Egyptian Journal of Biological Pest Control

RESEARCH

Open Access

Mass production of entomopathogenic fungi *Purpureocillium lilacinum* PL1 as a biopesticide for the management of *Amrasca devastans* (Hemiptera: Cicadellidae) in okra plantation

Hai Nguyen Thi[1], Quynh Nhu Nguyen[2,3], Nhu Quynh Dang Thi[1], Ngoc Lam Nguyen[1] and Anh Duy Do[4*]

Abstract

Background Effective management strategies are crucial in minimizing the adverse consequences associated with the leafhopper, *Amrasca devastans* (Dist.) (Hemiptera: Cicadellidae). Economic limitations to entomopathogenic fungi production present a substantial challenge, particularly in developing countries. This study aimed to investigate a cost-effective solid-state fermentation (SSF) for large-scale production of *Purpureocillium lilacinum* PL1 conidia to manage *A. devastans* infestations in okra cultivation.

Results Rice and maize were demonstrated as highly suitable substrates for producing conidia densities of over 2×10^{10} conidia g^{-1}. Furthermore, the influence of agricultural phytosanitary agents on the growth rates of *P. lilacinum* PL1 was evaluated. Certain pesticides were ineffective on the expansion of *P. lilacinum* PL1 colonies, while fungicides exhibited complete inhibition. The laboratory investigation revealed that 1×10^7 conidia ml^{-1} of *P. lilacinum* PL1 exhibited a success rate of 88.66% in decreasing the population of *A. devastans* nymphs in vitro. Furthermore, field investigations carried out in okra plantations demonstrated that the utilization of *P. lilacinum* PL1 at the concentration of 1×10^7 conidia ml^{-1} of resulted in a significant reduction of the pest nymph population by 72.87% subsequent to the 2 applications.

Conclusion In conclusion, the cost-effective mass production of *P. lilacinum* PL1 conidia through SSF presents a promising solution for managing *A. devastans* infestations in okra farming, particularly in economically challenged regions.

Keywords *Amrasca devastans*, Biopesticides, *Purpureocillium lilacinum*, Fermentation, Pest management

*Correspondence:
Anh Duy Do
daduy@ntt.edu.vn
Full list of author information is available at the end of the article

Background

Okra [*Abelmoschus esculentus* (L.) Moench. family Malvaceae], a vegetable crop primarily cultivated in warm regions of Africa and Asia including Viet Nam, is grown by smallholder farmers for its edible immature pods (Tong 2016). Leafhopper [*Amrasca devastans* (Dist.); Hemiptera: Cicadellidae] is a significant pest of okra, which has a high prevalence in regions characterized by tropical and subtropical climates (Rehman et al. 2015). The feeding behavior of the leafhopper on okra plants involves the consumption of phloem sap, resulting in the manifestation of symptoms such as leaf yellowing, curling, and stunting, leading to a notable decrease in crop yield. The severity of damage varies depending on the infestation level and okra variety susceptibility (Sarwar 2020). Insecticides, particularly chemical synthetic pesticides, are frequently used by farmers to control this pest. The application of pesticides has resulted in ecological contamination, the emergence of pest resistance, and potential dangers to human health (Pathak et al. 2022). Therefore, utilization of eco-friendly techniques, such as entomopathogenic fungi (EPFs), for the control of sap-sucking pests has become increasingly prevalent as a substitute for chemical insecticides (Skinner et al. 2014).

Purpureocillium lilacinum is a hyphomycete fungus known for its entomopathogenic properties, which has made it a popular biocontrol agent against various insect pests, including soil-dwelling and above-ground pests (Goffré and Folgarait 2015). The fungal synthesizes diverse enzymes that degrade organic compounds, such as proteases and chitinases, which play a crucial role in catalyzing and hydrolysis of the insect cuticle, thereby facilitating the successful infiltration and subsequent infection of the host organism (Ibrahim et al. 2016). Furthermore, it has been indicated that administration of *P. lilacinum* PL1 showed significant lethality against whitefly [*Bemisia tabaci* (Gennadius); Hemiptera: Aleyrodidae] nymphs, with mortality rates of 88.24% in the laboratory and 78.86% in the field, respectively (Nguyen et al. 2023). In addition, *P. lilacinum* is also known to control various other insects, including mosquitoes, beetles, thrips, fruit flies, whiteflies, aphids, and even nematodes, making it a valuable tool in integrated pest management strategies (Amala et al. 2013; Goffré and Folgarait 2015). Hence, the utilization of *P. lilacinum* as a biocontrol agent for *A. devastans* in okra farming holds promise as an eco-friendly and sustainable approach.

The high cost of producing EPFs for pest control poses a significant challenge, particularly in developing nations (Morán-Diez and Glare 2016). The employment of solid-state fermentation (SSF) has surfaced as a viable and uncomplicated approach for the economical generation of EPFs, particularly for the production of conidia (Rayhane et al. 2019). SSF utilizes various agro-industrial residues as substrates, such as cereal bran, sugarcane bagasse, and sawdust (Sadh et al. 2018). Various strains of EPFs, such as *Metarhizium anisopliae*, *Beauveria bassiana*, and *Paecilomyces fumosoroseus*, have been effectively cultivated through SSF by adjusting growth conditions, substrate composition, and inoculation rate to attain optimal spore production (Qiu et al. 2019).

This study aimed to investigate the feasibility of utilizing SSF to cultivate *P. lilacinum* PL1 conidia as a strategy for the leafhopper *A. devastans* management in okra fields. In addition, the compatibility of EPFs and chemical pesticides is important for integrated pest management (IPM) strategies. Certain chemical pesticides may reduce EPF efficacy, while others may enhance their activity (Abd-El-Khair et al. 2019). This study further examined the compatibility of *P. lilacinum* PL1 with synthetic fungicides and pesticides through cocultivation and investigated their impact on the growth of this fungus.

Methods

The study was designed using a flow diagram to visually depict the research methodology and its progression (Fig. 1). This study assessed the optimal propagation and solid fermentation mediums for the efficient production of *P. lilacinum* PL1 conidia. Besides that, the effect of phytosanitary substances on the growth of *P. lilacinum* PL1 was investigated. Additionally, the study examined the lethality of *P. lilacinum* PL1 on *A. devastans* nymphs under controlled laboratory conditions. Furthermore, the efficacy of *P. lilacinum* PL1 in the management of *A. devastans* nymph populations in okra fields was assessed (Fig. 1).

Propagation of A. devastans nymphs in the greenhouse

Greenhouse leafhopper propagation was conducted according to the protocol described by Manivannan et al. 2018. The leafhopper *A. devastans* population was obtained from okra plantations and subsequently cultivated in a greenhouse to facilitate population growth. The maintenance of the leafhopper culture was achieved through the cultivation of okra plants in pots that were enclosed with a cage made of a thin film of mylar (Manivannan et al. 2018). The confirmation of the final instar of *A. devastans* nymphs was conducted based on the pictorial guide (Nagrare et al. 2012).

Fungal strain

The fungal strain *P. lilacinum* PL1 was isolated from *B. tabaci* cadavers, obtained from a previous study (Nguyen et al. 2023), and was maintained in Potatoes dextrose agar (PDA) culture medium (HiMedia, India) at 30 °C.

