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Insecticidal activity of isolated *Purpureocillium lilacinum* PL1 against whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) on cassava plantations in southern Viet Nam

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Abstract

Background Whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) is a plant-damaging insect in tropical and subtropical regions that causes agricultural damage worldwide, including in Viet Nam. The abuse of pesticides derived from chemicals has resulted in the evolution of insect-resistant strains, polluting the environment and threatening human health. Using entomopathogenic fungi (EPF) for biological control is an alternative strategy in integrated pest management. Hence, an attempt was conducted to isolate, characterize and evaluate the efficacy of EPF, *Purpureocillium lilacinum* against whitefly *B. tabaci* under laboratory and field conditions.

Results *Purpureocillium lilacinum* PL1 (PL1) was isolated from the whitefly *B. tabaci* cadavers and subsequently identified using morphological study and internal transcribed spacer sequencing. *Purpureocillium lilacinum* PL1 had effectively grown and sporulated at temperatures ranging from 25 to 35 °C and throughout a broad pH range, which is particularly advantageous against the harsh tropical monsoon climate. Bioassay study indicated that 1×10^7 conidia/ml of *P. lilacinum* PL1 had a high lethality against the whitefly *B. tabaci* nymphs in vitro with efficiency was 88.24% after 7 days of treatment. The median lethal concentration (LC_{50}) of *P. lilacinum* PL1 to *B. tabaci* after 7 days of treatment was 1.24×10^5 conidia/ml. In field conditions, 1×10^7 conidia/ml of *P. lilacinum* PL1 lowered the population of *B. tabaci* nymphs with efficacy was 78.86% after 2 batches, 7 days after treatments.

Conclusion The findings indicated that *P. lilacinum* PL1 was effective in the biological control of *B. tabaci* nymphs, which could be a potential alternative to chemical pesticides for pest management.

Keywords *Purpureocillium lilacinum*, *Bemisia tabaci*, Entomopathogenic fungi, Chitinase, Protease

Background

Cassava (*Manihot esculenta* Crantz, Euphorbiaceae) is among the most important food crops in the world. It is cultivated on approximately 544,300 hectares in Viet Nam, with an annual production of 9.74 million tons (Kim et al. 2015). Cassava mosaic disease (CMD), which is caused by the begomovirus poses the greatest threat to global cassava productivity (Tokunaga et al. 2018). Cassava mosaic begomoviruses (CMB) are naturally

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transmitted by the whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), the primary vector of begomoviruses, which has a relatively high reproductive capacity and rapid dispersal ability (Islam et al. 2018). CMD was originally found in Southeast Asia in 2015 in Ratanakiri, Cambodia, with the causative agent later identified as the Sri Lankan cassava mosaic virus (SLCMV) (Wang et al. 2016). SLCMV was recorded in Tay Ninh province, Viet Nam, in 2017 and represents a new danger to cassava farming in Viet Nam, reducing its tuber yield and starch content by 16–33%, respectively (Uke et al. 2018).

Bemisia tabaci is a polyphagous insect pest that has caused widespread damage to agricultural productivity around the world (Cruz-Estrada et al. 2013). Adults and nymphs alike feed on the sap, and their bile (sugar excreta) promotes the growth of “soot mold” on the leaves and fruits, reducing crop productivity (Gangwar et al. 2018). *Bemisia tabaci* might play an essential role in the transmission of over 350 plant virus species, which can severely damage crops’ productivity (Götz et al. 2016).

Insecticides derived from synthetic chemicals such as neonicotinoids and insect growth regulators have played a major role in eradicating *B. tabaci* over the past 2 decades (Khalid et al. 2021). Rising applications of chemical pesticides have raised a variety of concerns about human health and environmental damage (Pathak et al. 2022). In recent years, there has been a surge of interest in using biological control agents, such as entomopathogenic fungi (EPF), as an alternative strategy to chemical insecticides (Skinner et al. 2014). EPFs are biological control agents used to eliminate sap-sucking pests, and pests with chewing mouthparts. Using EPFs have many benefits beyond its effectiveness, such as reduced pesticide residues, maintenance of other natural enemies, and increase biodiversity in human-managed habitats (Lacey et al. 2001).

Purpureocillium spp. (Hypocreales: Ophiocordycipitaceae), which was previously classified as *Paecilomyces*, is well-known for its effectiveness as an EPF in the management of arthropods and nematodes (Goffré et al. 2015). *Purpureocillium lilacinum* is a well-known species that has been employed to control aphids, thrips, whiteflies, fruit flies, beetles, mosquitoes, and plant parasitic nematodes (Amala et al. 2013; Goffré et al. 2015). In the previous study, *P. lilacinum* was shown to be highly lethal to *B. tabaci* in vitro with efficacy up to 98% (Desoky et al. 2022). However, different EPFs have been shown to have varying effectiveness levels when administered in tropical and subtropical areas (Indriyanti et al. 2017). EPFs can be adapted to their environmental conditions depending on various geographical considerations (Indriyanti et al. 2017). Therefore, isolation of local EPFs, which have long

been adapted to geographical and environmental conditions, is a useful strategy to exploit the potential for biological management of the whitefly *B. tabaci*. This study aimed to isolate an EPF strain and evaluate its efficacy in managing the whitefly, *B. tabaci* in cassava plantations in southern Vietnam, contributing to the development of an integrated pest management (IPM) approach for *B. tabaci*.

