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RESEARCH ARTICLE

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Biocontrol and Pathogenicity Analysis of *Phytophthora infestans* Affecting Potatoes in Brebes, Indonesia

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ABSTRACT

Late blight disease caused by *Phytophthora infestans*, remains a major constraint in potato (*Solanum tuberosum* L.) production in Indonesia, particularly in Brebes, Central Java, a key potato-growing region. The objectives of the study are a) to isolate and characterize *P. infestans* populations from potato fields in Brebes based on morphological, molecular, and pathogenic traits; and (b) to evaluate the efficacy of a biofungicide formulation combining selected yeast isolates and nanoformulated clove oil under field conditions. A factorial field experiment was conducted using two potato cultivars (Granola and Median), two yeast isolates (Y6 and Y8), and two nano-clove oil concentrations (3 mL/L and 5 mL/L), applied as foliar sprays or in combination with seed treatment. Results revealed that the yeast isolate Y6 and nano-clove oil at 5 mL/L showed the most effective suppression of late blight. Notably, the combination of seed treatment and foliar application significantly reduced disease severity compared to foliar application alone. Further characterization isolates showed that ten *P. infestans* isolates were collected from infected potato plants and cultured on Rye B agar medium, producing white, cottony colonies with sporangia measuring 12.30–55.50 µm in length and 11.40–33.80 µm in width, with a length-to-width ratio of 1.00–2.90. Pedicel and papilla lengths ranged from 1.40–7.50 µm and 1.20–4.20 µm, respectively. The detached leaf assay on the cultivar Golden revealed five pathogenicity groups, while molecular identification using ITS region-specific primers confirmed all isolates as *P. infestans*. These findings highlight the potential of integrating yeast-based biocontrol agents with plant-derived nanomaterials as an environmentally sound strategy for late blight management and demonstrate the considerable genetic and pathogenic diversity of *P. infestans* in Brebes.

Keywords: Biofungicide; Nano clove; Pathogenicity; Phytophthora infestans; Potato.

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INTRODUCTION

The potato (*Solanum tuberosum* L.) is one of the most important horticultural crops in Indonesia, contributing significantly to both the domestic food supply and the country's economic development. It is widely cultivated across key agricultural regions, including West Java, Central Java, East Java, North Sulawesi, and several provinces in Sumatra (Central Bureau of Statistics, 2021). Driven by population growth and expanding demand from fresh consumption and the food processing industry, the need for a stable and high-quality potato supply is increasing. However, the productivity of potato farming in Indonesia is severely threatened by late blight disease, caused by the oomycete *Phytophthora infestans,* a highly destructive pathogen responsible for substantial yield losses worldwide.

Globally, *P. infestans* imposes a severe economic burden on the potato sector. Annual combined costs of yield losses and disease management exceed €5 billion,

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with the total global impact estimated at US$14–20 billion per year. In Europe, the economic cost of late blight surpasses €900 million annually, largely due to intensive fungicide use and crop losses. In sub-Saharan Africa, the disease contributes to typical yield reductions of 15–30%, posing a major threat to smallholder livelihoods and food security. Across Asia, including major potato-producing regions in South and Southeast Asia, late blight remains a principal constraint, driving high production costs and recurrent epidemics (Savary et al., 2019; Bose et al., 2023; Maurya et al., 2025). In Indonesia, late blight can cause a yield loss of up to 80% under favourable environmental conditions (Utami & Ambarwati, 2017). These economic impacts highlight the global significance of *P. infestans* and the urgency of sustainable management strategies.

The disease affects all aerial parts of the plant, including leaves, stems, and tubers, and is especially aggressive when infections occur during the vegetative phase, leading to poor canopy development, wilting, and eventual plant death (Lambert et al. 1998). Disease outbreaks are most prevalent during Indonesia's rainy and foggy seasons, which offer cool and humid conditions ideal for pathogen proliferation. On a global scale, climate change is also influencing late blight epidemiology by extending conducive weather periods and altering the geographic distribution of aggressive clonal lineages (Adolf et al., 2020; Ivanov et al., 2021). In tropical potato-growing systems, climate change is further intensifying late blight pressure by altering rainfall distribution, increasing the frequency of extreme weather events, and prolonging periods of high humidity. Warmer temperatures combined with elevated nighttime dew, persistent cloud cover, and irregular rainfall create microclimates that favor rapid sporulation and infection cycles of *P. infestans*. Recent studies indicate that climate-driven shifts, including more frequent cool–wet spells and unseasonal rain events, are enabling earlier disease onset and faster epidemic development in tropical highland potato regions (e.g., Southeast Asia, East Africa, and the Andean tropics) (Sherwood et al., 2014; Criollo et al., 2025) These changing environmental conditions not only complicate forecasting and preventive spraying schedules but also accelerate the emergence of aggressive clonal lineages adapted to fluctuating climates.

Consequently, climate-resilient and sustainable disease management strategies—particularly those less reliant on weather-sensitive synthetic fungicides—are becoming increasingly critical for tropical potato production systems. Recent studies have revealed that *P. infestans* populations worldwide exhibit high genetic variability, rapid evolutionary change, and the emergence of fungicide-resistant genotypes (Guha Roy et al., 2021; Wang et al., 2025). These epidemiological changes complicate disease forecasting and contribute to recurrent late blight outbreaks that impose billions of dollars in annual losses worldwide. As a heterothallic organism with two mating types (A1 and A2), the pathogen can undergo sexual reproduction to form thick-walled oospores. These survival structures contribute to environmental persistence, enhanced adaptability, and long-distance spread (Utami & Ambarwati, 2017; Izarra et al., 2025).

The increasing complexity of *P. infestans* population biology underscores the need for region-specific monitoring, especially in major potato-producing regions. Despite its widespread occurrence in Indonesia, information on the local population structure, morphological diversity, and pathogenicity of *P. infestans*, particularly in critical production zones like Brebes, Central Java, remains limited. Brebes is one of the nation's leading potato-producing regions, yet baseline data on pathogen diversity in this area are scarce. A deeper understanding of *P. infestans* variability is essential to inform regional disease management strategies and support the development of resistant cultivars adapted to local pathogen populations.

Current management practices for late blight heavily rely on synthetic fungicides. Although effective in suppressing epidemics, their intensive and repeated use has led to environmental contamination, the development of fungicide-resistant strains, and concerns over chemical residues in food (Naqvi et al., 2024). Another pressing issue in the management of late blight is the rapid evolution of fungicide resistance in *P. infestans*. Repeated exposure to commonly used chemistries—especially phenylamides fungicides, and certain multi-site protectants—has led to the emergence of resistant or reduced-sensitivity strains in many potato-growing regions. This resistance evolution diminishes the effectiveness of standard spray programs and forces growers to increase application frequency or shift to more expensive fungicide options. For smallholder farmers in tropical regions, these challenges are even more pronounced due to limited financial resources, inconsistent access to high-quality fungicides, and constraints in implementing precise spray schedules. Consequently, fungicide resistance not only elevates production costs but also heightens crop vulnerability, making late blight a persistent and economically devastating threat for smallholder potato systems (Hussain, 2025).

Consequently, there is a growing global shift toward sustainable and environmentally friendly alternatives, including biological control agents and plant-derived antimicrobials. In recent years, several yeasts, bacteria, and fungal antagonists have shown promising biocontrol activity against *Phytophthora* spp. through competition, antibiosis, and mycoparasitism (Freimoser et al., 2019; Wang & Long, 2023). Recent research has expanded the use of biological control agents such as *Bacillus, Trichoderma, Pseudomonas*, and yeast-based antagonists, which have demonstrated significant suppressive activity and plant defense activation under greenhouse and field conditions (Léger et al., 2021; Kowalska et al., 2022; Mollah & Hassan, 2024). Likewise, yeasts have also gained attention as promising biocontrol agents due to their ease of cultivation, ability to colonize plant surfaces, and antagonistic mechanisms.