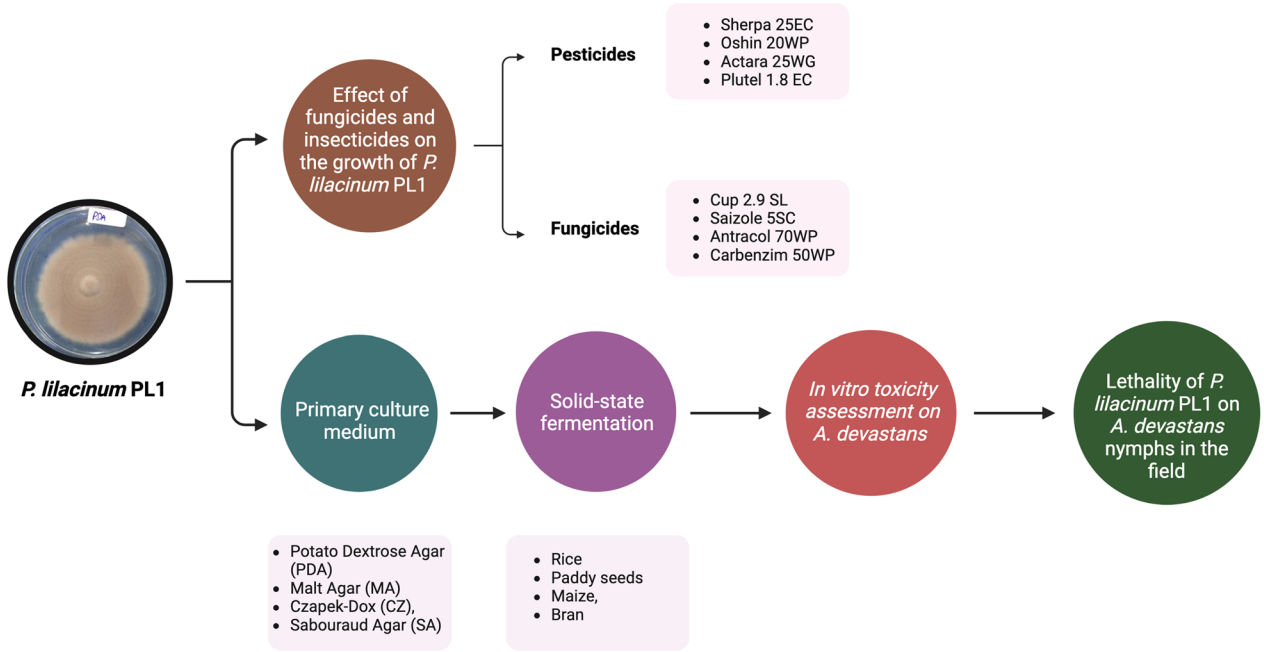

Fig. 1 Study flow diagram of the study. The figure was created with BioRender.com. Publication license number ZT25P4RANN

Effect of culture medium on primary multiplication of P. lilacinum PL1

The mycelia disk measuring 5 mm in diameter of *P. lilacinum* PL1 was subjected to cultivation on various nutrient media, including Potato Dextrose Agar (PDA), Malt Agar (MA), Czapek-Dox (CZ), and Sabouraud Agar (SA). The Petri dishes were subjected to incubation at a temperature of 28 °C for a duration of 7 days. The extent of radial growth of the fungus was quantified by measuring the diameter of the colony. The experiment was conducted in triplicate to ensure the accuracy of the results.

Effectiveness of fungicides and insecticides on the growth of P. lilacinum PL1

As shown in Table 1, the pesticides or fungicides were integrated into a PDA medium in this investigation. The PDA medium was sterilized at 121 °C for 20 min. Following, the recommended field doses (Table 1) of pesticides or fungicides were added and evenly distributed in Petri plates with a diameter of 90 mm, with a volume of 18 ml/plate. Plugs with a diameter of 5 mm were obtained from the periphery of the *P. lilacinum* PL1 colonies and then transferred to the PDA medium supplemented with pesticides or fungicides. The control group consisted of *P.*

Table 1 The phytosanitary products utilized in this study

Trade name	Active ingredient	Abbreviation	Recommended field dose
Pesticides			
Sherpa 25EC	Cypermethrin	CM	0.1% (v/v)
Oshin 20WP	Dinotefuran	DF	600 ppm (w/v)
Actara 25WG	Thiamethoxam	TM	600 ppm (w/v)
Plutel 1.8 EC	Abamectin	AM	250 ppm (v/v)
Fungicides			
Cup 2.9 SL	Copper nanoparticles	CuNPs	0.25% (v/v)
Saizole 5SC	Hexaconazole	HC	0.2% (w/v)
Antracol 70WP	Probineb	PP	2.5% (w/v)
Carbenzim 50WP	Carbenzim	CZ	0.2% (w/v)

lilacinum PL1 cultured on free-pesticide PDA culture medium (Mayo-Prieto et al. 2022). Subsequently, the plates were incubated at 25 °C in the absence of light for a duration of 7 days. The mycelial growth of *P. lilacinum* PL1 was quantified following a 7-day cultivation, and the experimental procedures were conducted in triplicate. The assessment of the initial toxicity of fungicides and insecticides on the growth of *P. lilacinum* PL1 was conducted utilizing a 4-level category system. The categories were defined as follows: 1—harmless, indicating an inhibition less than 50%; 2—slightly harmful, indicating an inhibition ranging from 50 to 79%; 3—moderately harmful, indicating an inhibition ranging from 80 to 99%; and 4—harmful, indicating an inhibition greater than 99% (Hassan et al. 1985).

Effect of substrate on the production of P. lilacinum PL1 conidia utilizing solid-state fermentation

Mass multiplication of *P. lilacinum* PL1 through solid-state fermentation was conducted using 4 different solid substrates: rice, paddy seeds, maize, and bran (Sadh et al. 2018). The experimental procedure involved the uniform allocation of 1 kg of distinct substrates onto a tray with dimensions of 40 cm × 40 cm. Subsequently, 500 ml of water was added, and the tray was subjected to sterilization through autoclaving. Conidia of *P. lilacinum* PL1 were acquired from PDA culture medium, then suspended in phosphate-buffered saline (PBS), and subsequently introduced to the sterilized substrate at a density of 1×10^6 conidia g^{-1}. Subsequently, the trays underwent an incubation process at 28 °C for 14 days. Following the incubation, the conidia density of *P. lilacinum* PL1 in each substrate was assessed through either serial dilution on PDA plates or enumeration with a hemocytometer (Hirschmann, MO, USA).

Lethality of P. lilacinum PL1 on A. devastans nymphs in vitro

A total of 50 last instar nymphs of *A. devastans* were gathered and transferred onto okra leaves, which were then arranged in a 150-mm-diameter Petri dish. Each dish contained 50 nymphs. The *P. lilacinum* PL1 conidia were obtained from rice substrate following 14 days of cultivation. These conidia were then suspended in sterile distilled water containing Tween 80 (0.02% v/v) and adjusted to a concentration of 1×10^7 conidia ml^{-1} (Nguyen et al. 2023). A toxicity investigation was conducted wherein *A. devastans* nymphs were administrated with a 5 ml of *P. lilacinum* PL1 conidia suspension. The group only received water supplemented with 0.02% Tween 80 was referred to as an untreated group. In contrast, the positive control group was subjected to a treatment consisting of 5 ml of Abamectin (AM) at a concentration of 250 ppm (v/v). Following the air-drying, the leaves were subsequently relocated to a separate Petri dish that was furnished with a layer of 1.5% agarose gel and incubated at 28 °C, accompanied by a relative humidity range of 50–60% (Shah et al. 2020). Daily monitoring of the nymph's mortality was conducted over a period of 7 consecutive days. The calculation of mortality was performed as a percentage utilizing 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 A. devastans nymphs under field conditions

Field studies utilizing a randomized complete blocks design (RCBD) were carried out at three distinct okra farms located in Cu Chi, Ho Chi Minh City, Vietnam (Fig. 2). Each treatment was replicated 3 times. The study utilized an experimental design consisting of plots with dimensions of 10 m by 5 m and a spacing of 1 m. Each plot was capable of accommodating a total of 50 okra plants. Before the treatment, the quantification of *A. devastans* nymphs was carried out on okra leaves situated at the top, middle, and bottom of each plant/plot. The experimental interventions comprised *P. lilacinum* PL1 at a concentration of 1×10^7 conidia ml^{-1}, Abamectin (AM) at a concentration of 250 ppm (v/v) as a positive control, and untreated plots sprayed with water as negative controls with spray volume of 300l/hectare were utilized to apply two sprays at 7-day intervals during the afternoon after 4 pm (Lavers 2001). The enumeration of *A. devastans* nymphs was carried out on the leaves located at the top, middle, and bottom portions of every plant/plot following a 7-day and 14-day treatment. The Henderson–Tilton formula was employed to assess the effectiveness of each treatment in managing the *A. devastans* nymph population (Henderson and Tilton 1955).