Methods

Isolation of entomopathogenic fungi from *B. tabaci* cadavers

The whitefly *B. tabaci* was obtained from a cassava field in Tan Chau, Tay Ninh province, Viet Nam. The whitefly population was maintained on cassava leaves in laboratory. EPFs were isolated following the protocol provided by (Sharma et al. 2018). Briefly, whitefly cadavers were obtained individually in sterile tubes and examined under a stereomicroscope (40×) to identify potential EPF based on the presence of hyphae surrounding the cuticle. The dead *B. tabaci* suspected of being infected with EPFs was surface sterilized using 70% ethanol for 1 min, followed by sterilized water twice for 1 min, dried on filter paper then transferred to potato dextrose agar (PDA) (Merck, Germany), supplemented with 0.1% chloramphenicol to prevent bacteria growth and incubated at 28 ± 1 °C, 50–60% RH for 14 days. The fungal colonies formed were then isolated by subculturing on PDA medium with the addition of 0.1% chloramphenicol and cultured at 28 °C (Sharma et al. 2018). After 3–7 days of cultivation, the microscopic characteristics of the isolated fungus strain were observed. The *B. tabaci* was then re-infected with fungal isolate to further investigation the causal relationship between the fungal isolate and the death of *B. tabaci* according to Koch’s postulate (Grimes 2006).

Morphological identification of fungal isolate

The 5 mm mycelia disk from the second isolation purification was inoculated on PDA monitored and recorded daily. After 7 days of cultivation, the color of colonies, and the presence of medium and incubated at 28 °C, 50–60% RH for 7 days. The radial expansion of the colonies and conidia were studied. Additionally, the microscopic of the fungal isolate was investigated. Briefly, the mycelia were collected by a sterile needle and placed in a drop of lactophenol cotton blue. The texture of hyphae, phialides, and conidial shape was observed at 1000× magnification using an Eclipse E100 microscope (Nikon Corp., Tokyo, Japan). The identification of morphological characteristics was based on the description provided by (Watanabe 2010) and (Humber 2012).

Molecular identification of fungal isolate

Molecular identification of fungal isolate used the internal transcribed spacer (ITS) region universal ITS primers: ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Tangthirasunun et al. 2010). The PCR reaction was performed on a T100 thermal cycler (Bio-Rad, Irvine, CA, USA) at 95 °C for 5 min (pre-denaturation), 35 cycles of 95 °C for 1 min (denaturation), 55 °C for 1 min (annealing), and 72 °C for 1 min (extension), followed by 72 °C for 10 min (final extension). The PCR products were observed on a 0.8% agarose gel electrophoresis, using GelRed Loading Buffer (TBR, Ho Chi Minh City, Viet Nam), then purified and sequenced by Nam Khoa Biotek (Ho Chi Minh city, Viet Nam). The Sanger sequencing findings were edited with Bioedit and then aligned with published full-length sequences in the NCBI Basic Local Alignment Search Tool (BLAST) databases to collect fungal species references. Phylogenetic analysis based on ITS-5.8S rDNA sequence was performed with Clustal-W function and neighbor-joining method using MEGA 6.0.

Evaluation of chitinase activity of *P. lilacinum* PL1

Colloidal chitin was prepared using Hsu and Lockwood method (Hsu et al. 1975). About 5 g of chitin from shrimp shells (Sigma-Aldrich, Darmstadt, Germany) was mixed with 30 ml of HCl acid (35.5%) and incubated overnight at 4 °C. The colloidal chitin was precipitated by slowly adding 250 ml of chilled ethanol (50%), with constant stirring at 4 °C and left for overnight. The colloidal chitin was centrifuged at 10,000 g for 20 min, and sterile distilled water was used to wash the pellets until the pH value was neutral. The chitin medium contained 1% colloidal chitin, NaNO₃ 3.5 g/l, K₂HPO₄ 1.5 g/l, MgSO₄·7H₂O, 0.5 g/l, KCl 0.5 g/l, and FeSO₄·7H₂O 0.01 g/l, and agar 20 g/l. The mycelia disk (5 mm) of *P. lilacinum* PL1 were cultured on 1% colloidal chitin agar and incubated at 28 °C, 50–60% RH. After 1–4 days of cultivation, chitin degradation ability was monitored by flooding *P. lilacinum* PL1 colonies in 1.5% Lugol solution and measuring the diameter of the halo around the colonies.

Evaluation of protease activity of *P. lilacinum* PL1

The casein medium contained casein 10 g/l, glucose 1 g/l, yeast extract 1 g/l, K₂HPO₄ 1 g/l, KH₂PO₄ 0.5 g/l, MgSO₄·7H₂O, and agar 20 g/l. The mycelia disk (5 mm) of *P. lilacinum* PL1 was cultured on casein medium agar and incubated at 28 °C, 50–60% RH for 1–4 days. The diameter of the bright halo surrounding the colonies was monitored as a characteristic of the biodegrade casein activity (Zhang et al. 2021).

Effect of temperature on the growth of *P. lilacinum* PL1

Suitability of temperature was investigated for the growth of *P. lilacinum* PL1 mycelia. The mycelia disk (5 mm) of *P. lilacinum* PL1 was cultured on PDA medium then incubated at different temperatures, viz. 25, 30, 35, and 40 °C, 50–60% RH for 14 days. The growth of *P. lilacinum* PL1 mycelia was investigated by measuring the diameter of the colony.

Effect of pH on the growth of *P. lilacinum* PL1

PDA culture medium was adjusted a pH 5, 6, 7 and 8 levels for this study, by using NaOH (0.1N) and HCl (0.1N) solution. The mycelia disk (5 mm) of *P. lilacinum* PL1 was cultured on PDA medium with a range of pH value from 5 to 8, then incubated at 28 °C, and subsequently monitored the colony diameter after 14 days of cultivation.