Essential oils—particularly from clove (*Syzygium aromaticum*), thyme, and oregano have also been widely reported for their antifungal efficacy against late blight and other oomycetes (Alonso-Gato et al., 2021; Hashem et al., 2023). However, conventional essential oil applications face

issues such as low stability, rapid volatilization, and limited field persistence. Advances in the development of nanoformulations including nanoemulsions of essential oils, polymer-based nanocarriers, and metallic nanoparticles have further strengthened biological and botanical product performance by enhancing stability, controlled release, and environmental persistence, leading to improved late blight suppression under variable field conditions (Luna et al., 2021; Machado et al., 2022; Kaur et al., 2023; Dávila Costa & Romero, 2025). Collectively, these studies demonstrate that integrating modern epidemiological insights with biological control and nano-enabled formulations offers a promising direction for reducing fungicide dependence while achieving more resilient and sustainable management of potato late blight worldwide (Arif et al., 2025).

To overcome these limitations, nanoencapsulation and nanoemulsion technologies are increasingly utilized to enhance the bioavailability, stability, and delivery efficiency of botanical fungicides (Kaur et al., 2024; Barath et al., 2025). Several recent studies have demonstrated the improved antifungal performance of nanoformulated plant oils against *Phytophthora* species and other phytopathogens, indicating their potential for integration into disease management systems (Nayak, 2024; Rodrigues et al., 2025). Despite these advances, the combined use of yeasts and nanoemulsified clove oil remains underexplored, particularly for field-level application in tropical Indonesian agricultural systems.

This study was conducted with two main objectives: (a) to evaluate the efficacy of a biofungicide formulation combining selected yeast isolates and nanoformulated clove oil under field conditions, and (b) to isolate and characterize *P. infestans* populations from potato fields in Brebes based on morphological, molecular, and pathogenic traits. Specifically, the study compared the effectiveness of different application methods (foliar vs. combined seed and foliar treatments), tested the performance of two yeast strains, and assessed the impact of varying nano-clove oil concentrations. The findings are expected to contribute to the development of integrated, location-specific, and sustainable management strategies for late blight in Indonesian potato farming systems.

MATERIALS & METHODS

Study Location

This study was conducted in two main phases. Field trials for biofungicide application were carried out on a farmer's land in Pandansari Village (7°16′18″S, 109°7′25″E), Brebes District, Central Java, Indonesia, an area endemic to late blight disease. The pathogen isolation and laboratory analyses were performed at the Center for Research and Development of Agricultural Biotechnology and Genetic Resources, Bogor, West Java, Indonesia, in 2021.

Biofungicide Formulation

Yeast Isolates and Preparation

Yeast isolates Y6 and Y8, obtained from the Biogen Culture Collection (Biogen CC), were cultured in potato dextrose broth and incubated for 5 days. For liquid formulation, cultures were further incubated for 7 days at room temperature following Dymond (2013), then stored in a sealed plastic container under refrigeration until use (Fig. 1A).

Nano-Clove Oil Preparation

Clove oil nanoemulsion was prepared following the spontaneous emulsification method described by Bouchemal et al. (2004). Equal volumes of clove oil and emulsifiers (Tween, glycerol, turpentine) were mixed in a tank and stirred for 2 hours at 1,000 rpm. The resulting nanoemulsion was stored in plastic bottles for field application (Kaur et al., 2024) (Fig. 1B).

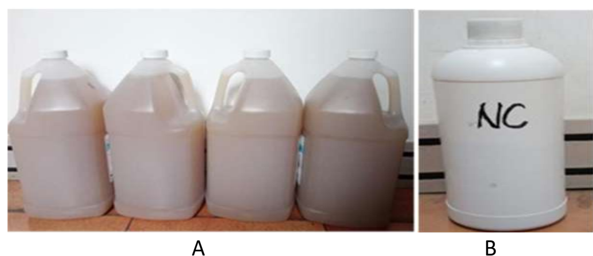

Fig. 1: A) Preparation of yeast-biofungicide formulation, and B) nanoformulation-biofungicide of clove oil.

Biofungicide Efficacy Evaluation in Field Trial

Field testing was conducted using a factorial randomized block design with two factors: (1) potato cultivar (cv.) (Granola and Median) and (2) biofungicide treatment (10 treatment combinations of yeast and nano-clove oil). Each treatment was replicated three times (Table 1). Plots measured 2 m × 3 m with 80 cm spacing between rows and 30 cm within rows. Basal manure fertilizer (10 tons/ha) and Nitrogen, Phosphorus, and Potassium (NPK) 16:16:16 (200 kg/ha) were applied at planting and 45 days after planting.

Table 1: Treatment for biofungicide application

Code of treatment	Means application and rate
T1: Y6	Seed treatment 100 mL/L + foliar spraying 50 mL/L
T2: Y8	Seed treatment 100 mL/L + foliar spraying 50 mL/L
T3: Y6	Foliar spraying 50 mL/L
T4: Y8	Foliar spraying 50 mL/L
T5: NC	Seed treatment 3 mL/L + foliar spraying 3 mL/L
T6: NC	Seed treatment 5 ml/L + foliar application 5 mL/L
T7: NC	Foliar spraying 3 mL/L
T8: NC	Foliar spraying 5 mL/L
T9:Fungicide (a.i. mancozeb 64% + mefenoxam 4%)	Seed treatment 1.25 g/L+ foliar spraying 1.25 g/L
T10: Control	without application

Y6, Y8= code of yeast isolate, NC = nano clove oil.

(A)

(B)

(C)

Fig. 2: A) Preparation of potato planting during biofungicide application in the field trial; B) seed treatment application, and C) foliar spraying application.

Biofungicide application consisted of two methods: seed treatment by soaking tubers in the formulation before planting and foliar spraying starting at 3 weeks after planting (WAP), continued weekly for five weeks (Fig. 2A-C). Disease progression was monitored weekly.

Pathogenicity

Isolation and Identification

Infected potato leaves exhibiting late blight symptoms were collected from fields in the Paguyangan (7°16′56″S, 109°3′9″E) and Sirampog (7°14′5″S, 109°7′3″E) subdistricts of Brebes. Samples were wrapped in sterile tissue, transported in plastic containers, and processed immediately. Leaves were surface sterilized in 70% ethanol for 30 seconds, rinsed with sterile water, air-dried, and dissected into symptomatic and asymptomatic areas.

Leaf fragments were placed on previously sterilized potato tubers. After 7–14 days of incubation at 15°C, emerging mycelia were subcultured on Rye B agar medium supplemented with pimaricin (10 ppm) and rifampicin (30 ppm) to prevent bacterial contamination. Further purification was conducted using V8 juice agar and Rye B media. For morphological characterization, one-month-old cultures grown on V8 juice agar were examined using an Olympus BX51 microscope to observe colony features, sporangia, hyphae, papillae, and pedicels.

Molecular identification involved genomic deoxyribonucleic acids (DNA) extraction using the Quick-DNA™ Miniprep kit (Zymo Research), followed by polymerase chain reaction (PCR) amplification using internal transcribed spacer (ITS) 6 and ITS4 primers. PCR products were run on 1% agarose gels, stained with ethidium bromide, visualized using an ultraviolet (UV) transilluminator (Bio-Rad, UK), and sequenced at First Base, Malaysia. Nucleotide sequences were compared using BLASTn (https://blast.ncbi.nlm.nih.gov/), and phylogenetic trees were constructed using the Molecular Evolutionary Genetics Analysis (MEGA) 6 software with the maximum likelihood method and 1,000 bootstrap replications (Tamura et al., 2013).

Pathogenicity Assay

Detached leaves of potato cultivar ‘Golden’ (5 weeks old) were surface sterilized with 1% sodium hypochlorite (NaOCl), rinsed with sterile water, and placed abaxial side up in petri dishes lined with moist sterile tissue paper. For each treatment, five leaves were used per replicate, with three independent replicates (a total of 15 leaves per treatment). Leaves were wounded with a sterile needle and inoculated with a 0.5 cm² plug of *P. infestans* culture. while control leaves received a plug of sterile V8 agar. Plates were sealed with plastic wrap and incubated at 15 °C. Lesion development and symptom severity were recorded daily.