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

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

Results were calculated using the mean of triplicate readings.

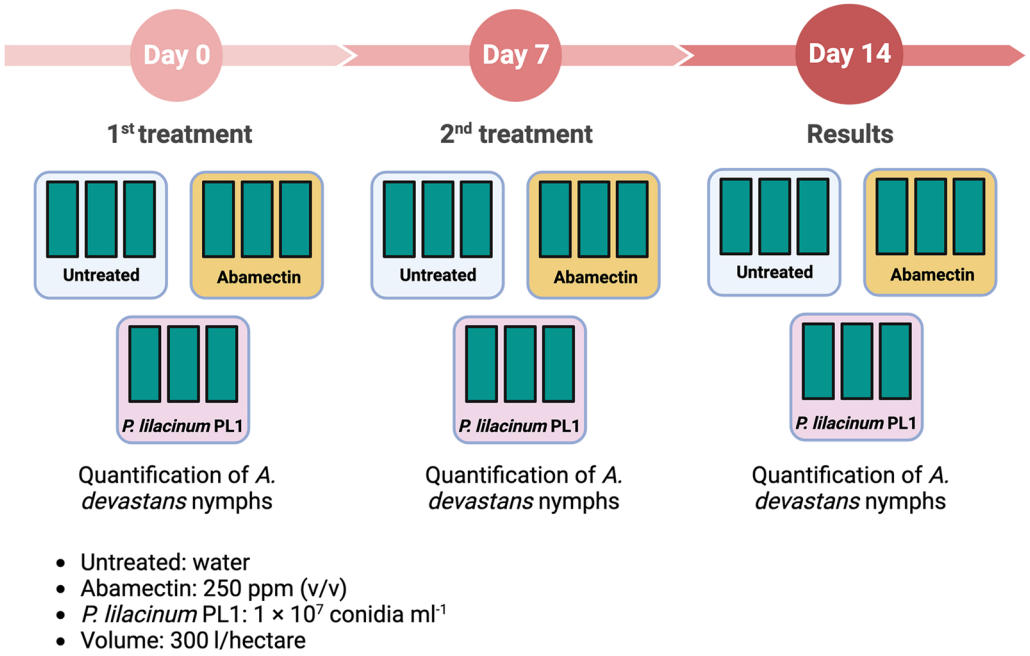

Fig. 2 Field experimental design using *Purpureocillium lilacinum* PL1 in the management of *Amrasca devastans* population on okra plantations. The figure was created with BioRender.com. Publication license number DZ25P4HFOE

Statistical Analysis

The study utilized a completely randomized design (CRD) with 3 replicates for each treatment. Data were analyzed using SAS 9.4 software (SAS, Inc., Cary, NC, USA) and presented as the mean ± standard error of the mean based on triplicate readings. Statistical significance between groups was determined using Duncan's test, with $p < 0.05$ indicating significance. Probit analysis was conducted using SAS 9.4 software to calculate LT_{50} and LT_{90}.

Results

Effect of culture medium on the growth of P. lilacinum PL1 mycelia

The primary multiplication of *P. lilacinum* PL1 was conducted using four nutrient media including PDA, MA, CZ, and SA. The growth rate of *P. lilacinum* PL1 mycelia was assessed by measuring its radial expansion after 7 days of cultivation. The results obtained indicate that there were significant variations in the growth rates of *P. lilacinum* PL1 among the culture media that were investigated ($p < 0.05$). Significantly, the most substantial growth rates were observed on MA and PDA media, with mycelia diameters ranging from 69.7 to 77.0 mm. In contrast, the CZ medium exhibited a comparatively lower growth rate, as evidenced by a mycelial diameter of 45.5 mm (Fig. 3A, B).

Effectiveness of fungicides and insecticides on the growth of P. lilacinum PL1 mycelia

The effectiveness of fungicides and insecticides on the growth of *P. lilacinum* PL1 was evaluated over a period of 14 days. The results, as depicted in Fig. 4A, B, showed that HC, PP, and CZ had a considerable effect on fungal growth, with complete inhibition of the growth of *P. lilacinum* PL1 colonies. The level of inhibition was categorized as level 4, indicating the highest level of impact—harmful (Table 2). In contrast, CuNPs alone had a minimal effect on the proliferation of fungi, resulting in an inhibition rate of approximately 7.24%, which was categorized as level 1or harmless (Table 2). In case of insecticides, AM had a slight effect on the growth of *P. lilacinum* PL1 (Fig. 4A, B), with a 67.29% inhibition rate of the fungal colony growth, categorized as level 2 or slightly harmful (Table 2). On the other hand, CM, DF, and TM had minimal effects on fungal growth (Fig. 4A, B), with less than 50% inhibition, classified as level 1 or harmless (Table 2).

Effect of solid substrate on the mass production of P. lilacinum PL1 conidia

The effect of solid substrate on the mass propagation of *P. lilacinum* PL1 conidia was investigated through the utilization of 4 organic substrates, namely rice, rice husk, maize, and paddy seeds. The fermentation was carried