Mass production of *P. lilacinum* PL1 using solid state fermentation

Solid-state fermentation was applied for mass multiplication of *P. lilacinum* PL1, using broken rice as substrate (Dawar et al. 2003). Briefly, 1 kg of broken rice was spread on a tray with a size of 40 cm by 40 cm, supplement with 500 ml water, then sterilized by autoclaving. *Purpureocillium lilacinum* PL1 conidia was then harvested from PDA culture medium, resuspended at a density of 1×10^6 conidia/g with the sterilized substrate, then incubated at 28 °C for 14 days. After 14 days of cultivation, the *P. lilacinum* PL1 conidia density in broken rice substrate were determined using serial dilutions on PDA plates or counting by hemocytometer (Hirschmann, MO, USA).

Propagation of *B. tabaci* nymphs in the greenhouse

Greenhouse whitefly propagation was carried out according to the protocol described by (Mascarin et al. 2013). Briefly, a population of whitefly *B. tabaci* that had colonized cassava plantations was collected and reared in a greenhouse. Whitefly-free plants were planted in close proximity to cassava seedlings afflicted with adult whiteflies for 72 h to facilitate horizontal transmission. This method might produce approximately 30–50 eggs/leaf (Mascarin et al. 2013). The adult whiteflies were then removed and the plants carrying eggs were transported to a different glasshouse at 26–30 °C, 50–60% RH for 12–13 days, until the nymphs molted to the second instar. The second instar of *B. tabaci* nymphs were confirmed according to the pictorial guide (Naranjo et al. 2017).

Laboratory evaluation of the lethality of *P. lilacinum* PL1 on *B. tabaci* nymphs

Leaves contained second-instar *B. tabaci* nymphs were collected and placed with abaxial surface up in a 150-mm diameter Petri plate. The number of nymphs was adjusted to 50 individual/plate using a needle. *Purpureocillium lilacinum* PL1 conidia were harvested in broken rice substrate after 14 days of cultivation with a sterile spatula and suspended in sterile distilled water with Tween 80 (0.02% v/v), then and the conidia concentration was determined using a hemocytometer (Hirschmann, MO, USA). For the pathogenic assay, *P. lilacinum* PL1 conidia suspension was adjusted by a series of aqueous dilutions (1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , and 1×10^9 conidia/ml). Bioassays were conducted by treating *B. tabaci* nymphs with 5 ml of *P. lilacinum* PL1 conidia solutions. *B. tabaci* nymphs were treated with sterile distilled water with Tween 80 (0.02% v/v), were assigned as the control group. The

cassava plants. Before treatment, *B. tabaci* nymphs' population was investigated on leaves from the top, middle, and bottom of each plant/plot. Insecticides were applied as foliar applications with 3 treatments: *P. lilacinum* PL1 at a concentration of 1×10^7 conidia/ml; fenobucarb (Nicotex Joint Stock, Binh Duong, Viet Nam) at a concentration of 2 mg/ml (as recommended by the manufacturer) as positive controls; and untreated plots were sprayed with water as negative control. *Purpureocillium lilacinum* PL1 or fenobucarb were applied as foliar sprays in the afternoon after 4 pm, with two sprays were performed at 14-day intervals. The spray volumes were subject to 300 l/hectares, according to the guideline of Food and Agriculture Organization of the United Nations (Lavers 2001). *Bemisia tabaci* nymphs were counted on leaves from the top, middle, and bottom of each plant/plot after 7 and 14 days after treatment. The efficacy of each treatment in the management of *B. tabaci* nymphs' population was calculated using the Henderson–Tilton methodology (Henderson et al. 1955).

$$\text{Efficiency (\%)} = \left(1 - \frac{n \text{ in } T \text{ after treatment} \times n \text{ in Co before treatment}}{n \text{ in } T \text{ before treatment} \times n \text{ in Co after treatment}} \right) \times 100$$

leaves were then air-dried and transferred to another 150-mm diameter Petri plate containing a layer of 1.5% agarose gel, then incubate at 28 °C, 50–60% RH (Shah et al. 2020). Nymphs' mortality rate was recorded daily for 9 days, starting on day 3rd after inoculation. Data on nymphs mortality were converted to the percentages of nymphs mortality at each time interval and dilution according to Abbott's formula (Abbott 1925) as follows:

$$\text{Effectiveness (\%)} = [(m - n)/(100 - n)] \times 100$$

where *m* and *n* are for the percentages of dead nymphs in the treatment group and untreated group, respectively.

Evaluation of the lethality of *P. lilacinum* PL1 on *B. tabaci* nymphs under field conditions

Field trials were performed in 3 different cassava plantations in Tan Chau, Tay Ninh, Viet Nam. This location was chosen due to the abundance of the whitefly *B. tabaci*, and cassava plants showing symptoms of CMD. This study utilized the Randomized complete blocks design (RCBD) with 3 replicates for each treatment. The plot size was 10 m × 5 m × 1 m spacing, and each plot allocated 50

where *n* insect population; *T* treated; *Co*: control.

Results were calculated using the mean of triplicate readings.

Statistical analysis

The experiment was conducted using a completely randomized design (CRD) with 3 replicates for each treatment. Results were calculated using the mean of triplicate readings and presented as the mean ± standard error of the mean data were analyzed using SAS 9.4 software (SAS, Inc., Cary, NC, USA). Statistical significance was assessed between groups, using F-test and Duncan's test. *p* < 0.05 was considered to indicate statistical significance. *LC*₅₀ and *LC*₉₀, as well as *LT*₅₀ and *LT*₉₀, were calculated with Probit analysis, using SAS 9.4 software (SAS, Inc., Cary, NC, USA).

Results

Isolation and characterization of isolate fungi from infected-whitefly *B. tabaci*

The cadavers of naturally infected whitefly *B. tabaci* with mycelia on the cuticle were collected for isolation of the

(See figure on next page.)