Disease Assessment and Data Analysis

Disease incidence was determined using the formula:

$$DI\ (disease\ incidence\ ) = \frac{number\ of\ infected\ plants}{number\ of\ healthy\ plants} x\ 100\%$$

Disease severity (DS) was rated on a 1–9 scale (Mandal et al., 2021):
0 = 0% infection, 1 = 1–10%, 3 = 10–20%, 5 = 20–30%, 7 = 30–50%, 9 = >50%.
Severity of the disease (DS) is estimated using the formula:

$$DS = \sum_{k=0}^{n} \frac{ni\ x\ vi}{N\ x\ V}\ x\ 100\%$$

Where *n_i* = number of plants at score *i*, *vi* = severity score, *N* = total plants observed, and *V* = maximum score. The area under the disease progress curve (AUDPC) (Campbell and Maden, 1990) is calculated using the formula as follows:

$$AUDPC\ = \sum_{i=1}^{Ni-1} \frac{Xi + Xi + 1}{2}\ (ti + 1 - ti)$$

Where *X* = disease severity at time *i*, and *t* = time of observation.
The infection rate (r) was calculated using the formula:

$$r = \frac{e}{t}(\frac{1}{1 - Xi + 1} - log\,\frac{1}{1 - Xi})$$

Where *e* is the natural logarithm base, *t* is the time interval between observations, and *X* is disease severity. All data were analyzed using analysis of variance (ANOVA). If significant differences were found (F-test, $p < 0.05$), means were compared using Duncan’s Multiple Range Test (DMRT).

RESULTS

Effect of Biofungicide Application on the Incidence of Potato Leaf Blight

According to the observation's findings, the two

potato cultivars employed in this investigation, cv. Median and Granola exhibited comparable resistance responses to the leaf blight disease (*P. infestans*) ($P<0.05$). The interaction between variety and biofungicide/synthetic fungicide treatment (variety x treatment) did not alter the incidence or severity of blight disease in this study ($P< 0.05$). Only variations in the use of biofungicide or synthetic fungicide affected the disease's incidence and severity.

Over time, the incidence of blight increased rapidly in areas that were not treated (control). The disease incidence in the control group increased from 46.0% at the start of observation 4 weeks after planting (WAP) to 100.00% at 7 WAP (Table 2). In contrast, the disease incidence in the cv. Granola control group was 31.30% at 4 WAP and reached 100.00% at 7 WAP (Table 3). In the meantime, disease incidence might be reduced in the plots treated with fungicide formulations, yeast, and nano-clove oil. Nevertheless, all treatments demonstrated a disease incidence of 100.00% after final observation at eight WAP.

Observations during the 4th WAP and the 7th WAP demonstrated the impact of synthetic and biofungicides on disease control. In comparison to the untreated control group, plants treated with these disease control techniques showed a noticeably lower disease incidence rate during this time. This suggests that both synthetic fungicides and biofungicides (yeast and nano clove oil) were essential in reducing the onset and spread of leaf blight disease.

A distinct pattern became apparent while comparing the disease incidence of the Median and Granola potato cultivars. Plants treated with synthetic and biofungicides consistently showed a significantly lower disease rate than the control group in both cases. This demonstrates how effectively these treatments reduce the severity of the disease and prevent it from spreading rapidly throughout the crops. With a disease rate value of 0.02, the T1 treatment showed the lowest disease incidence for the cv. Median among all the yeast-based biofungicide treatments. With a disease infection rate of 0.02 (Table 3), the T1 treatment for the cv. Granola also had the lowest disease incidence rate among yeast-based biofungicide treatments, nearly matching the outcomes observed in the cv. Median.

Effect of Biofungicide Application on the Severity of Leaf Blight Disease

In potato plants cv. Median and Granola, the use of synthetic fungicide treatments and biofungicides (yeast and nano cloves) not only delays the beginning of the disease but also lessens its severity. Both synthetic and biofungicides successfully reduced the severity of the disease in the case of cv. Median. The leaf blight severity in treated plots at 4 WAP ranged from 20.00% to 42.70%, which is substantially less than the disease severity in the control, which reached 45.30%. At 8 WAP, the control plot had a significantly higher disease severity of 82.00%, whereas the treatment plots had a range of 58.00% to 70.00% (Table 4). This indicates that, in comparison to untreated plants, the use of synthetic and biofungicides can considerably slow the disease's progression.

Likewise, biofungicide and synthetic fungicide treatments have shown significant effectiveness in managing the disease for the cv. Granola. The untreated control had a severity of 48.70% at 4 WAP, but the severity in treated plots varied from 24.70 to 45.30%. In contrast to the control group, which reached 81.30% by 8 WAP, the severity in treated plots varied from 61.30 to 70.00% (Table 5).

Table 2: Effect of biofungicide application on leaf blight disease incidence in potatoes cv. Median

Treatment	Incidence (%)					Infection rate
	4 WAP	5 WAP	6 WAP	7 WAP	8 WAP	
T1: Y6 (Seed treatment 100 mL/L + foliar spraying 50 mL/L)	19.90	27.80	41.10	84.10	100.00	0.02^{bc}
T2: Y8 (Seed treatment 100 mL/L + foliar spraying 50 mL/L)	23.70	31.10	50.30	92.00	100.00	0.03^{bc}
T3: Y6 (Foliar spraying 50 mL/L)	42.00	48.60	68.90	93.70	100.00	0.04^{bc}
T4: Y8 (Foliar spraying 50 mL/L)	45.30	51.90	75.80	95.40	100.00	0.06^{b}
T5: NC (Seed treatment 3 mL/L + foliar spraying 3 mL/L)	16.50	25.60	45.60	85.20	100.00	0.03^{bc}
T6: NC (Seed treatment 5 mL/L + foliar application 5 mL/L)	17.10	26.40	43.10	79.60	100.00	0.03^{bc}
T7: NC (Foliar spraying 3 mL/L)	31.70	35.00	56.30	89.20	100.00	0.03^{bc}
T8: NC (Foliar spraying 5 mL/L)	34.30	35.60	38.20	82.20	100.00	0.01^{c}
T9: Fungicide (a.i mancozeb 64% + mefenoxam 4%) (Seed treatment 1.25 g/L + foliar spraying 1.25 g/L)	13.40	26.50	32.80	63.80	100.00	0.02^{c}
T10: Control (without application)	46.0	58.8	93.7	100.0	100.0	0.15^{a}

The DMRT ($P<0.05$) found no significant difference between means in one column followed by the same letter; WAP represents weeks after planting.

Table 3: Effect of biofungicide application on leaf blight disease incidence in potatoes cv. Granola

Treatment	Incidence (%)					Infection rate
	4 WAP	5 WAP	6 WAP	7 WAP	8 WAP	
T1: Y6 (Seed treatment 100 mL/L + foliar spraying 50 mL/L)	17.50	25.20	38.20	76.80	100.00	0.02^{de}
T2: Y8 (Seed treatment 100 mL/L + foliar spraying 50 mL/L)	20.80	27.60	46.50	84.30	100.00	0.03^{bcd}
T3: Y6 (Foliar spraying 50 mL/L)	20.70	27.90	51.80	82.70	100.00	0.04^{bc}
T4: Y8 (Foliar spraying 50 mL/L)	27.30	32.90	59.20	97.60	100.00	0.04^{b}
T5: NC (Seed treatment 3 mL/L + foliar spraying 3 mL/L)	20.10	28.40	38.40	75.90	100.00	0.02^{de}
T6: NC (Seed treatment 5 mL/L + foliar application 5 mL/L)	24.20	24.20	36.00	74.40	100.00	0.01^{e}
T7: NC (Foliar spraying 3 mL/L)	26.10	30.20	48.10	78.10	100.00	0.02^{cd}
T8: NC (Foliar spraying 5 mL/L)	29.50	31.30	44.70	77.20	100.00	0.02^{de}
T9: Fungicide (a.i mancozeb 64% + mefenoxam 4%) (Seed treatment 1.25 g/L + foliar spraying 1.25 g/L)	21.30	26.30	30.90	73.30	100.00	0.01^{e}
T10: Control (without application)	31.30	47.00	74.60	100.0	100.00	0.07^{a}

The DMRT ($P<0.05$) found no significant difference between means in one column followed by the same letter; WAP represents weeks after planting.