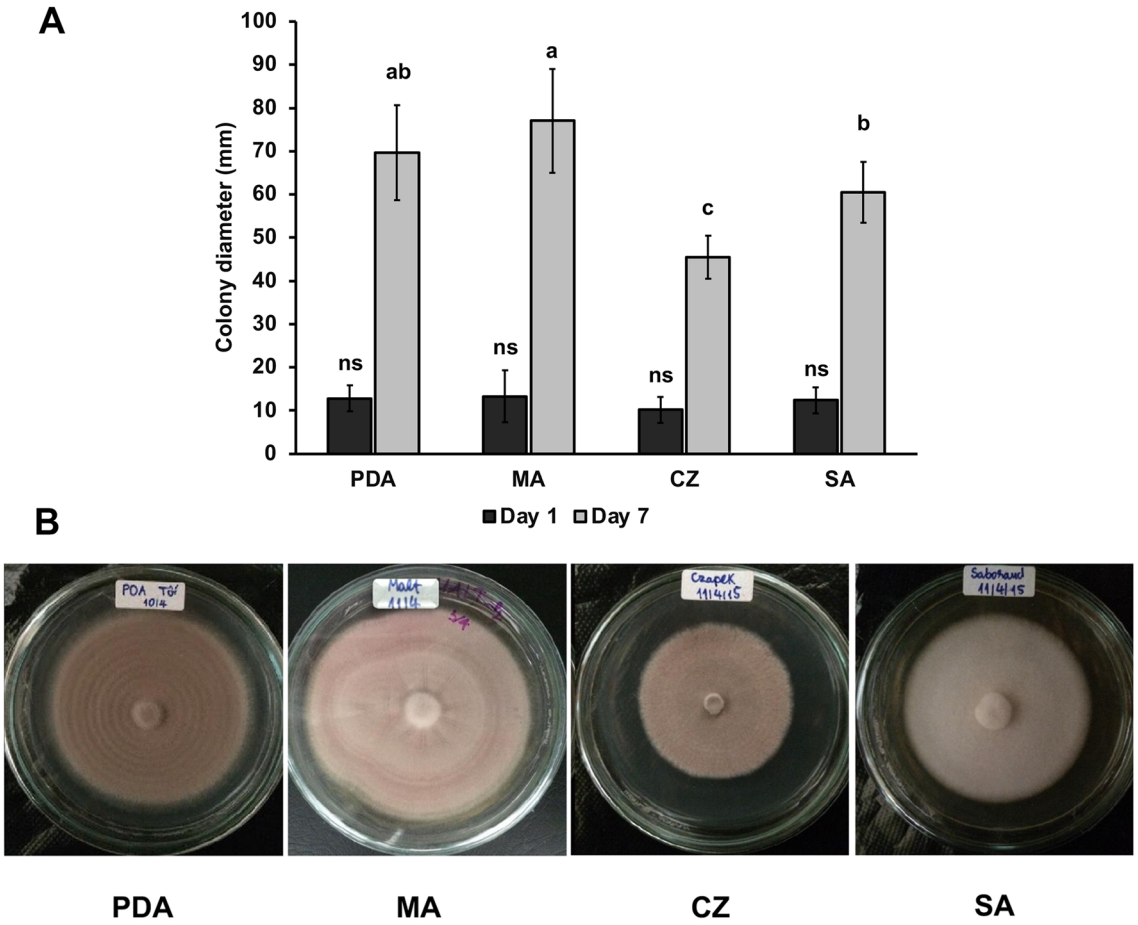

Fig. 3 **A** Radial growth of cultured *Purpureocillium lilacinum* PL1 mycelia after 7 days in difference culture mediums. **B** Representative photographs of PL1 mycelia after 7 days of cultivation in various culture mediums. PDA: Potato Dextrose agar, MA: Malt agar, CZ: Czapek-Dox agar, and SA: Sabouraud agar. Data are presented as the means of triplicate analysis ± standard deviation. "ns" indicates non-significant statistical differences, while lowercase letters **a**–**c** indicate significant differences in the colony diameter of PL1 across culture media. The statistical analysis was conducted using ANOVA followed by Duncan's test ($p < 0.05$)

out for 14 days, wherein the growth rate of *P. lilacinum* PL1 conidia was observed. The results as shown in Fig. 5 suggested that the substrate significantly affects the biomass yield of *P. lilacinum* PL1 conidia. Obtained results revealed that the growth of *P. lilacinum* PL1 was most favorable on rice and maize substrates, yielding approximately 2×10^{10} conidia g^{-1}, following a 14-day cultivation period (Fig. 5). In contrast, a significantly low conidial mass of 2.2×10^{9} conidia g^{-1} was seen in paddy seeds substrate (Fig. 5).

Lethality of P. lilacinum PL1 on A. devastans nymphs in vitro

The effect of *P. lilacinum* PL1 on *A. devastans* mortality was examined through an in vitro study. The data in Fig. 4A demonstrated that the application of *P. lilacinum* PL1 at the concentration of 1×10^{7} conidia ml^{-1} resulted in a significant reduction in the population of *A. devastans* nymphs within a 7-day treatment. The treatment exhibited an efficacy rate of 86.66%, which did not exhibit a significant difference compared to the efficacy rate of the AM treatment group. Despite *P. lilacinum* PL1 taking longer to eliminate *A. devastans* nymphs than AM, with LT_{50} values of 4.21 and 2.14 days, respectively (Table 3), their lethality became comparable after 7 days of treatment. Microscopic examination of *A. devastans* nymph cadavers in the *P. lilacinum* PL1 treatment group revealed the presence of hyphae on the cuticle, displaying microscopic characteristics consistent with indications of *P. lilacinum* infection, as depicted in Fig. 6B and C (Nguyen et al. 2023).

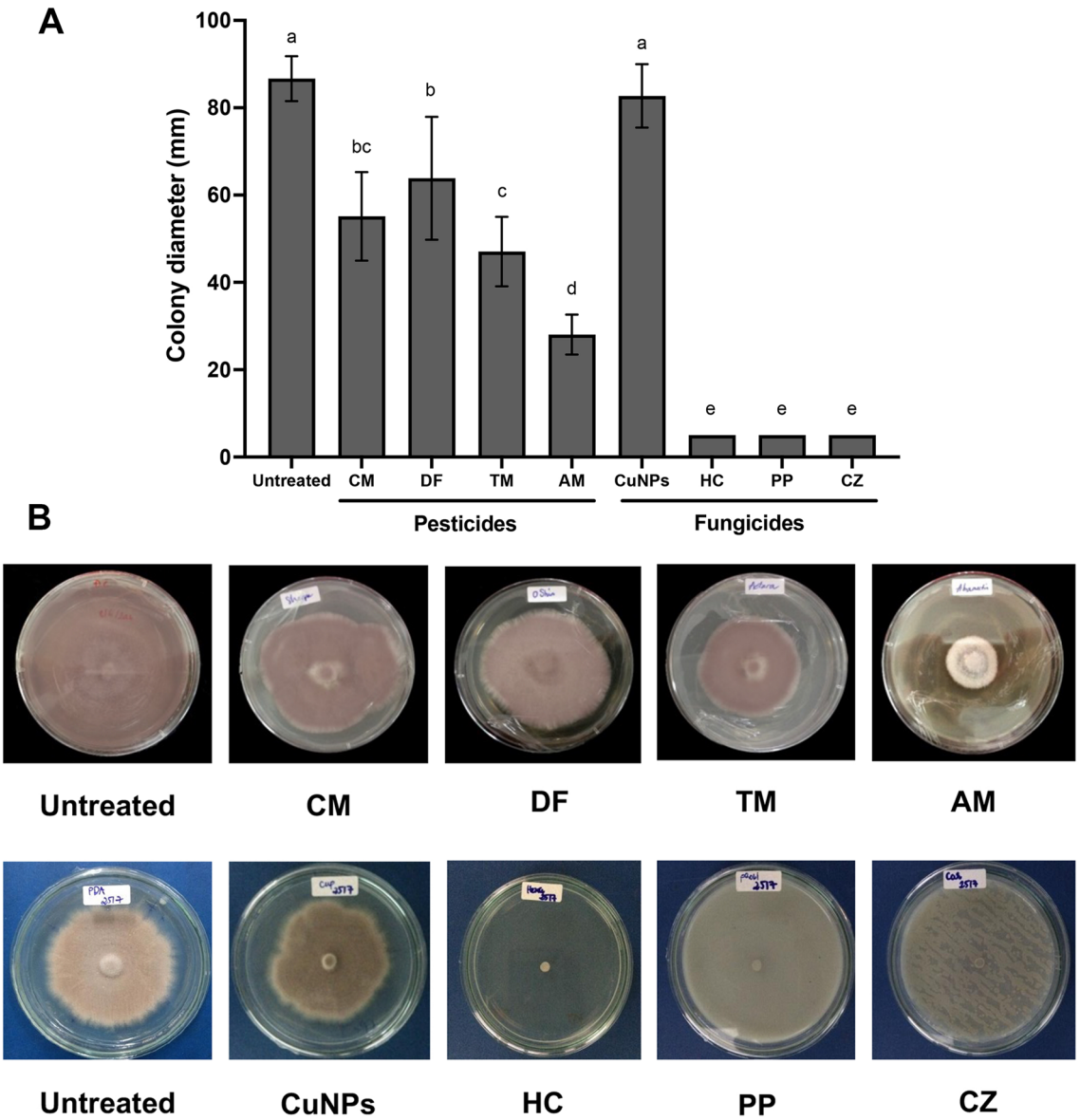

Fig. 4 **A** Radial growth of cultured *Purpureocillium lilacinum* PL1 mycelia in PDA mediums supplemented with insecticides or fungicides. **B** Representative photographs of PL1 mycelia after 7 days of cultivation in PDA mediums supplemented with phytosanitary products. AM: Abamectin, CM: Cypermethrin, CuNPs: Copper nanoparticles, CZ: Carbenzim, DF: Dinotefuran, HC: Hexaconazole, TM: Thiamethoxam. Data are presented as the means of triplicate analysis ± standard deviation. Different lowercase letters **a**–**c** indicate statistical differences in the colony diameter of PL1 among insecticides or fungicides treatment. The statistical analysis was conducted using ANOVA followed by Duncan's test ($p < 0.05$)