Fig. 1 Characterization of *Purpureocillium lilacinum* PL1 isolated from *Bemisia tabaci*. **A** Dead *B. tabaci* with the presence of mycelia on the cuticle. **B** *Bemisia tabaci* cadavers under stereomicroscope at 40× magnification. **C** Macroscopic morphology of *P. lilacinum* PL1 isolates in PDA culture medium. **D** Microscopic morphology of *P. lilacinum* PL1 isolates under microscope at 1000× magnification. **E** Macroscopic morphology of *P. lilacinum* PL1 re-isolated from *B. tabaci* using Koch postulates **F** Microscopic morphology of *P. lilacinum* PL1 re-isolated from *B. tabaci* using Koch postulates. **G** Phylogenetic tree based on the ITS gene sequences of *P. lilacinum* PL1 in this study and other known *P. lilacinum* strains in the GenBank database. The *P. lilacinum* PL1 in this study are marked in bold

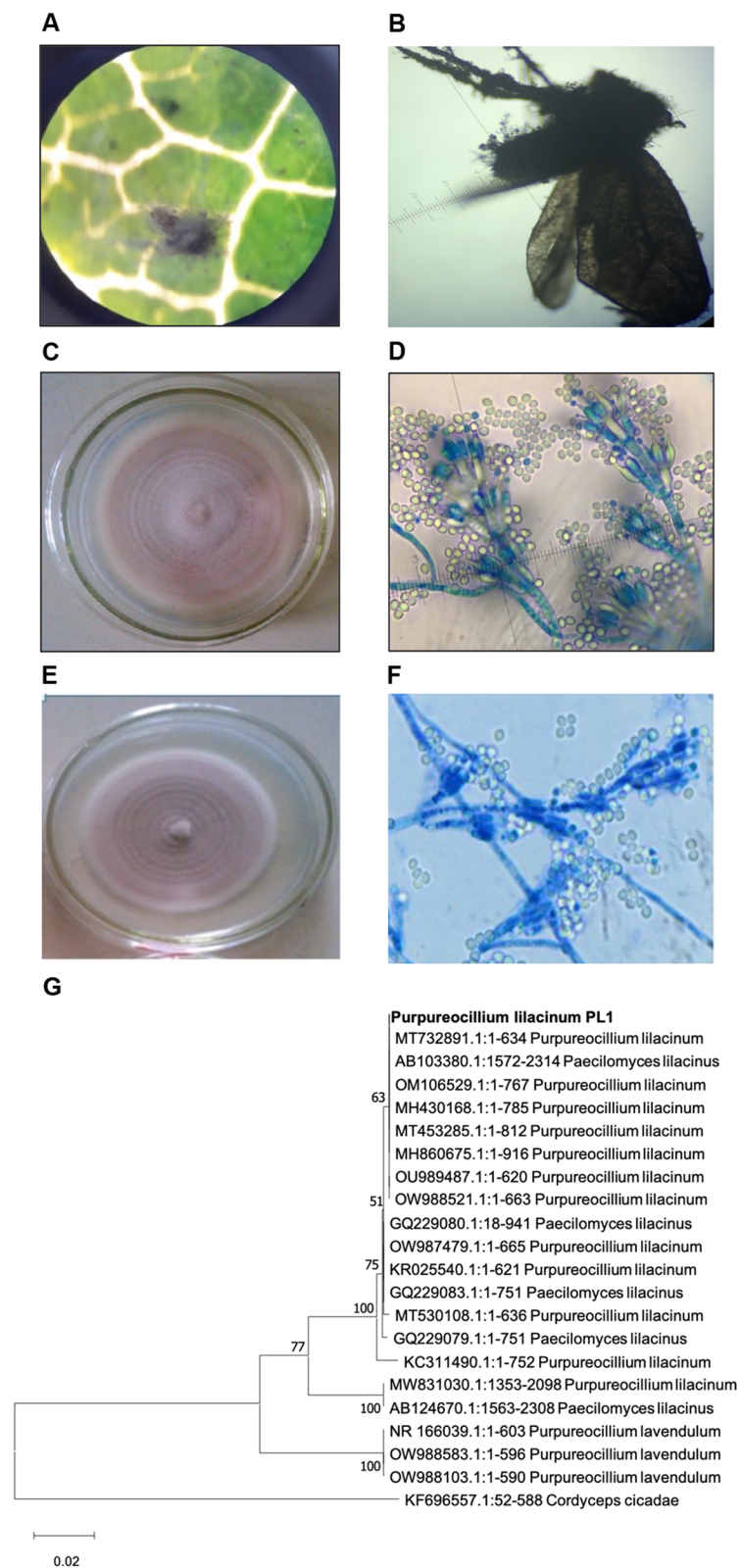


Fig. 1 (See legend on previous page.)

EPF (Fig. 1A, B). The macroscopic portion of the isolated fungus strain had a purple appearance (Fig. 1C). Under a microscope at 1000× magnification, the conidia were unicellular, ovoid, and formed chains (Fig. 1D). Phialides are distinguished by a long, tapering neck and a bulbous base (Fig. 1D). Koch's postulates were applied to indicate the causal relationship between the fungal isolates and the death of *B. tabaci*. The fungus isolated was responsible for the death of healthy whitefly *B. tabaci* and exhibited macroscopic and microscopic similarities with the isolated fungus strain (Fig. 1E, F). These data indicated that the fungal isolate was the cause of death in whiteflies *B. tabaci*, potentially serving as an EPF.

Molecular identification of isolated fungi

The rDNA region PCR product was further amplified and then purified to evaluate the nucleotide sequence of the fungus isolate. A phylogenetic analysis was performed using *Purpureocillium* reference fungal species from GenBank to determine the taxonomic identity of isolates. BLAST analysis revealed that the isolated fungus strain was 100% homologous to *P. lilacinum*. The phylogenetic tree in (Fig. 1G) was developed using MEGA 4.1. The phylogenetic tree based on ITS rDNA indicated that the isolated fungus strain and *P. lilacinum* belong to the same branch, while other *Paecilomyces* species indicated a greater gap among them (Fig. 1G). Thus, the fungus isolate strain was identified as *P. lilacinum* and referred as *P. lilacinum* PL1.