Table 4: Effect of biofungicide application on leaf blight disease severity and AUDPC in potatoes cv. Median

Treatment	Disease severity (%)					AUDPC
	4 WAP	5 WAP	6 WAP	7 WAP	8 WAP	
T1: Y6 (Seed treatment 100 mL/L + foliar spraying 50 mL/L)	20.00	34.00	37.30	48.00	63.30	1127.00^{c}
T2: Y8 (Seed treatment 100 mL/L + foliar spraying 50 mL/L)	37.30	39.30	46.00	54.70	68.00	1348.70^{bc}
T3: Y6 (Foliar spraying 50 mL/L)	36.00	38.70	44.00	54.00	64.70	1309.00^{bc}
T4: Y8 (Foliar spraying 50 mL/L)	42.70	44.00	50.70	56.70	70.00	1453.70^{ab}
T5: NC (Seed treatment 3 mL/L + foliar spraying 3 mL/L)	24.00	32.00	36.00	48.00	58.70	1101.30^{c}
T6: NC (Seed treatment 5 mL/L + foliar application 5 mL/L)	25.30	30.70	35.30	50.00	61.30	1115.30^{c}
T7: NC (Foliar spraying 3 mL/L)	33.30	33.30	37.30	54.70	59.30	1201.70^{bc}
T8: NC (Foliar spraying 5 mL/L)	32.00	32.00	35.30	52.70	65.30	1180.70^{bc}
T9: Fungicide (a.i mancozeb 64% + mefenoxam 4%) (Seed treatment 1.25 g/L + foliar spraying 1.25 g/L)	20.70	32.00	34.70	47.30	58.00	1073.30^{c}
T10: Control (without application)	45.30	48.00	55.30	70.70	82.00	1663.70^{a}

The DMRT (P<0.05) found no significant difference between means in one column followed by the same letter; WAP represents weeks after planting.

Table 5: Effect of biofungicide application on the leaf blight disease severity and AUDPC in potatoes cv.Granola

Treatment	Disease severity (%)					AUDPC
	4 WAP	5 WAP	6 WAP	7 WAP	8 WAP	
T1: Y6 (Seed treatment 100 mL/L + foliar spraying 50 mL/L)	28.00	33.30	36.70	42.70	62.00	1103.70^{cd}
T2: Y8 (Seed treatment 100 mL/L + foliar spraying 50 mL/L)	35.30	40.00	46.70	50.00	68.00	1318.30^{bc}
T3: Y6 (Foliar spraying 50 mL/L)	38.70	42.70	44.70	48.70	66.70	1320.70^{bc}
T4: Y8 (Foliar spraying 50 mL/L)	45.30	47.30	49.30	52.70	70.00	1449.00^{b}
T5: NC (Seed treatment 3 mL/L + foliar spraying 3 mL/L)	24.70	34.70	36.00	44.00	65.30	1117.70^{cd}
T6: NC (Seed treatment 5 mL/L + foliar application 5 mL/L)	32.00	33.30	34.70	42.00	66.00	1113.00^{cd}
T7: NC (Foliar spraying 3 mL/L)	40.00	45.30	45.30	48.70	66.70	1348.70^{b}
T8: NC (Foliar spraying 5 mL/L)	39.30	42.00	43.30	46.70	67.30	1297.30^{bc}
T9: Fungicide (a.i mancozeb 64% + mefenoxam 4%) (Seed treatment 1.25 g/L + foliar spraying 1.25 g/L)	28.00	32.70	33.30	40.70	61.30	1059.30^{d}
T10: Control (without application)	48.70	53.30	56.00	64.00	81.30	1668.30^{a}

The DMRT (P<0.05) found no significant difference between means in one column followed by the same letter; WAP represents weeks after planting.

Both synthetic and biofungicide treatments demonstrated a similar suppression of late blight disease, with AUDPC values that did not differ substantially (Table 4). The fungicide treatment (T9) and the disease suppression response, particularly in treatments T1, T5, and T6, were similar. The AUDPC measurements have been used to assess how well disease suppression works. According to the results, the Y6 formulation showed a similar disease suppression response to the T3 treatment, which only featured foliar spraying without seed treatment, when applied using a combination of seed treatment and foliar spraying (T1).

This implies that, irrespective of the application method, the Y6 formulation was quite successful in managing the disease. Additionally, as seen by its lower AUDPC levels, the Y6 formulation showed better disease suppression than the Y8 formulation. A lower AUDPC result highlights the improved effectiveness of the Y6 formulation in reducing the impact of the disease by indicating decreased disease severity and progression over time.

Isolate Collection

The study found that using potato tuber bait to isolate *Phytophthora* was beneficial in encouraging its growth. Mycelium grew on potato tubers inoculated with symptomatic leaves. The potato tuber mycelia exhibited a large-non-septate mycelium that produced sporangia, similar to *Phytophthora*. (Fig. 3A).

Ten isolates of *Phytophthora* were found to be the only cause of leaf blight disease in potato plants in Brebes, Central Java. Table 6 shows that eight isolates were obtained from the Paguyangan district and two from the Sirampog district. These isolates grew well on V8-juice agar media, forming white, cottony colonies without patterns (Fig. 3B).

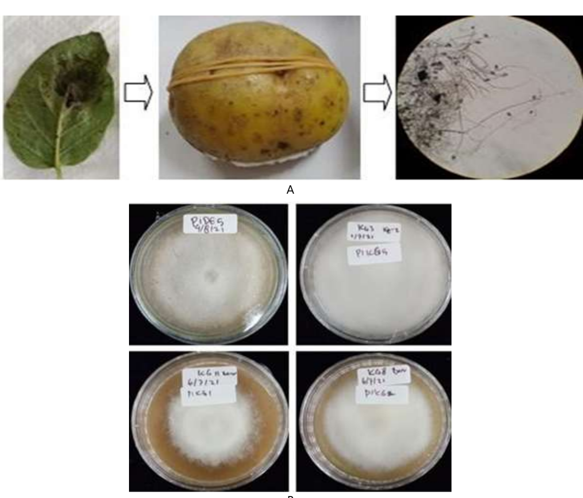

Fig. 3: A) Inducing *Phytophthora* growth on potato tubers containing isolates of potato leaf blight symptoms from the field; B). Representative pictures of a colony isolate of *Phytophthora* from Brebes, grown on V8-Juice agar.

Table 6: Homology of the 10 isolates *P. infestans* from the collection with GenBank accessions based on ITS sequences

No	Isolates code	Species GenBank	Accession No	Query Cover (%)	E-value	Identity (%)
1	PiKG1	*P. infestans*	MH401206.1	98.00	0.00	100.00
2	PiKG2	*P. infestans*	MH401206.1	98.00	0.00	100.00
3	PiKG5	*P. infestans*	MH401206.1	97.00	0.00	100.00
4	PiKG6	*P. infestans*	MH401206.1	98.00	0.00	100.00
5	PiDE3	*P. infestans*	KT363862.1	98.00	0.00	100.00
6	PiDE5	*P. infestans*	KT363862.1	98.00	0.00	100.00
7	PiDE9	*P. infestans*	MH401206.1	98.00	0.00	100.00
8	PiDE11	*P. infestans*	KT363862.1	97.00	0.00	99.89
9	PiS1	*P. infestans*	KT363860.1	98.00	0.00	100.00
10	PiS2	*P. infestans*	MH401206.1	98.00	0.00	99.89

Morphological Characteristics

In the current investigation, morphological analysis of ten *Phytophthora* isolates from Brebes revealed major taxonomic traits that are congruent with recognized

diagnostic criteria for *Phytophthora infestans*. All isolates had non-septate, hyaline hyphae averaging 6.40 µm in diameter and produced sporangia borne singly on branching or unbranched sporangiophores, which is characteristic of the genus (Fig. 4).