Application of P. lilacinum PL1 reduced A. devastans nymph's populations on okra plantations

The efficient usage of *P. lilacinum* PL1 in the control of *A. devastans* nymphs was further investigated on okra plantations in Cu Chi, Ho Chi Minh City, Vietnam (Fig. 7). The investigation was conducted within defined environmental parameters, including a temperature range of 28–36 °C, with air humidity levels were 55–70% RH, and no precipitation during the study. Before treatment, the *A. devastans* nymph densities on okra leaves ranged from 6.45 to 6.90 nymphs/leaf (Table 4), highlighting the necessity for prompt implementation of control strategies. The fungus *P. lilacinum* PL1 was administered at a concentration of 1×10^7 conidia ml^{-1}, and AM was utilized as a positive control at a concentration of 250 ppm (v/v), in accordance with the instructions provided by the manufacturer.

Table 2 Effect of phytosanitary on the growth of *Purpureocillium lilacinum* PL1 mycelia

Active ingredient	% Inhibition	Inhibition index	Evaluation categories
Pesticides			
CM	36.67[d]	1	Harmless
DF	26.48[d]	1	Harmless
TM	46.59[c]	1	Harmless
AM	67.27[b]	2	Slightly harmful
Fungicides			
CuNPS	7.24[e]	1	Harmless
HC	100.00[a]	4	Harmful
PP	100.00[a]	4	Harmful
CZ	100.00[a]	4	Harmful

Different superscript letters (a–e) within a column indicate statistically significant differences between groups ($p < 0.05$). The statistical analysis was conducted using ANOVA followed by Duncan's test ($p < 0.05$)

The application of AM and *P. lilacinum* PL1 sprays resulted in a significant reduction in the population density of *A. devastans* nymphs than the control group. Following 7 days subsequent to the initial treatment, *P. lilacinum* PL1 exhibited greater efficacy in the control of *A. devastans* nymphs in comparison with AM, with efficacy rates of 64.46 and 32.22%, respectively (Table 4). Subsequent treatment was conducted 7 days after the initial treatment. According to the findings presented in Table 4, the density of *A. devastans* nymphs in the untreated group exhibited an increase to 7.96 nymphs/leaf after 7 days of the second treatment. However, in the groups that received AM and *P. lilacinum* PL1 treatment, the nymph density was observed to be 4.95 and 2.27 nymphs/leaf, and corresponding efficiencies were 34.67 and 72.87%, respectively (Table 4).

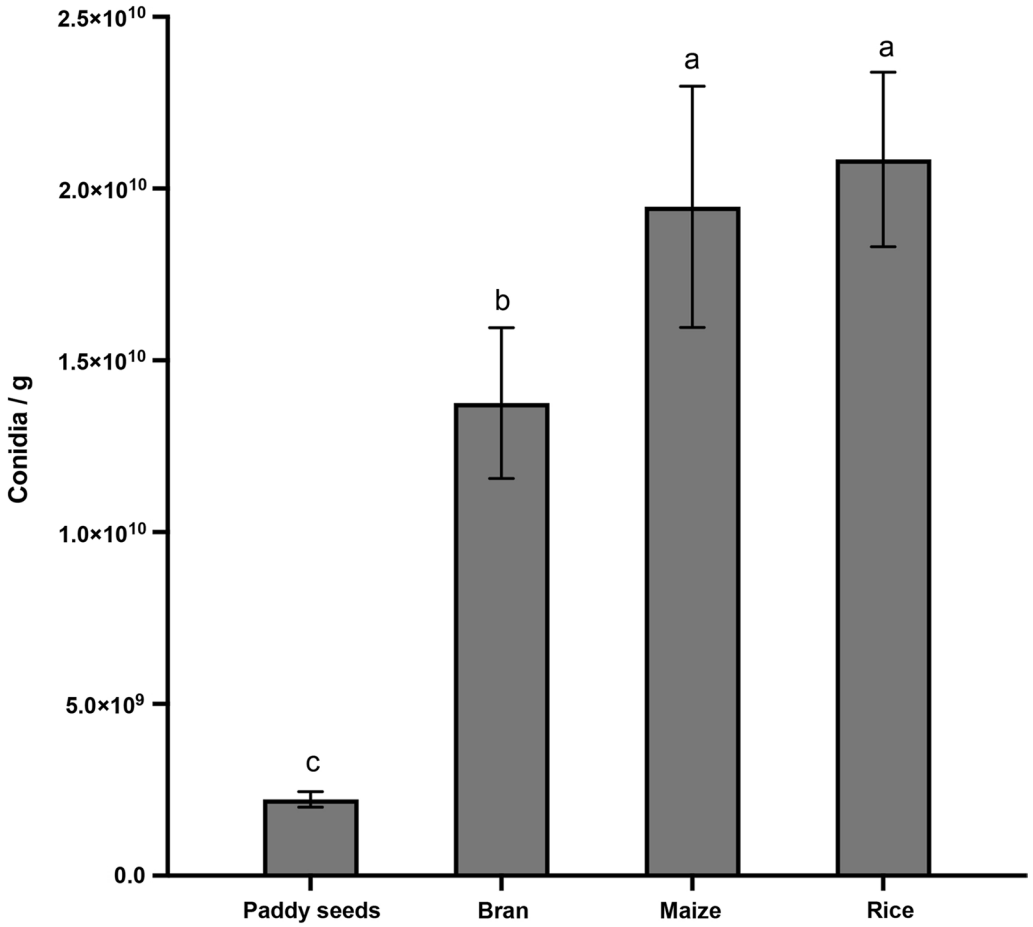

Fig. 5 Effect of substrate on the mass production of *Purpureocillium lilacinum* PL1 conidia. Data are presented as the means of triplicate analysis ± standard deviation. Different lowercase letters **a**–**c** indicate statistical differences in PL1 conidia density among solid media. The statistical analysis was conducted using ANOVA followed by Duncan's test ($p < 0.05$)

Table 3 Lethal time of 1×10^7 conidia/ml of *Purpureocillium lilacinum* PL1 or 250 ppm of abamectin on the *Amrasca devastans* nymph populations

	Values	95% confidence limits		Slope	Chi-square	Pr > Chi-square
		Lower limit	Upper limit			
Abamectin						
LT_{50} (days)	2.14	1.46	2.61	13.23 ± 1.25	30.17	< 0.0001
LT_{90} (days)	6.28	5.37	8.34			
Purpureocillium lilacinum PL1						
LT_{50} (days)	4.21	3.97	4.44	13.56 ± 1.87	130.97	< 0.0001
LT_{90} (days)	6.83	6.31	7.58			