Chitinase and protease activity

The biosynthesis of extracellular chitinase or protease in *P. lilacinum* PL1 was also evaluated using an agar plate assay for the degradation of chitin or casein. The results in (Fig. 2) demonstrated that *P. lilacinum* PL1 might synthesize chitinase and protease. The chitin degrading halo zone diameter was 13.3 mm on day 1st and increased to 28.70 mm after 4 days of cultivation (Fig. 2A, B). Furthermore, the protein degrading ability, as indicated by the casein degrading halo zone, gradually increased from 13.30 to 30.00 mm after 4 days of cultivation (Fig. 2A, C). Thus, *P. lilacinum* PL1 might synthesize extracellular protease and chitinase, satisfying the candidate criteria of an EPF.

Effect of pH and temperature on the growth of *P. lilacinum* PL1

The effect of pH and temperature on the growth of *P. lilacinum* PL1 was further investigated. *Purpureocillium lilacinum* PL1 was cultivated in PDA culture medium with a pH range of 5.0, 6.0, 7.0, and 8.0. The mean of colony diameters was measured with the whole culture after 14 days of cultivation. The results presented in (Fig. 3A,

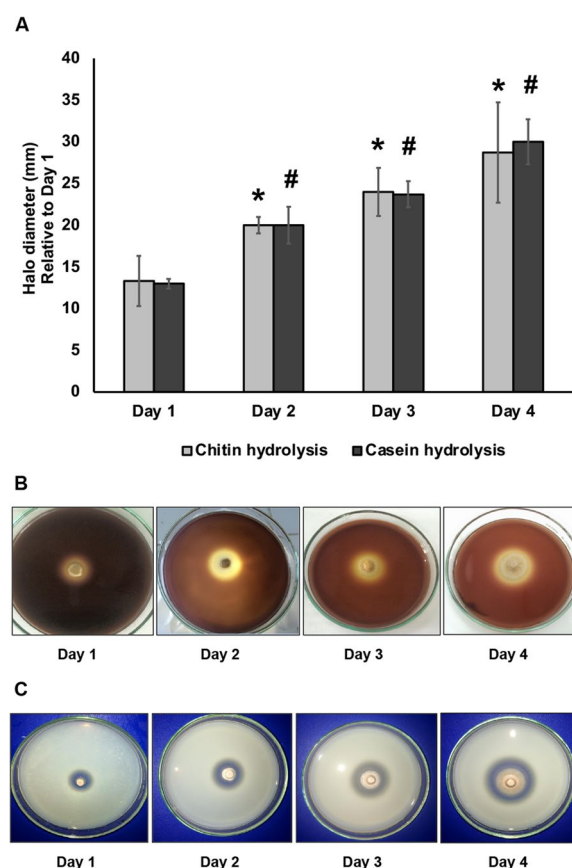


Fig. 2 Chitinase and protease activity of *Purpureocillium lilacinum* PL1 on agar plate. **A** Halo diameter of chitin (black) and casein (gray) degradation of *P. lilacinum* PL1 at different time intervals. **B** Representative images of the chitin degradation ability of *P. lilacinum* PL1 on agar plate. **C** Representative images of the casein degradation ability of *P. lilacinum* PL1 on agar plate. * or # Indicates statistically significant differences relative to the day 1 group

B) indicated that there was non-significant difference between the pH values on the growth of *P. lilacinum* PL1 mycelia. Furthermore, the effect of temperature on the growth of *P. lilacinum* PL1 mycelia was also screened. *P. lilacinum* PL1 mycelia had effectively grown, and there was non-significant difference in the diameter of colonies between 25 and 35 °C (Fig. 3C, D). However, the growth of *P. lilacinum* PL1 mycelia was attenuated by 25% at 40 °C (Fig. 3C, D).

Laboratory evaluation of the lethality of *P. lilacinum* PL1 on *B. tabaci* nymphs

The effect of *P. lilacinum* PL1 on the mortality of *B. tabaci* nymphs was investigated in vitro with a range of concentrations from 1×10^4 to 1×10^9 conidia/ml for 9 days. The results in (Table 1) demonstrated that *P. lilacinum* PL1 at a concentration of 1×10^7 conidia/ml decreased the *B. tabaci* nymphs' population in vitro after