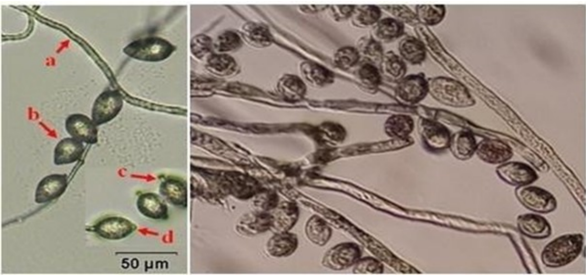

Fig. 4: Micromorphology of *P. infestans* from Brebes, Central Java. (a) A sporulating mycelium producing numerous ovoid to lemon-shaped sporangia, (b) sporangium, (c) pedicel, and (d) papilla (200x magnification). A scale bar of 50 µm indicates that the sporangia are approximately 30–50 µm in length, consistent with many plant-pathogenic oomycetes.

Molecular Characteristics

The amplification of DNA fragments measuring around 900 base pairs (bp) from all 10 isolates (Fig. 5) verifies the primer set's specificity and the purity of the extracted DNA. Sequencing and comparing the amplicons to the National Center for Biotechnology Information (NCBI's) GenBank database yielded identity values ranging from 99.89% to 100,00%, indicating that all ten isolates are conspecific with *Phytophthora infestans* (Table 6). This high degree of sequence similarity reflects a close genetic relationship with reference strains and validates the morphological and pathogenicity-based diagnosis. The phylogenetic analysis based on ITS ribosomal deoxyribonucleic acid (rDNA) sequences confirmed that all 10 isolates obtained from infected potato plants in Brebes clustered monophyletically with *Phytophthora infestans* reference sequences in the GenBank (Fig. 6).

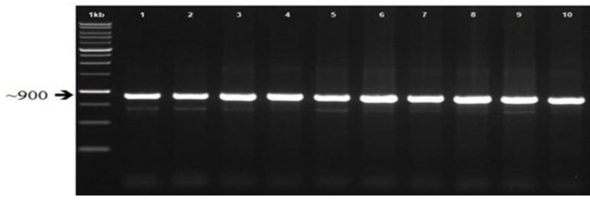

Fig. 5: *Phytophthora* DNA amplification results using ITS6/ITS4 primers. Lanes no. 1-10 indicates the isolate code.

Pathogenicity of Isolates on the Golden Potato Cultivar

All ten *Phytophthora infestans* isolates may cause blight symptoms on detached leaves of the Golden potato cultivar, which reflect the symptoms reported in field samples, confirming their pathogenicity (Fig. 7). The pathogenicity test showed substantial variance in lesion sizes, ranging from 3.90 to 180.90 cm² ($P<0.05$), and infection rates ranging from 0.07 to 0.25 (Table 7). Higher lesion areas indicate more aggressive colonization and faster tissue necrosis, resulting in greater yield losses in the field. The presence of five unique pathogenicity groups among the 10 isolates (Group I > II > III > IV > V) demonstrates the considerable pathogenic variability of *P. infestans* in the Brebes region.

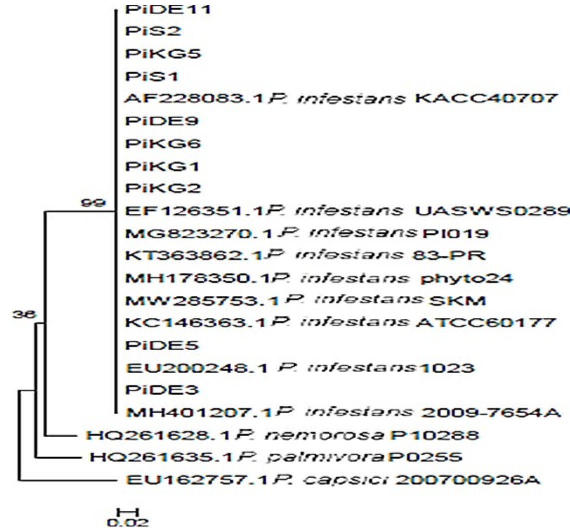

Fig. 6: A phylogenetic tree was constructed based on ITS sequences with the maximum likelihood method using the Hasegawa-Kishino-Yano model (Hasegawa et al., 1985) with a bootstrap of 1,000×.

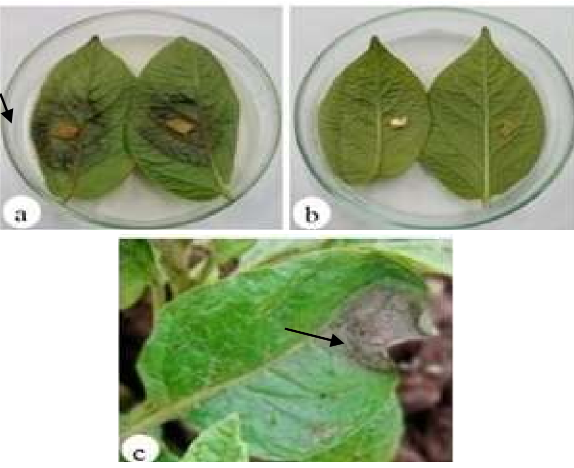

Fig. 7: Representative symptoms of blight on detached potato leaves: (a) inoculated with PiDE11 isolate, (b) control (uninoculated with pathogen), and (c) field symptoms of leaf blight.

Table 7: Pathogenicity of *P. infestans* isolates from Brebes, Central Java, based on the extent of symptoms and infection rate on the Golden potato cultivar

Isolates	Leaf area infection(cm^2)	Infection rate (r)
PiKG1	42.50 ^{cdef}	0.18
PiKG2	3.90 ^{ef}	0.07
PiKG5	158.90 ^{ab}	0.22
PiKG6	82.20 ^{bcd}	0.21
PiDE3	78.20 ^{cde}	0.21
PiDE5	107.60 ^{abc}	0.23
PiDE9	24.50 ^{def}	0.15
PiDE11	180.90 ^{a}	0.25
PiS1	104.30 ^{bc}	0.22
PiS2	102.90 ^{bc}	0.22
Control	0.00 ^{f}	0.00

The DMRT ($P<0.05$) found no significant difference between means in one column followed by the same letter; WAP represents weeks after planting.

DISCUSSION

Late blight, caused by *P. infestans*, is one of the most

economically damaging diseases in potato production in Indonesia. Control of this pathogen has traditionally relied on synthetic fungicides. However, the rise of fungicide-resistant *P. infestans* strains and increasing environmental concerns necessitate a transition toward sustainable disease management strategies (Matson et al., 2015; Nicolopoulou-Stamati et al., 2016). Overuse of fungicides not only accelerates resistance development but also contributes to soil and water contamination, non-target toxicity, and increased production costs (Haverkort et al., 2016; Murtaza et al., 2026).

At an elevation of around 1,400 meters above sea level, the area used for this test had been rotated and planted with carrots and potatoes, respectively, the season before. Pre-research observations revealed that late blight disease (*P. infestans*) severely ravaged the potato field surrounding the testing site; as a result, it may be a perfect source of natural inoculum. Environmental factors that were highly permissive to the development of leaf blight disease throughout the test included excessive rainfall and frequently foggy circumstances, particularly in the third to eighth week following planting. According to the results, late blight disease in potatoes can be effectively managed with both synthetic and biofungicides. Nonetheless, one benefit of biofungicides is their greater environmental friendliness. Additionally, by lowering the need for conventional synthetic fungicides while preserving disease control, both kinds of treatments help to promote more sustainable potato production. The findings suggest that applying synthetic and biofungicides can be a practical way to enhance the health of potato crops, especially when it comes to preventing late blight (Etik, 2025).

