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Efficacy of indigenous *Beauveria bassiana* and *Purpureocillium lilacinum* for controlling *Planococcus minor* (Maskell) in durian fruits

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Abstract *Planococcus minor* (Maskell) spread in durian fields has a great impact on durian farmers. Because *P. minor* can damage durian at almost every stage, from fruit setting to harvest. Therefore, it is necessary to select indigenous entomopathogenic fungi that have the potential to control *P. minor* in durian fruits. The efficacy of indigenous *B. bassiana* and *P. lilacinum* for controlling *P. minor* (Maskell) in durian friuts was investigated. Insect pest samples were collected from durian orchards in three provinces: Chanthaburi, Trat, and Rayong. The collected samples were morphological identified to confirm the presence of *B. bassiana* and *P. lilacinum.* The results indicated that both spore suspension of *B. bassiana* and *P. lilacinum* at 10^4, 10^6, and 10^8 spore/ml were able to inhibit all growth stages of all *P. minor*. The concentration of 10^8 spore/ml resulted in the highest mortality rates for *P. minor* nymphs, achieving 97% and 100%, respectively. In conclusion, indigenous *B. bassiana* and *P. lilacinum* should be applied to *P. minor* at the nymph stage rather than during the adult stage, as the nymphs are more susceptible affected entomopathogenic fungi.

Keywords: Indigenous, *Beauveria bassiana*, *Purpureocillium lilacinum*, *Planococcus minor* (Maskell), Durian friuts

Introduction

Currently, durian farmers are facing problems with the spread of pest infestations that destroy durian crops from the initial stage of leaf development to the harvest. The pests causing significant damage include thrips, scale insects, mealybugs, psyllids, shot hole borer, longhorn beetles, and durian seed borers. According to Hodkinson (2009), in durian-growing areas in the eastern region, mealybugs (Hemiptera: *Pseudococcidae*) *Planococcus minor* (Maskell) are the primary pest. Mealybugs are found to cause damage throughout the year. They attack by sucking sap from branches, flower clusters, young fruits, and mature

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fruits, with red ants and black ants helping to transport them to various parts of the plant (Perez-Rodriguez *et al.,* 2021). The affected areas become stunted and stop growing. Additionally, mealybugs excrete honeydew, which attracts black mold, further reducing photosynthesis (Fabrice *et al.,* 2020). If mealybugs infest young durian fruits, the fruits will become stunted and will not grow. Although larger durian fruits may not suffer internal damage, their quality will deteriorate, leading to lower prices and rejection by consumers.

Due to this, durian farmers must find methods to prevent and control the spread of mealybugs, often by spraying chemical insecticides (Tudi *et al.,* 2021). However, there are limitations to using chemicals against mealybugs. Even when sprayed directly, the chemicals may not penetrate the mealybug's body because they are covered with a waxy coating that makes it difficult for the insecticide to be absorbed (Tong *et al.,* 2022). Additionally, mealybugs are small and can easily evade chemical spray (Hodkinson and Brid, 2006). As a result, chemical spraying offers limited or ineffective control (Tong *et al.,* 2024). Given these limitations, researchers have explored the use of entomopathogenic fungi, such as *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces lilacinus*, as an alternative approach (Zapata *et al.,* 2020; Ullah *et al.,* 2018; Mascarin and Jaronski, 2016). Currently, the use of entomopathogenic fungi has become very popular due to its ability to target a wide range of insect pests. These fungi can attach to insects and penetrate their internal tissues (Mascarin *et al.,* 2019), then grow and reproduce inside the insect's body, causing it to slow down, stop feeding, and eventually die (Luangsa-ard *et al.,* 2011). The dead insects are often covered with fungal mycelium and conidia of *B. bassiana*, which later spread to infect other insects (Erler and Ates, 2015). However, to effectively use entomopathogenic fungi, it is necessary to discover new strains with high efficacy or use them as a guideline for insect control. The advantage of using local fungi is that they can naturally coexist in the environment without needing to adapt to local conditions. Most importantly, they do not harm crops, allowing the plants to grow healthily and reducing pest damage (Anutrakunchai *et al.,* 2019).

Therefore, the objective was to test the effectiveness of indigenous entomopathogenic fungi in controlling mealybugs in durian-growing areas of eastern Thailand, providing an alternative method for managing mealybugs in these regions.

Materials and methods

Entomopathogenic fungal isolates

The insects were collected from a total of 3 orchards in the eastern region (Table 1). The insect samples included *Curculionidae*, *Scarabaeidae*, *Cerambycidae*, *Psyllidae*, mealybugs, thrips, shot hole borer, durian seed borer and fruit borer. After collection, the insects are kept individually in petri dishes in the laboratory and fed with young durian leaves to observe their behavior, including feeding and movement. If fungal growth is observed on the joints or body of an insect, it was isolated and placed in a humid environment to encourage the growth and multiplication of fungal structures such as mycelium or spores. Only fungi that grew rapidly on the insects were selected to minimize contamination by other microorganisms that grow later. These fungi were then be used in the process of isolating pure fungal cultures.