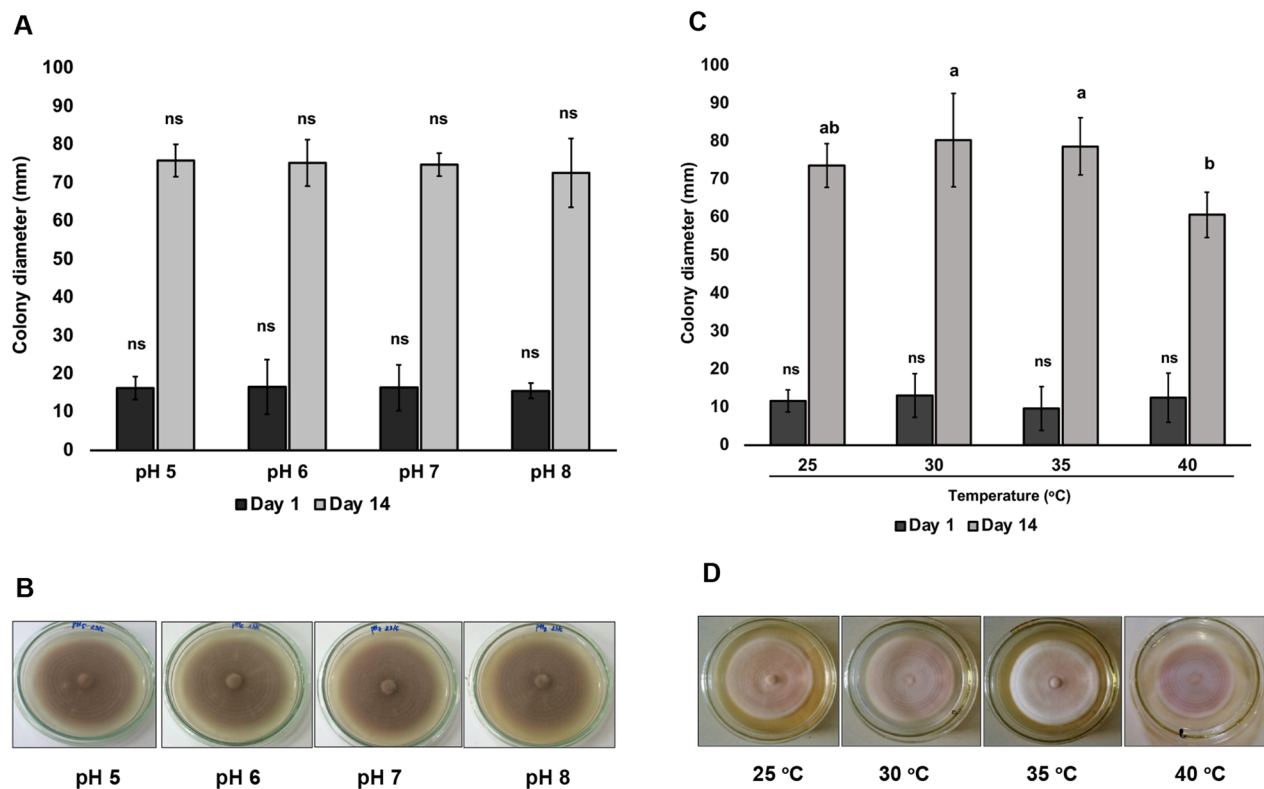


Fig. 3 Effect of pH (A) and temperature (B) on the growth of *Purpureocillium lilacinum* PL1 mycelial indicated by radial growth after 4 days of cultivation. C Representative images of *P. lilacinum* PL1 mycelial on PDA culture medium at different pH value after 14 days of cultivation. D Representative images of *P. lilacinum* PL1 mycelial on PDA culture medium at different temperature after 14 days of cultivation. Different superscript lowercase letters (a–c) within a line and capital letters (A–D) within a column indicate statistically significant differences between groups ($p < 0.05$)

Table 1 Efficacy of *Purpureocillium lilacinum* PL1 at different concentrations on the mortality of *Bemisia tabaci* nymphs in vitro at different time intervals

% Mortality of <i>Bemisia tabaci</i> nymphs						
Days after treatments	<i>Purpureocillium lilacinum</i> PL1 concentration (conidia/ml)					
	1×10^4	1×10^5	1×10^6	1×10^7	1×10^8	1×10^9
Day 1	0.00 ^{aC}	0.00 ^{aD}	0.00 ^{aC}	0.00 ^{aC}	0.00 ^{aD}	0.00 ^{aD}
Day 3	5.33 ^{cC}	9.33 ^{bcD}	13.33 ^{bc}	12.00 ^{bc}	24.67 ^{aC}	28.67 ^{aC}
Day 5	29.30 ^{bB}	27.18 ^{bC}	44.06 ^{bB}	68.82 ^{aB}	79.79 ^{aB}	81.08 ^{aB}
Day 7	33.36 ^{cB}	45.12 ^{cB}	66.18 ^{baB}	88.24 ^{abA}	91.04 ^{abA}	91.08 ^{aA}
Day 9	57.82 ^{ba}	63.93 ^{ba}	77.30 ^{abA}	94.14 ^{aA}	96.36 ^{aA}	95.93 ^{aA}

Different superscript lowercase letters (a–c) within a line and capital letters (A–D) within a column indicate statistically significant differences between groups ($p < 0.05$)

7 days of treatment (Table 1). The elimination efficiency of *B. tabaci* nymphs was 88.24% after 7 days of treatment with *P. lilacinum* PL1 at the concentration of 1×10^7 conidia/ml and there was non-statistically significant difference between the lethality caused by the concentration of 1×10^8 conidia/ml and 1×10^9 conidia/ml as well as efficiency after 9 days of treatment (Table 1). Therefore,

P. lilacinum PL1 at the concentration of 1×10^7 conidia/ml was further applied to the management of *B. tabaci* nymphs under field conditions.

The highest median lethal concentration (LC_{50}) and LC_{90} of *P. lilacinum* PL1 on *B. tabaci* nymphs after 7 days of treatment was recorded as 1.24×10^5 conidia/ml and 1.08×10^8 conidia/ml, respectively (Table 2).

Table 2 Toxicity of *Purpureocillium lilacinum* PL1 isolate at the concentration of 1×10^7 conidia/ml against *Bemisia tabaci* nymphs after 7 days of treatment

	Values	95% Confidence limits		Slope	Chi-square	Pr > Chi-square
		Lower limit	Upper limit			
LC ₅₀ (conidia/ml)	1.24×10^5	5.08×10^4	2.01×10^5	0.13 ± 0.01	137.58	< 0.0001
LC ₉₀ (conidia/ml)	1.08×10^8	4.67×10^7	3.25×10^8			
LT ₅₀ (days)	4.36	4.22	4.88	13.23 ± 1.25	208.05	< 0.0001
LT ₉₀ (days)	6.79	6.46	7.20			

Furthermore, the highest median lethal time (LT₅₀) of 1×10^7 conidia/ml of *P. lilacinum* PL1 on *B. tabaci* nymphs was recorded as 4.36 days (Table 2).