Biofungicides based on natural compounds, such as plant essential oils and microbial antagonists, present a promising alternative. Clove oil (*Syzygium aromaticum*), rich in eugenol, has demonstrated strong antifungal activity against a wide spectrum of pathogens, including *Candida* sp, *Phytophthora* spp., and *Colletotrichum* spp., by disrupting cell membrane integrity and inhibiting spore germination (Pavela & Benelli, 2016; Murtiastutik et al., 2023; Kaur et al., 2023). The use of nanoemulsion technology in clove oil delivery improves stability, penetration, and bioavailability under field conditions (Nazzaro et al., 2017). Regarding the nano clove oil treatment, there was a general pattern that both foliar spraying and seed treatment (T5 and T6) resulted in greater levels of disease suppression than that of just foliar spraying (T7 and T8) treatments. This pattern supports broader findings that nanoemulsion essential oils perform best when applied early, allowing systemic interaction with the plant (Kaur et al., 2024; Pandey et al., 2022). Furthermore, the efficiency of clove oil was significantly influenced by concentration, with 5 mL/L providing stronger suppression, consistent with dose-dependent activity reported in nano-essential oil formulations (Pavela & Benelli, 2016).

Yeasts have also gained traction as biocontrol agents due to their ability to colonize plant surfaces and compete for nutrients and space, produce inhibitory volatile organic compounds (VOCs), and induce host resistance mechanisms (Sipiczki, 2006; Robiglio et al., 2011). Several antagonistic yeasts release VOCs—such as alcohols, aldehydes, esters, and organic acids—that inhibit pathogen growth even without direct physical contact. VOCs disrupt membrane integrity, inhibit mitochondrial respiration, or interfere with signaling pathways required for sporangial germination or zoospore motility. Species such as *Metschnikowia*, *Candida*, and *Saccharomyces* are known to emit VOCs like ethyl acetate, 2-phenylethanol, and acetaldehyde, which exert fungistatic or fungicidal activity (Liu et al., 2013). Components of yeast cell walls—such as β-glucans, mannoproteins, and chitin-like molecules—are recognized by plant pattern-recognition receptors (PRRs), triggering defense signaling networks. These include increased production of reactive oxygen species (ROS), activation of phenylpropanoid pathways, upregulation of defense-related genes (e.g., PR-1, PAL), and enhanced accumulation of phenolics and phytoalexins. The result is stronger basal resistance to *P. infestans* infection and slower lesion expansion (Ferraz et al., 2016). Their ease of cultivation and compatibility with other biocontrol agents make them suitable for field-scale application. The results of this study suggest that dual-application methods (seed + foliar treatment) of biofungicides were more effective than foliar application alone. This highlights the importance of early colonization and systemic protection. Furthermore, higher concentrations of clove oil (5 mL/L) consistently resulted in stronger suppression of disease symptoms than lower concentrations (3 mL/L), in line with previous findings on dose-dependent activity of essential oils (Murtiastutik et al., 2022).

The T1 treatment was the most successful in lowering infection levels. The T8 treatment, on the other hand, had the lowest disease rate among plants treated with nano-clove oil, suggesting that this particular application technique or concentration was the most successful in suppressing disease in the cv. Median. The T6 treatment, however, had the lowest disease incidence rate for the cv. Granola-nano clove oil treatment, suggesting that this specific therapy was the most effective at blight management. The two kinds of varying ideal treatment efficacy raise the possibility that a variety of factors, including plant physiology, genetic resistance, or interactions with applied biofungicides, could affect the results of disease suppression. The effectiveness of biofungicide was further supported by statistical analysis, which revealed that the late blight disease infection rate in some treatments employing yeast and nano-clove oil did not differ significantly from those using synthetic fungicides. This observation is especially significant since it shows that the amount of disease control offered by biofungicide formulations (yeast and nano-clove oil) was on par with that of synthetic fungicides.

Comparable AUDPC values between Y6 and the synthetic fungicide (T9) are consistent with recent regional and global reports showing that well-formulated biologicals, particularly combinations of microbial antagonists and nano-essential oil formulations, can achieve disease suppression levels approaching those of

chemical fungicides under natural inoculum pressure (Abbasi et al., 2025). Nanoemulsions of essential oils improve stability, foliar retention, and penetration, thereby enhancing in-planta efficacy relative to bulk oils, while microbial antagonists contribute by competition, VOCs, and induced resistance (Troussieux et al., 2022). Nevertheless, as observed elsewhere, heavy rainfall and persistent leaf wetness reduce persistence and rain fastness, explaining the decline in protection after ≈8 WAP and indicating a need for improved slow-release carriers or adjusted application frequency in high-rain environments.

The results of this study indicate that although biofungicide formulations (yeast and clove oil) are efficient in preventing the emergence of leaf blight disease in the early to mid-growth stages of the crop, their protective effects seem to have a short half-life. According to the study, disease suppression persisted for around 8 WAP. The disease persisted even when biofungicides were applied, suggesting that their efficacy gradually decreased. This may be attributed to heavy rainfall, persistent leaf wetness, and frequent fog, which create optimal conditions for *P. infestans* infection and sporulation. Moreover, excessive moisture can degrade or wash off biofungicide residues, especially if they are not designed for prolonged field persistence (Judelson & Blanco, 2005). These findings are consistent with other studies showing that biofungicide effectiveness can be highly variable under fluctuating environmental conditions (Abd-Elsalam & Khokhlov, 2015; Trivedi et al., 2020; Ayaz et al., 2023). The enhanced disease suppression observed in the combined seed and foliar applications can be attributed to the complementary mechanisms of nano-clove oil and yeast antagonists. Nanoemulsified clove oil, rich in eugenol, provides rapid fungitoxic activity by disrupting pathogen cell membranes, inhibiting spore germination, and forming a stable antimicrobial film that improves leaf coverage and penetration relative to bulk oils. In parallel, yeasts establish early and persistent colonization of the phyllosphere, where they compete for nutrients and infection sites, secrete inhibitory VOCs and lytic enzymes, and may trigger plant defense pathways. The structural damage inflicted by nano-clove oil can increase the susceptibility of *P. infestans* to yeast-derived hydrolytic enzymes and VOCs, thereby creating a multilayered inhibitory environment. Moreover, early colonization through seed treatment enhances microbial exclusion before pathogen arrival, while foliar-applied nano-clove oil reduces initial inoculum pressure, resulting in additive or synergistic suppression. Together, these biochemical, ecological, and plant-mediated mechanisms explain the superior performance of dual-application treatments and highlight the potential of oil–microbe combinations as robust alternatives to synthetic fungicides for late blight management (Ferraz et al., 2016; Troussieux et al., 2022).

Climatic conditions during the trial played a substantial role in shaping the performance of the biofungicide treatments. While the plant growth stage contributes to the susceptibility of potato foliage, the environmental conditions present during the study were highly conducive to the rapid development and spread of late blight. The trial site experienced heavy and frequent rainfall, which is known to promote the dissemination of *P. infestans* sporangia and to prolong periods of leaf wetness—both critical requirements for infection. Persistent rainfall also poses a major challenge for biofungicide formulations, as contact-based microbial agents and nano-essential-oil films are prone to wash-off, reducing their residual activity on the leaf surface. In addition, frequent fog events and consistently high humidity likely intensified disease pressure by maintaining saturated leaf surfaces for extended periods. Such conditions not only accelerate pathogen sporulation and lesion expansion but can also decrease the longevity and stability of biological products, either by diluting their active compounds or by accelerating microbial degradation. Collectively, these climatic (temperature and high humidity) stresses created an environment in which *P. infestans* could thrive while simultaneously diminishing the persistence and protective capacity of the biofungicide treatments, thereby limiting their field efficacy over time (Trivedi et al., 2020; Vero et al., 2023).

In light of these difficulties, the study's findings imply that the biofungicide formulations examined in their current application method were unable to suppress disease over the long term in such harsh environmental circumstances. Other approaches to disease control might be required to increase the efficacy of biofungicide treatments. For example, increasing application frequency may contribute to consistent disease protection throughout crop development. Similarly, changing the concentration of biofungicide applications, for example, by adding additional yeast or clove oil, may improve their capacity to inhibit the growth of pathogens. Further improving disease control results may be achieved by combining the use of biofungicides with other cultural practices like choosing crop varieties resistant to disease, maximizing plant spacing for improved air circulation, and modifying planting schedules to avoid periods of high disease-favorable weather (Shaukat, 2025).

