Ltd. The Kasetsart University Research and Development Institute (KURDI), Bangkok, Thailand provided assistance with English editing.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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