Evaluation of the efficacy of *P. lilacinum* PL1 for the management of *B. tabaci* nymphs in cassava field in Tay Ninh, Viet Nam

The effectiveness of *P. lilacinum* PL1 in the management of *B. tabaci* nymphs was evaluated in a cassava farm in Tan Chau district, Tay Ninh, Viet Nam in April 2022 with temperature range between 26 and 35 °C, air humidity of approximately 50–65% RH, and an absence of raining. The densities of whitefly *B. tabaci* nymphs on cassava leaves were also investigated, the results in (Table 3) indicated that *B. tabaci* nymphs appeared at densities ranging from 5.94 to 6.12 nymphs/leaf, and an urgent strategy was required to restrict the spread of *B. tabaci*.

In the above assays, *P. lilacinum* PL1 was identified, having a high mortality rate against *B. tabaci* nymphs in vitro. Therefore, *P. lilacinum* PL1 was the leading candidate for the management of whitefly in cassava fields. The following experimental conditions were included for the field conditions: *P. lilacinum* PL1 was obtained at a concentration of 1×10^7 conidia/ml. The chemical insecticide fenobucarb at the recommended concentration of 2 mg/ml according to the manufacturer, was used as a positive control. *Bemisia tabaci* nymphs' densities

varied significantly after 7 days of the first treatments. The results in (Table 3) indicated that both *P. lilacinum* PL1 and fenobucarb significantly reduced the density of *B. tabaci* nymphs relative to the untreated group. Under field conditions, *B. tabaci* nymphs' population showed variability in both the control and treatment groups, which differed than the controlled laboratory conditions. In order to evaluate the efficacy of *P. lilacinum* PL1 in the percentage of nymph *B. tabaci* reduction under field conditions, the Henderson–Tilton formula, which is a modified version of Abbott's formula (Abbott 1925), was employed to compare the untreated (control) and treated groups before and after the treatment was applied (Henderson et al. 1955). The results in (Table 3) indicated that after 7 days of the first treatment, *P. lilacinum* PL1 was more effective than fenobucarb in the management of *B. tabaci* nymphs, with efficacy rates of 77.46 and 56.08%, respectively. The second treatment was performed 7 days following the first treatment in order to improve the efficacy of *B. tabaci* nymphs' management under field conditions. Results shown in (Table 3) indicated that 7 days after the second treatment, the *B. tabaci* nymphs' density in the control groups increased to 13.2 nymphs/leaf. However, there were only 6.57 *B. tabaci* nymphs/leaf in the fenobucarb treatment group and 2.27 nymphs/leaf in the *P. lilacinum* PL1 treatment group (Table 3), for respective efficiencies of 50.27 and 78.86% (Table 3).

Table 3 Efficacy of *Purpureocillium lilacinum* PL1 and fenobucarb on the management of *Bemisia tabaci* nymphs in cassava field after 2 spraying batches 7 days after treatments

Treatments	Before treatment	1st treatment		2nd treatment	
	Number of <i>Bemisia tabaci</i> nymphs/leaf	Number of <i>B. tabaci</i> nymphs/leaf	Percentage of nymph reduction (%)	Number of <i>B. tabaci</i> nymphs/leaf	Percentage of nymph reduction (%)
Untreated	5.94 ± 1.85^a	6.98 ± 2.03^a	–	13.20 ± 4.50^a	–
<i>Purpureocillium lilacinum</i> PL1	5.53 ± 2.62^a	1.21 ± 0.28^c	77.46 ^a	2.27 ± 0.38^c	78.86 ^a
Fenobucarb	6.12 ± 1.64^a	3.03 ± 1.05^b	56.08 ^b	6.57 ± 1.69^b	50.27 ^b

Different superscript letters (a–c) within a column indicate statistically significant differences between groups ($p < 0.05$)

Discussion

Numerous studies have indicated that *Purpureocillium* spp. isolated from infected insect cadavers might be used as a biological agent for pest management (Johnny et al. 2012). The macroscopic morphology of *P. lilacinum* is characterized by the purple-colored and suede-like floccose on the colony surface. Conidiophores are erect, bearing branches with densely clustered phialides. Phialides are swollen at their bases, gradually tapering into a slender neck. Conidia are ellipsoidal to fusiform and are produced in divergent chains (Sun et al. 2021). In this study, *P. lilacinum* PL1 isolated from infected *B. tabaci* developed colonies with a similar morphological appearance on PDA culture medium. Pairwise comparisons of ITS sequence data showed that *P. lilacinum* PL1 isolated was 100% homologous with the previously described *P. lilacinum* strains (GenBank MT732891.1:1–634). Thus, based on colony characteristics, microscopic morphology, spore morphology, and molecular characteristics, the fungal propagules were identified as *Purpureocillium lilacinum*.

Southern Viet Nam has a tropical monsoon climate with high temperatures, humidity, and abundant rainfall throughout the year (Kurbatova et al. 2015). These environmental conditions are suitable for the growth of EPFs (Akner et al. 2020). Our findings indicated that *P. lilacinum* PL1 mycelia and sporulation were optimally grown at 25–35 °C. Colony radial growth was roughly 25% lower at 40 than 30 °C. Furthermore, the sporulation of *P. lilacinum* PL1 characterized by purple-colored colonies was still maintained at 40 °C. This contrasts with the previous study, which indicated that *Purpureocillium* spp. isolated in Germany could not be grown and sporulated above 36 °C (Kiewnick 2006). Native EPFs have been shown to be superior for pest management since they are biologically compatible with their location and habitat type (Bidochka et al. 2002). Thus, the *P. lilacinum* PL1 isolate in this study was appropriate for the tropical monsoon climate in Viet Nam, characterized by high temperatures (Kosanec et al. 2019). Furthermore, pH is one of the abiotic variables impacting EPFs activity in both laboratory survival and efficacy in field conditions. As an agricultural application, *P. lilacinum* PL1 had effectively grown and sporulated across a large pH range (from 5 to 8), which was highly beneficial as it would be amenable to application in various habitats with different pH ranges.