The differential efficacy across potato cultivars (e.g., T6 for Granola vs. T8 for Median) also suggests that host resistance traits and physiological responses influence the success of biological control. Potato cultivar resistance to *P. infestans* is a quantitative trait involving multiple loci, and its expression may interact with both biocontrol efficacy and pathogen aggressiveness (Vleeshouwers et al., 2011; Sedláková et al., 2011).

These findings imply that biofungicides may be practical, environmentally friendly substitutes for traditional synthetic fungicides in the management of late blight disease, given the growing concerns regarding chemical residues, environmental sustainability, and pathogen resistance to synthetic fungicides. To fully realize their potential in effectively managing late blight disease, additional improvements in application techniques and disease management strategies are required.

Traditional *Phytophthora* species classification has long been based on morphological traits, particularly the characteristics of their sporangia (spore-producing structures), which are classified as papillate, semi-papillate,

or non-papillate based on the presence and prominence of an apical thickening (Han et al., 2013; Erwin & Ribeiro, 1996). The identified sporangia were diverse, including ovoid, lemonoid, and subspherical forms, which is consistent with previously reported variety in *P. infestans* morphology (Cooke et al., 2000). Semi-papillate apices measuring 1.20-4.20 µm corroborate their designation. *P. infestans* has been shown to generate semi-papillate, readily detached sporangia, allowing for aerial dissemination and rapid spread in humid field settings (Erwin & Ribeiro, 1996; Wang et al., 2025). The sporangia's length (12.30-55.50 µm) and width (11.40-33.80 µm), along with a variable length-to-width ratio (1.00-2.90), may suggest intra-species variation and the impact of environmental circumstances or isolate-specific genetics. Sporangia morphology is important not only for species identification but also for understanding epidemiological behavior. For example, elongated lemonoid sporangia have been linked to more aggressive strains with better dispersal efficiency (Judelson & Blanco, 2005).

This study's observation of pedicels of 1.40-7.50 µm helps identify *P. infestans*, which normally has small but distinct pedicels on sporangiophores. While morphological identification is still an important step, it might be misleading due to overlapping features among *Phytophthora* species or environmental plasticity affecting morphology. Thus, combining morphology with molecular analysis, as done in this study, is strongly suggested for reliable pathogen diagnosis (Cooke et al., 2000; Lévesque & de Cock, 2004). This integrative technique improves species identification reliability and distinguishes *P. infestans* from physically similar species such as *P. mirabilis* or *P. ipomoeae*, which can coexist but have distinct host ranges and virulence profiles. The range in sporangial structure and size in this collection may potentially represent underlying genotypic heterogeneity, a well-documented feature in *P. infestans*, particularly in areas where sexual recombination occurs or multiple clonal lineages coexist (Goodwin, 1997; Wang et al., 2025). Such morphological variety has significant consequences for epidemic development, fungicide response, and resistance breeding, as pathogen populations with higher phenotypic plasticity may adapt more quickly to environmental constraints or host resistance genes.

The morphological characterization performed in this study not only supports the correct identification of *P. infestans* as the causal agent of late blight in Brebes, Central Java, but also emphasizes the importance of continuing to monitor phenotypic traits as part of integrated pathogen surveillance efforts. These findings, together with genetic and pathogenicity assessments, provide a solid platform for managing late blight through informed breeding, cultural practices, and chemical control techniques.

P. infestans is a pathogenic organism that reproduces quickly. This microbe can go through multiple reproductive cycles in a single agricultural season. Under ideal circumstances, the full life cycle is completed in five days. The cycle begins when sporangia or zoospores adhere to plant surfaces, germinate, and penetrate host tissues. *P. infestans* begins as a hemibiotroph, relying on living host cells for survival before shifting to necrotrophic growth on decaying plant materials. Infected foliage and stems develop sporangial structures, each carrying 20-40 motile zoospores that emerge under favorable environmental conditions. These freed zoospores act as the principal inoculum source for subsequent infections (Kiiker et al., 2018; López-Orona et al., 2013). Precipitation promotes the downward dispersal of sporangia into the soil, where they can infect growing tubers. Wind-driven rain events promote aerial distribution, allowing for interplant and cross-field transmission. The practice of sowing diseased seed tubers results in persistent transmission across multiple seasons.

This study validates the isolates' molecular identities and adds to the evidence from morphological and pathogenicity studies. The phylogenetic tree shows excellent bootstrap support, indicating considerable genetic similarity between the Brebes isolates and reference *P. infestans* strains, implying that these isolates represent the same species and may belong to the same or closely related clonal lineages. Identifying *Phytophthora* species simply based on physical features is difficult, especially given the isolates' remarkable phenotypic flexibility under different environmental and culture circumstances.

Although diagnostic morphological features such as sporangia shape, papillation, and colony characteristics provide preliminary information, these traits frequently overlap across species and can vary within a single species depending on growth media, temperature, and isolate age (Erwin & Ribeiro, 1996; Cooke et al., 2000). The current investigation found significant heterogeneity in sporangial shape and size among the ten isolates, highlighting the limitations of morphology-based taxonomy in *Phytophthora*. To address this, molecular identification based on DNA sequence analysis was used. This method is now widely accepted as a viable way to identify and distinguish *Phytophthora* species and strains (Lévesque & de Cock, 2004).

The ITS6/ITS4 primer pair, which is commonly used in oomycete diagnostics, was used to target the internal transcribed spacer (ITS) region of ribosomal DNA because of its conserved flanking regions and variable internal sequences, which provide species-level resolution (White et al., 1990; Robideau et al., 2011). Previous research on *P. infestans* and other *Phytophthora* spp. has thoroughly documented the use of ITS-based molecular identification, providing a solid framework for phylogenetic analysis and species delimitation (Arafa et al., 2018; Lin et al., 2025). Furthermore, molecular approaches enable early and accurate pathogen detection, which is crucial for disease management, particularly when dealing with quickly changing and widely spread pathogens such as *P. infestans* (Judelson & Blanco, 2005).

While ITS is useful for species-level identification, it is important to highlight that intraspecific variation, such as clonal lineages or mating types, cannot be resolved using ITS alone. Additional markers for finer-scale genotyping, such as simple sequence repeats (SSRs), mitochondrial

haplotypes, or effector gene profiles (e.g., AVR genes), are frequently utilized in population genetics investigations. Nonetheless, ITS sequencing remains a useful first step in diagnostic procedures and biodiversity studies. The successful application of ITS-based molecular identification in this study reinforces the necessity of integrated diagnostic approaches, combining morphological, pathogenicity, and molecular data to accurately characterize *P. infestans*. This comprehensive strategy ensures reliable identification and facilitates surveillance, epidemiology, and resistance breeding programs targeting late blight.

Phylogenetic reconstructions, particularly those based on conserved regions such as ITS, are commonly employed for oomycete species identification as well as for effectively resolving evolutionary relationships within the *Phytophthora* genus (Cooke et al., 2000; Lin et al., 2025). In the current study, *Phytophthora* isolates formed a single clade distinct from closely related genera such as *Pythium, Peronospora*, and *Halophytophthor*a, supporting earlier taxonomic revisions that separated these genera based on both molecular and morphological traits (Cooke et al., 2000; Lévesque & de Cock, 2004). The clear separation of *P. infestans* from other *Phytophthora* spp. also demonstrates the specificity of the ITS region in distinguishing species within the Peronosporales order.

The presence of Brebes isolates in a monophyletic cluster implies a common evolutionary origin, but it does not rule out the potential of genotypic variation within the population. Previous research has demonstrated that significant genetic and phenotypic heterogeneity can occur within clonal lineages of *P. infestans* due to mitotic recombination, genome plasticity, and high mutation rates (Goodwin, 1997; Haas et al., 2009). *P. infestans*' innate plasticity allows it to change rapidly in response to host resistance genes and fungicide pressure, frequently resulting in localized outbreaks of very aggressive strains (Ivanov et al., 2021). Han et al. (2013) underlined that the composition and pathogenicity features of such dynamic populations can change fast, especially in response to changing environmental circumstances and agricultural practices. This highlights the need to conduct regular genetic surveillance of *P. infestans* populations in endemic areas such as Brebes. The detection of genetic homogeneity in ITS does not preclude broader genomic variation in other regions such as mitochondrial haplotypes, SSR markers, or effector gene profiles, which have been shown to reveal much finer-scale population structure (Rekad et al., 2017).