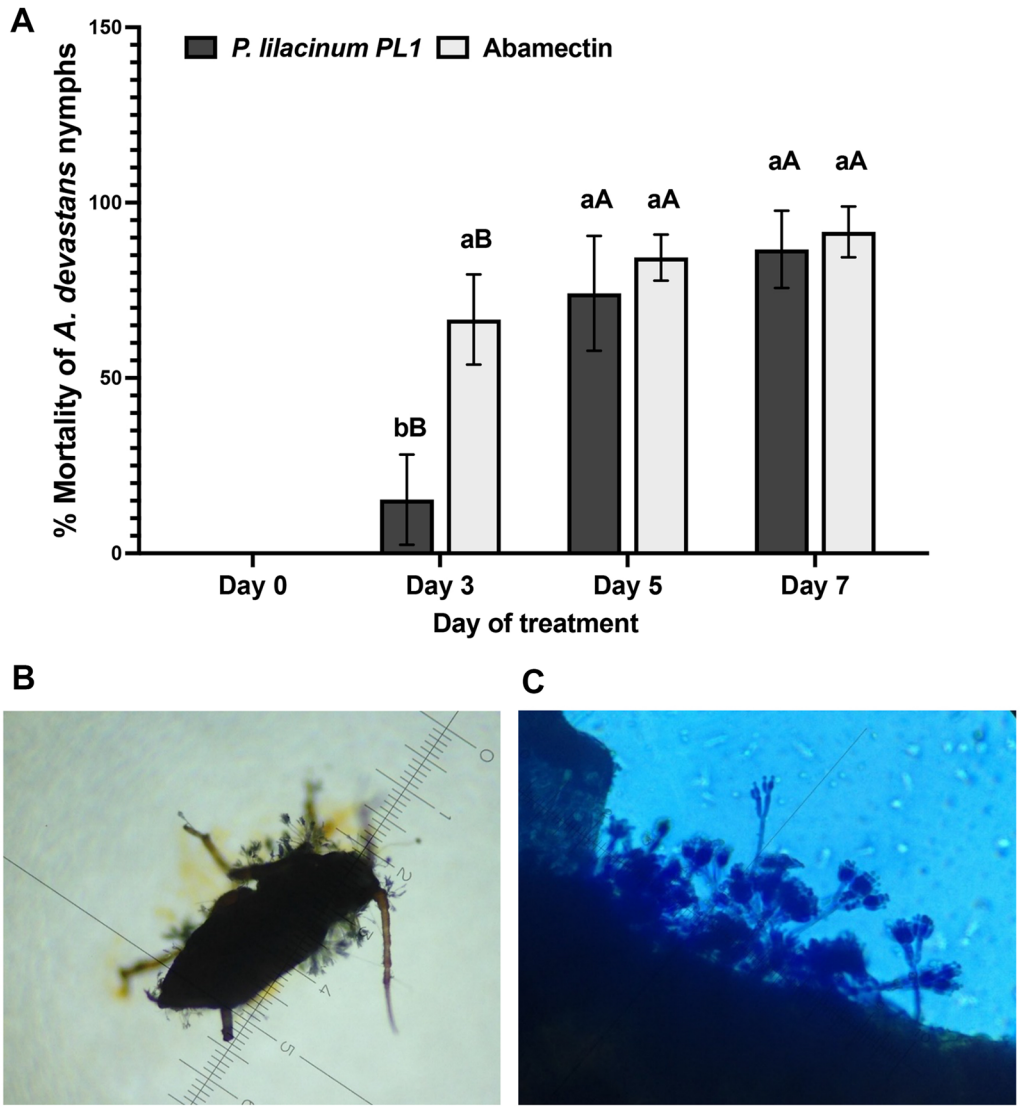

Fig. 6 **A** Effect of 1×10^7 conidia ml^{-1} of *Purpureocillium lilacinum* PL1 or 250 ppm of Abamectin on the mortality of *Amrasca devastans* nymphs after 7 days of treatment. Representative photographs of dead *A. devastans* nymphs cause by PL1 with mycelia on the cuticle under the light microscope at 40 × magnification (**B**) and 400 × magnification (**C**). Data are presented as the means of triplicate analysis ± standard deviation. Different lowercase letters **a**, **b** indicate statistical differences in the mortality of *A. devastans* nymphs between PL1 and Abamectin treatment groups. Capital letters **A**, **B** indicate statistical differences in the mortality of *A. devastans* nymphs at different time intervals after treatment by PL1 or Abamectin. The statistical analysis was conducted using ANOVA followed by Duncan's test ($p < 0.05$)

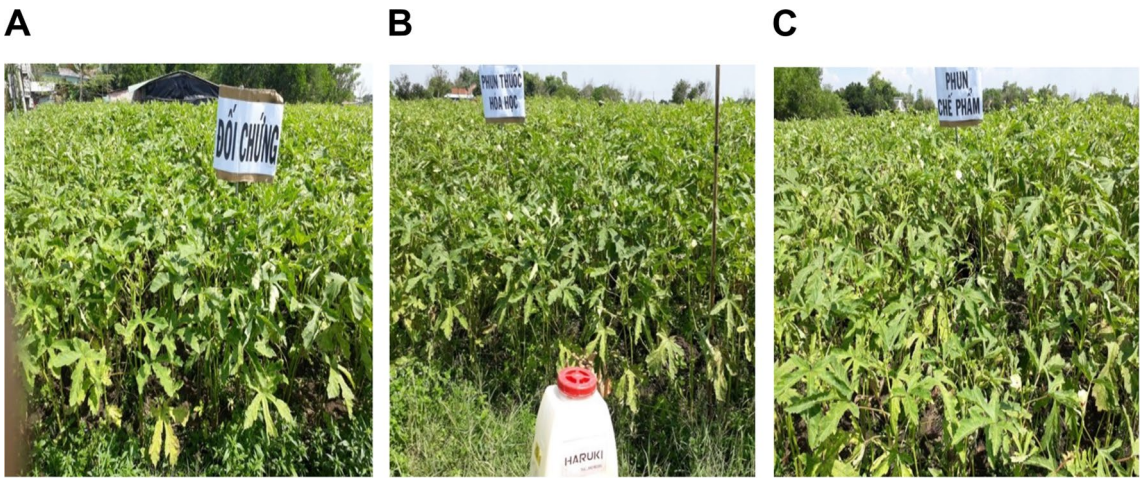

Fig. 7 Field assessment of the effectiveness of *Purpureocillium lilacinum* PL1 on the management of *Amrasca devastans* nymph's populations on okra plantations. **A** *A. devastans* nymph infected okra plants in the untreated plot, **B** *A. devastans* nymph infected okra plants in the abamectin treatment plot, **C** *A. devastans* nymph infected okra plants in the *P. lilacinum* PL1 treatment plot

Table 4 Effectiveness of *Purpureocillium lilacinum* PL1 and abamectin on the control of *Amrasca devastans* nymphs in okra field following 2 spraying treatments for 7 days

Treatments	Before treatment	1st treatment		2nd treatment	
	Number of *A. devastans* (nymphs/leaf)	Number of *A. devastans* nymphs/leaf	Percentage of nymph reduction (%)	Number of *A. devastans* (nymphs/leaf)	Percentage of nymph reduction (%)
Untreated	6.45 ± 2.12^{a}	6.61 ± 1.05^{a}	–	7.96 ± 2.31^{a}	–
Purpureocillium lilacinum PL1	6.90 ± 1.61^{a}	2.40 ± 1.98^{c}	64.46^{a}	2.27 ± 1.25^{c}	72.87^{a}
Abamectin	6.87 ± 1.33^{a}	4.50 ± 1.05^{b}	32.22^{b}	4.95 ± 2.03^{b}	34.67^{b}

Different superscript letters (a–c) within a column indicate statistically significant differences between groups ($p < 0.05$). The statistical analysis was conducted using ANOVA followed by Duncan's test ($p < 0.05$)