Entomopathogenic fungi are well-known for parasitizing arthropods by adhering to and interacting with the insect's epicuticular layer through the formation of physical or enzymatic activity after penetrating the insect cuticle (Ortiz-Urquiza et al. 2013). EPFs establish a pathogenic connection with the insect by producing an infective structure known as the appressorium (Sandhu

et al. 2012) then further penetrate the insect cuticle via mechanical pressure and cuticle-degrading enzymes such as proteases and chitinases (Ibrahim et al. 2016; Sunitha et al. 2013). The present findings suggested that *P. lilacinum* PL1 may produce the cuticle-degrading enzymes protease and chitinase, which are required for insect parasitism.

In the present study, the effect of *P. lilacinum* PL1 isolated against the whitefly *B. tabaci* under laboratory conditions, followed by field experiments was examined. From the data presented, it is apparent that a dilution of *P. lilacinum* PL1 at 1×10^7 conidia/ml considerably reduced the *B. tabaci* nymphs' populations in vitro after 7 days of treatment, with an efficiency of 88.24%. This result corroborated a previous study showing that *P. lilacinum* isolate XI-1 caused the highest adult mortality rate of whitefly *B. tabaci* (86.81%) within 7 days of treatment at a concentration of 1×10^7 conidia/ml (Sun et al. 2021), lending further credence to our proposal of using EPF as a suitable agent for pest management. The LC_{50} of *P. lilacinum* PL1 on whitefly *B. tabaci* nymphs deduced from the bioassay studies was 1.24×10^5 conidia/ml at day 7th after treatment. Thus, *P. lilacinum* PL1 expressed the same pattern in the management of whitefly *B. tabaci* in the comparison to *P. lilacinum* isolate XI-1 in the previous study (Sun et al. 2021).

The field evaluation study in assessing the lethality of *P. lilacinum* PL1 at concentration of 1×10^7 conidia/ml, in the present study revealed the percentage of *B. tabaci* nymphs' reduction as 77.46% at day 7th after the first spraying. *B. tabaci* nymphs' mortality was considerably higher in the fungal treatment plots after the second spraying (78.86%) than the untreated group, demonstrating that the *B. tabaci* nymphs' population was suppressed, following 2 batches of 7-day after treatment of *P. lilacinum* PL1. The concentration of 1×10^7 conidia/ml used in this study was comparable to previous studies for insect management (Cabanillas et al. 2009). In previous study, *P. fumosoroseus* and *P. lilacinus* lowered the glasshouse whitefly *T. vaporariorum* over 70% (Gökçe et al. 2005). *P. fumosoroseus* Apopka-97, on the other hand, reduced the *T. vaporariorum* population by 48% (Alma et al. 2007). Thus, the efficacy of *P. lilacinum* PL1 is considered to be equivalent to or higher than several previous studies. Furthermore, the *P. lilacinum* PL1 application was more effective than the fenobucarb treatment plots at managing the *B. tabaci* nymphs' population.

Pesticides are currently the mainstay of whitefly management. Considering the threats associated with pesticide abuse, *B. bassiana*, *M. anisopliae*, and *I. fumosorosea* are effective EPFs that play a role in the management of whitefly nymphs as part of an integrated pest management strategy (Gul et al. 2014; Mora et al. 2016). Thus, *P.*

lilacinum PL1 identified in this investigation was found to be very efficient and possesses exceptional potential as a biopesticide. Despite the fact that EPFs have significant advantages over conventional pesticides, there are several limitations to their application for pest management. The insecticidal activity of EPF in open fields in the tropics can be negatively affected by environmental conditions such as temperature, humidity and UV exposure (Loong et al. 2013). Therefore, further studies on the effects of humidity, temperature, and UV protection agents are required to enhance the efficacy of *P. lilacinum* PL1 in the management of sucking insects.

Conclusions

A highly pathogenic strain of *P. lilacinum* PL1 was isolated from *B. tabaci* cadavers infected with EPFs. The lethality of *P. lilacinum* PL1 against *B. tabaci* nymphs was assessed in the laboratory and in the field conditions. According to a biological assay, *P. lilacinum* PL1 was highly lethal to *B. tabaci* nymphs under laboratory conditions. In addition, *P. lilacinum* PL1 was superior to the chemical agent fenobucarb for the management of *B. tabaci* nymphs in cassava fields. Our findings authenticate the potential of *P. lilacinum* PL1 in the management of the whitefly *B. tabaci*; consequently, *P. lilacinum* PL1 might be utilized as a biopesticide as part of an integrated pest management (IPM) strategy to manage whiteflies in a sustainable manner.

Abbreviations

CMB	Cassava mosaic begomoviruses
CMD	Cassava mosaic disease
EPF	Entomopathogenic fungi
<i>g</i>	Gravity force or relative centrifugal force
ITS	Internal transcribed spacer
LC	Lethal concentration
LT	Lethal time
PDA	Potato dextrose agar
RCBD	Randomized complete blocks design
SLCMV	Sri Lankan cassava mosaic virus
PCR	Polymerase chain reaction
RH	Relative humidity

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Author contributions

HNT and ADD designed this study; KYPL, NDTT, and TDN performed experiments; ADD wrote the paper. All authors approved this final manuscript. All authors have read and agreed to the published version of the manuscript.

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Competing interests

The authors have no conflict of interest to declare.

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