Given that the *Phytophthora* genus contains over 150 species divided into multiple phylogenetic clades (now known as clades 1-10 and subclades), correctly classifying and placing *P. infestans* within clade 1c is critical for understanding its evolutionary history, ecological niche, and host range (Lin et al., 2025; Yang et al., 2017). Its close relatives, *P. mirabilis* and *P. phaseoli*, have been found on other solanaceous hosts and exhibit comparable epidemiological characteristics, implying that host-driven speciation may play a role in clade divergence.

The molecular phylogeny in this study supports the taxonomic identity of *P. infestans* in Brebes and emphasizes the importance of integrated genetic surveillance to detect potential shifts in the pathogen's population dynamics. Incorporating multilocus genotyping or whole-genome sequencing techniques into future studies would provide more information on the evolutionary factors generating *P. infestans* diversity and improve late blight management strategies. The pathogenicity test findings show a wide range of pathogenicity among the isolates, which is a common feature of *P. infestans* populations, especially in areas with different environmental circumstances and many potato cropping cycles. In general, the severity of late blight infection is proportional to both lesion size and infection rate, which represent the pathogen's aggressiveness (Cohen, 2024). This heterogeneity is especially concerning since different pathogenicity patterns hinder efforts to manage the disease with traditional fungicides and resistance breeding. Variation in *P. infestans* pathogenicity is one of the most difficult obstacles in breeding for long-term resistance, because a cultivar that is immune to one isolate may be vulnerable to another. Furthermore, this diversity is most likely due to the pathogen's ability to reproduce both sexually and asexually, allowing it to rapidly generate novel genotypes through mutation, mitotic recombination, or sexual recombination when both mating types are present (Knapova et al., 2002; Grünwald et al., 2011).

The prevalence of varied pathogenicity levels among isolates implies local evolutionary forces such as host genotype selection and fungicide use, which can lead to the establishment of more aggressive strains (Yuen & Andersson, 2013). Other potato-growing regions have reported high pathogenic diversity in *P. infestans* populations, including India (Guha Roy et al., 2021), Algeria (Rekad et al., 2017), Mexico (López-Orona et al., 2013), and Guatemala (Izarra et al., 2025), implying that the Brebes phenomenon is consistent with global trends. Understanding pathogenicity diversity is critical not only for developing effective disease control strategies but also for guiding host resistance screening in potato breeding projects. The results reported in this study provide a solid foundation for developing integrated disease management (IDM) strategies that are suited to the local pathotype composition. According to Rietman et al. (2012), matching host resistance genes (R genes) to local pathogen races is crucial for long-term resistance.

Furthermore, the classification of isolates into pathogenicity groups may serve as a foundation for the creation of differential host cultivars used to track *P. infestans* race composition over time. This would enable early detection of alterations in population pathogenicity, allowing breeding and management strategies to be updated on a timely basis.

This is the first full morphological, genetic, and pathogenicity characterization of *Phytophthora infestans* isolates from Pandansari village, Brebes, Central Java—a key potato-producing area in Indonesia that has never been well examined. The identification of ten *P. infestans* isolates distributed across five distinct pathogenicity

groups highlights a substantial degree of intra-population variability in aggressiveness, infection efficiency, and morphological traits. Such heterogeneity is a well-documented characteristic of *P. infestans* populations and reflects the pathogen's exceptional evolutionary flexibility. Differences in lesion expansion rate, latent period, sporulation capacity, and host tissue colonization among groups indicate that the isolates do not behave uniformly, even when collected from the same geographic region.

Several biological processes likely underlie this variation. *P. infestans* is known for its genomic plasticity, driven by high mutation rates, mitotic recombination, and the presence of rapidly evolving effector genes that modulate host–pathogen interactions. These processes generate diverse pathotypes capable of overcoming different levels of host resistance. Environmental selection pressures, such as fungicide exposure, variation in host cultivar genetics, and fluctuating climatic conditions, can further shape the composition of pathogenicity groups by favoring more aggressive or better-adapted isolates. Morphological differences, such as variation in sporangial size, shape, and degree of papillation, reinforce the presence of phenotypic diversity within the population. These traits often correlate with epidemiological fitness; for instance, isolates with larger or more efficiently detached sporangia may disperse more readily under humid or rainy conditions. Similarly, isolates with shorter pedicels or higher sporulation rates may have enhanced capacity for generating secondary infections during favorable weather. The presence of multiple pathogenicity groups within a single production area has important implications for disease management. It suggests that the local pathogen population is dynamic, capable of producing outbreaks of varying severity depending on which pathotypes dominate in a given season (Andrivon, 1994). This variability also complicates breeding programs, as resistance effective against one pathogenicity group may be insufficient against others. For integrated disease management, understanding the distribution and behavior of these groups becomes critical for selecting cultivars, optimizing fungicide regimes, and designing targeted biocontrol strategies. The integration of pathogenicity profiling and molecular identification provides fresh baseline data for Brebes, which is critical for developing region-specific late blight management methods and potato breeding programs focused on long-term resistance. In conclusion, the identification of discrete pathogenicity groups and a wide range of infection capacities among *P. infestans* isolates in Brebes emphasizes the critical necessity for ongoing pathogen surveillance, molecular typing, and pathogenic tracking. Such data are critical for effective resistance breeding, fungicide management, and the development of sustainable potato production systems in Indonesia and elsewhere.

Conclusion

Field trials demonstrated that a biofungicide formulation combining yeast isolates (Y6 and Y8) with nanoformulated clove oil effectively suppressed late blight disease, particularly when applied through an integrated seed treatment and foliar spraying approach. This combined method provided more consistent, systemic, and longer-lasting protection compared to foliar applications alone. Weekly applications maintained effective disease control for up to eight weeks after planting, although efficacy gradually declined thereafter, likely due to environmental degradation and reduced persistence of the bioactive components. The study also confirmed *Phytophthora infestans* as the causal agent of late blight in potato crops in Pandansari Village, Brebes, Central Java, based on morphological and molecular analyses of ten isolates. Substantial variability in sporangial morphology and pathogenicity among the isolates reflects the adaptive and diverse nature of local *P. infestans* populations. The grouping of isolates into five distinct pathogenicity categories provides valuable baseline data for future resistance breeding, epidemiological surveillance, and regionally tailored disease management programs. Overall, the findings highlight the promise of yeast–nano-clove oil biofungicides as sustainable and environmentally friendly alternatives to conventional chemical fungicides. Such biobased solutions offer multiple advantages, including reduced chemical residues, lower risk of resistance development, improved ecological safety, and compatibility with integrated pest and disease management (IPDM) systems. However, realizing their full potential will require further refinement of formulation stability, optimization of application intervals, and assessment under varying agroecological conditions and seasons. Future research should focus on (i) improving nanoformulation delivery systems to enhance field persistence, (ii) exploring synergistic combinations with other biological agents or plant extracts, (iii) integrating biofungicides with host resistance and cultural practices such as canopy management and disease forecasting tools, and (iv) evaluating long-term impacts on soil microbiota, non-target organisms, and overall ecosystem health. Strengthening these research directions will help advance the development of resilient, climate-adapted, and environmentally sustainable late blight management strategies for potato farming in Indonesia and other tropical production systems.

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Author's Contribution: W, AA, TH, RR, RN conceived and designed the experiment, and performed the study. W, DNS, YS conducted laboratory analyses writing the original draft; supervised and coordinated the experiments methodology, and investigation; W, YS, DNS, ED prepared the draft of the manuscript and edited final preparation of the manuscript; W, YS performed statistical analyses of experimental data, and validation, IMS, W contributed to investigation and resources; H, NG, EK, contributed to resources and manuscript preparation. All authors critically revised the manuscript and approved the final version.

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