Discussion

Biopesticides derived from microbes are becoming more popular worldwide due to concerns about agrochemicals and demand for organic food. However, to ensure sustainable agricultural production, effective mass production and field application of biopesticides are necessary (Olson 2015). The selection of growth medium significantly impacts the primary cultivation of biocontrol agents, including *P. lilacinum* PL1. The growth rates of *P. lilacinum* PL1 mycelia were significantly affected by the nutrient media utilized, according to our research. MA and PDA media exhibit superior growth rates, whereas CZ medium yields weaker growth, indicating inadequate nutrient levels or unfavorable environmental conditions for the fungus. Furthermore, suitable fermentation substrates must also be considered for conidia production, taking into account factors such as availability and cost for conidia production (Hynes et al. 2006). Previous studies indicated that low-cost substrates such as rice, corn, and wheat brans can be used to mass production of different *Paecilomyces* spp. conidia, while other substrates like dried banana leaf, sorghum grains, used tea leaves, wheat bran-sawdust, and wheat bran-malt sprout mixture were suitable for high-density propagule production of *Trichoderma* spp. (Rini and Sulochana 2008). In this study, the growth of *P. lilacinum* PL1 on different organic materials in Viet Nam was investigated. It was indicated that *P. lilacinum* PL1 grew on all 4 solid substrates, but the level of colonization and biomass production varied. Rice and maize were the most suitable substrates for *P. lilacinum* PL1 conidia production, with densities exceeding 2×10^{10} conidia g^{-1}. This finding was consistent with a previous study that found *P. fumosoroseus*, isolated from dead lepidopteran caterpillars in India, produced more conidia when cultivated on sorghum than on corn, rice, pearl millet, or wheat (Sahayaraj and Namasivayam 2008).

Currently, agriculture utilizes an integrated approach that combines chemical compounds, cultural measures, resistant varieties, and biocontrol agents to achieve environmental sustainability. However, it is crucial to assess the potential interactions between chemical products and the development of biological agents. The present study investigated the impact of different phytosanitary agents on the growth of *P. lilacinum* PL1, revealing both positive and negative effects depending on the agent application. Specifically, the pesticide AM, a secondary metabolite of *Streptomyces avermitilis* that is biodegradable by microorganisms (Abd-Elgawad 2020), had a negative effect on *P. lilacinum* PL1 growth. This suggests that *P. lilacinum* PL1 may not be able to degrade AM. Similarly, a previous study reported that the growth and sporulation of *Trichoderma* spp. were also suppressed by AM (Mayo-Prieto et al. 2022). Furthermore, other insecticides such as CM, TM, and DF, which act on the neurotransmitter systems of insects (Wakita 2011), did not inhibit the growth of *P. lilacinum* PL1. CuNPs, which are used as fertilizers and antibacterial (Rojas et al. 2021), did not affect the growth of *P. lilacinum* PL1, as observed in previous studies indicated that *T. harzianum* had increased sporulation in the supplement of CuNPs (Banik et al. 2017). However, the present study found that broad-spectrum triazole fungicides such as HC, CZ, and PP completely inhibited the expansion of *P. lilacinum* PL1 colonies (Zhou et al. 2022). Thus, using *P. lilacinum* PL1 in conjunction with pesticides may be an effective pest management technique, but the use of fungicides in tandem with *P. lilacinum* PL1 should be strictly regulated to prevent unintended consequences.

The efficient usage of *P. lilacinum* PL1 to fight against *A. devastans* nymph was further examined in laboratory and field conditions. The efficacy of *P. lilacinum* PL1 was evaluated by assessing the population of *A. devastans* nymphs subsequent to a 7-day treatment at a density of 1×10^7 conidia ml^{-1}. These findings indicate that the utilization of *P. lilacinum* PL1 led to a noteworthy decrease in the population of *A. devastans* nymph, exhibiting an efficacy rate of 88.66% in vitro. The effectiveness demonstrated in this study is similar to the previous investigation, in which *P. lilacinum* PL1 was found to be successful in reducing *B. tabaci* nymph populations by a rate of 88.24% in vitro (Nguyen et al. 2023). Additionally, the results align with a previous study that demonstrated the efficacy of *P. lilacinum* XI-1 caused a lowering of 86.81% in adult whitefly populations within 7-day treatment (Sun et al. 2021). These findings demonstrated that *P. lilacinum* PL1 illustrates potential as a viable tool for pest management.

The efficacy of *P. lilacinum* PL1 in lowering the number of *A. devastans* nymphs in an okra plantation was also assessed in this study. The application of 1×10^7 conidia ml-1 of *P. lilacinum* PL1 resulted in a 64.46% reduction in nymph population after the first spraying, which further increased to 72.87% after the second spraying. These findings demonstrate the ability of P. lilacinum PL1 to effectively decrease *A. devastans* nymphs for a duration of up to 7 days. In the laboratory condition, AM was found to be more lethal to *A. devastans* nymphs than *P. lilacinum* PL1. However, under field conditions, the efficacy of AM dropped to 34.67%, significantly lower than the 72.87% mortality effect observed with *P. lilacinum* PL1. Sunlight, rising temperatures, and high soil moisture are all factors that might degrade AM and reduce its effectiveness under field conditions (Dionisio and Rath 2016). In the previous study, we indicated that *P. lilacinum* PL1 isolates thrive in tropical monsoon climates, as they can mature and sporulate at high temperatures up to 40 °C (Nguyen et al. 2023). Citrus psyllid (*Diaphorina citri*) populations may be lowered in the field by administering 1×10^7 conidia ml^{-1} of *Isaria fumosorosea* (Hoy et al. 2010). In addition, the *A. biguttula* population was reduced by 71.77–74.85%, when administrated with various biopesticides such *M. anisopliae, B. bassiana,* and *V. lecanii* (Janghel 2015). The results indicated that biopesticides are a viable alternative to chemical pesticides in managing sucking insects in okra. Integrated pest management involves combining chemical and biopesticide treatments for effective pest control. This study emphasizes the importance of exploring alternative methods for pest control to mitigate the negative impact of pesticides. Future studies are required to investigate the long-term effects of these interventions on the surrounding flora, soil, and ecological systems.

Conclusion

Utilization of microbial biopesticides, like *P. lilacinum* PL1, in sustainable agriculture is increasingly favored due to apprehensions regarding agrochemicals and the desire for organic food. The usage of cost-effective substrates for the production of *P. lilacinum* PL1 was crucial due to its contribution to sustainable pest management practices and economic benefits in developing countries. The present study indicated that rice and maize substrates were appropriated for enhancing the production of *P. lilacinum* PL1 conidia, particularly in an agricultural-oriented country like Vietnam. Furthermore, the effectiveness of *P. lilacinum* PL1 in reducing *A. devastans* nymph populations has been demonstrated in this study, including laboratory and field conditions. The promising findings highlight the potential of *P. lilacinum* PL1 as an effective and eco-friendly pest control agent, contributing to agriculture sustainable development. Nevertheless, the co-application of phytosanitary products might significantly

affect the growth of *P. lilacinum* PL1, yielding either positive or negative efficacy in pest management. Therefore, it is advisable to exercise caution and regulate the application of fungicides in conjunction with *P. lilacinum* PL1 to avoid any adverse outcomes.

Abbreviations

AM	Abamectin
CM	Cypermethrin
CuNPs	Copper nanoparticles
CZ	Carbenzim
CZ	Czapek-Dox
DF	Dinotefuran
EPF	Entomopathogenic fungi
HC	Hexaconazole
LT	Lethal time
MA	Malt agar
PDA	Potato dextrose agar
RCBD	Randomized complete blocks design
RH	Relative humidity
SA	Sabouraud agar
TM	Thiamethoxam

Acknowledgements
The authors are especially grateful to Nguyen Tat Thanh University and HUTECH University for providing all the resources needed for this study.

Author contributions
HNT and ADD designed this study; QNN, NQDT, and NLN 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.

Funding
Not applicable.

Availability of data and materials
All data generated or analyzed during this study are included in this manuscript.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Consent for publication was taken from the co-authors.

Competing interests
The authors have no competing interests to declare.

Author details
[1]Department of Biotechnology, Hutech Institute of Applied Sciences, HUTECH University, Ho Chi Minh City, Viet Nam. [2]Burnet School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, USA. [3]School of Global Health Management and Informatics, University of Central Florida, Orlando, USA. [4]Department of Biotechnology, NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Viet Nam.

Received: 1 June 2023 Accepted: 22 August 2023
Published online: 29 August 2023

References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18(2):265–267
Abd-Elgawad MM (2020) Optimizing biological control agents for controlling nematodes of tomato in Egypt. Egypt J Biol Pest Control 30(1):1–10
Abd-El-Khair H, Elshahawy I, Haggag H (2019) Field application of Trichoderma spp. combined with thiophanate-methyl for controlling Fusarium solani and Fusarium oxysporum in dry bean. Bull Natl Res Centre 43(1):1–9
Amala U, Jiji T, Naseema A (2013) Laboratory evaluation of local isolate of entomopathogenic fungus, Paecilomyces lilacinus Thom Samson (ITCC 6064) against adults of melon fruit fly, Bactrocera cucurbitae Coquillett. J Trop Agric 51(1):132–134
Banik S, Pérez-de-Luque A (2017) In vitro effects of copper nanoparticles on plant pathogens, beneficial microbes and crop plants. Span J Agric Res 15(2):e1005–e1005
Dionisio AC, Rath S (2016) Abamectin in soils: analytical methods, kinetics, sorption and dissipation. Chemosphere 151:17–29
Goffré D, Folgarait PJ (2015) Purpureocillium lilacinum, potential agent for biological control of the leaf-cutting ant Acromyrmex lundii. J Invertebr Pathol 130:107–115
Hassan S, Bigler F, Blaisinger P, Bogenschütz H, Brun J, Chiverton P, Dickler E, Easterbrook M, Edwards P, Englert W (1985) Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS working group 'pesticides and beneficial organisms.' Eppo Bulletin 15(2):214–255
Henderson CF, Tilton EW (1955) Tests with acaricides against the brown wheat mite. J Econ Entomol 48(2):157–161
Hoy MA, Singh R, Rogers ME (2010) Evaluations of a novel isolate of Isaria fumosorosea for control of the Asian citrus psyllid, Diaphorina citri (Hemiptera: Psyllidae). Florida Entomol 93(1):24–32
Hynes RK, Boyetchko SM (2006) Research initiatives in the art and science of biopesticide formulations. Soil Biol Biochem 38(4):845–849
Ibrahim A, Mohamed H, El-Naggar S, Swelim M, Elkhawaga O (2016) Isolation and selection of entomopathogenic fungi as biocontrol agent against the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae). Egypt J Biol Pest Control 26(2):249
Janghel M (2015) Efficacy of bio-pesticides against the jassid, Amrasca biguttula biguttula in okra at Bhubaneswar, Odisha. India Plant Archiv 15(2):833–836
Lavers A (2001) Guidelines on good practice for ground application of pesticides. Food and Agricultural Organization (FAO) of the United Nations, Rome
Manivannan A, Sridhar RP, Kamalakannan A, Ganapathy N, Karthikeyan S (2018) In vitro efficacy of bio control agents against cotton leafhopper, Amrasca biguttula biguttula (Ishida) (Homoptera: Cicadellidae). Int J Curr Microbiol App Sci 7(9):2026–2031
Mayo-Prieto S, Squarzoni A, Carro-Huerga G, Porteous-Álvarez AJ, Gutiérrez S, Casquero PA (2022) Organic and conventional bean pesticides in development of autochthonous Trichoderma strains. J Fungi 8(6):603
Morán-Diez ME, Glare TR (2016) What are microbial-based biopesticides? Microb Based Biopestic Methods Protoc 1477:1–10
Nagrare V, Bisane K, Deshmukh A, Kranthi S (2012) Studies on life cycle parameters of cotton leafhopper, Amrasca biguttula biguttula (Ishida). Entomon 37(1/4):93–99
Nguyen TH, Le Phung KY, Thai Thien ND, Nguyen TD, Do AD (2023) Insecticidal activity of isolated Purpureocillium lilacinum PL1 against whitefly, Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae) on cassava plantations in southern Viet Nam. Egypt J Biol Pest Control 33(1):1–11
Olson S (2015) An analysis of the biopesticide market now and where it is going. Outlooks Pest Manag 26(5):203–206
Pathak VM, Verma VK, Rawat BS, Kaur B, Babu N, Sharma A, Dewali S, Yadav M, Kumari R, Singh S (2022) Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: a comprehensive review. Front Microbiol 13:2833
Qiu L, Li J-J, Li Z, Wang J-J (2019) Production and characterization of biocontrol fertilizer from brewer's spent grain via solid-state fermentation. Sci Rep 9(1):480
Rayhane H, Josiane M, Gregoria M, Yiannis K, Nathalie D, Ahmed M, Sevastianos R (2019) From flasks to single used bioreactor: Scale-up of solid state fermentation process for metabolites and conidia production by *Trichoderma asperellum*. J Environ Manag 252:109496
Rehman A, Razaq M, Muhammad W (2015) Incidence and population dynamics of leaf hopper, *Amrasca bigutulla* (Cicadellidae: Homoptera) on four

varieties of okra (*Abelmoschus esculentus*) crop in Multan, Pakistan. Pak J Zool 47(3):763
Rini C, Sulochana K (2008) Substrate evaluation for multiplication of Trichoderma spp. J Trop Agric 45(1):55–57
Rojas B, Soto N, Villalba M, Bello-Toledo H, Meléndrez-Castro M, Sánchez-Sanhueza G (2021) Antibacterial activity of copper nanoparticles (Cunps) against a resistant calcium hydroxide multispecies endodontic biofilm. Nanomaterials 11(9):2254
Sadh PK, Duhan S, Duhan JS (2018) Agro-industrial wastes and their utilization using solid state fermentation: a review. Bioresour Bioprocess 5(1):1–15
Sahayaraj K, Namasivayam SKR (2008) Mass production of entomopathogenic fungi using agricultural products and by products. Afr J Biotechnol. https://doi.org/10.5897/AJB07.778
Sarwar M (2020) Insects as transport devices of plant viruses. In Applied Plant Virology. Elsevier, pp 381–402
Shah R, Al-Sadi AM, Nasser Al-Sabahi J, Al-Raeesi AA, Khamis Said Al-Rawahi K, Saud Al-Rashdi A, Fadhil Madad Al-Hinai S, Velazhahan R (2020) Efficacy of an Omani strain of Cordyceps javanica and its culture filtrate against whitefly (*Bemisia tabaci*) under laboratory conditions. All Life 13(1):615–622
Skinner M, Parker BL, Kim JS (2014) Role of entomopathogenic fungi in integrated pest management. Integr Pest Manag. https://doi.org/10.1016/B978-0-12-398529-3.00011-7
Sun T, Wu J, Ali S (2021) Morphological and molecular identification of four Purpureocillium isolates and evaluating their efficacy against the sweet potato whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae). Egypt J Biol Pest Control 31(1):1–9
Tong P (2016) Okra (*Abelmoschus esculentus*)–a popular crop and vegetable. UTAR Agric Sci J 2(3)
Wakita T (2011) Molecular design of dinotefuran with unique insecticidal properties. J Agric Food Chem 59(7):2938–2942
Zhou T, Guo T, Wang Y, Wang A, Zhang M (2022) Carbendazim: ecological risks, toxicities, degradation pathways and potential risks to human health. Chemosphere 314:137723

Publisher's Note