The method for isolating entomopathogenic fungi involved the Single Spore and Tissue Transplanting techniques. Spores or mycelia from the fungi growing on the insect are transferred using a needle onto water agar (WA) culture media and incubated for 3 days. Once the fungal mycelium grows to about 1-2 centimeters, it is transferred to Potato Dextrose Agar (PDA) for further growth. After 7 days, the fungi are examined under a microscope to study their morphology and classification at the molecular level were conducted by selecting primers and determining the optimal reaction temperature using primers targeting the rDNA region (ITS5 and ITS4). The primers ITS5 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.,* 1990) were used. The nucleotide sequence in the ITS region was analyzed using the maximum likelihood (ML) method with the MEGA 11.0 program, setting the bootstrap analysis value to 1000. The results were reported as a phylogenetic tree, following the standard methods of Samson *et al.* (1988) and Humber (1997). These fungi were then be used to test their effectiveness in controlling insect pests.

Table 1. Sources of insect sample on durian orchard in this study

Insect sample	Place (durian orchard)
Curculionidae, Scarabaeidae, Cerambycidae, Psyllidae, mealybugs, thrips, shot hole borer, durian seed borer, fruit borer	100 rai orchard, Kung Krabaen Bay, Chanthaburi
Shot hole borer, durian seed borer, fruit borer	Suan somboon, Rayong
Psyllidae, shot hole borer	Suan bo rai, Trat

Life cycle of mealybug

The mealybugs of stages 2 and 3 were collected which reared on pumpkin fruits and raise them on 120-day-old durian fruits, using 15-20 individuals per

fruit (Figure 1a). The mealybugs were divided and raised in black rectangular trays measuring 37 x 49 x 15 centimeters, with 3 fruits per tray, totaling 5 trays (Figure 1b). The growth of mealybugs in each stage was observed by counting the days of the mealybugs from the egg stage to the nymph and adult stages.

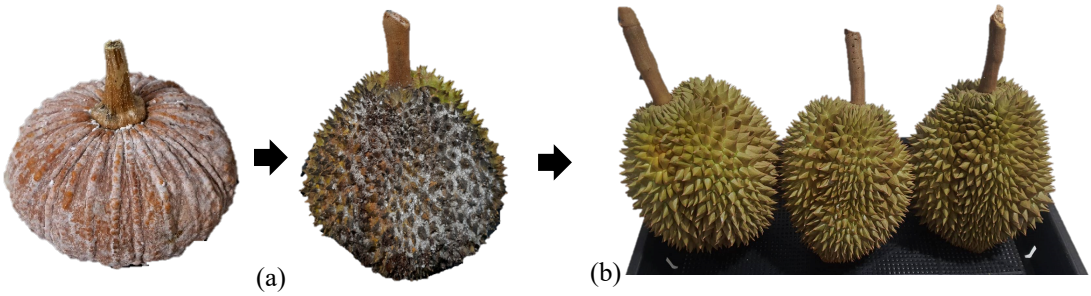

Figure 1. Cultivation of mealybugs for life cycle studies: (a) Pumpkin and durian fruits utilized as host plants for rearing mealybugs. (b) Durian fruits provided as food for mealybugs during the treatment phase

Effect of Beauveria bassiana and Purpureocillium lilacinus in controlling mealybugs on Durian fruits

B. bassiana and *P. lilacinus* were sprayed using a 2-milliliter glass spray bottle. Before spraying the fungi *B. bassiana* and *P. lilacinus*, the durian fruits were covered with a plastic container where the mealybugs released. The lid of the container was opened by hand to an extent that allowed the fungal spraying to spread and reached the area of the durian fruits with the mealybugs. The three concentration levels were 10^4, 10^6, and 10^8 spores per milliliter (spraying *B. bassiana* and *P. lilacinus* at 2 milliliters per durian fruit). The experiment was done using a Completely Randomized Design (CRD) consisting of 7 treatments, each with 3 repetitions, as follows: Treatment 1 was spray of sterilized water (Healthy control), Treatment 2 was spray of *B. bassiana* (10^4 spore/ml), Treatment 3 was spray of *B. bassiana* (10^6 spore/ml), Treatment 4 was spray of *B. bassiana* (10^8 spore/ml), Treatment 5 was spray of *P. lilacinus* (10^4 spore/ml), Treatment 6 was spray of *P. lilacinus* (10^6 spore/ml) and Treatment 7 was spray of *P. lilacinus* (10^8 spore/ml).

The experimental results were recorded by counting the number of dead mealybugs in each treatment. Data were calculated the mortality percentage of the mealybugs and conducted a statistical analysis for comparison. The growth of *B. bassiana* and *P. lilacinus* were observed the fungal mycelia growing on the mealybugs under a Stereomicroscope, and took photographs.

Results

Identification and characterization of Entomopathogenic fungals

Entomopathogenic fungi from 9 species of insect pests—namely, *Curculionidae*, *Scarabaeidae*, *Cerambycidae*, *Psyllidae*, mealybugs, thrips, shot hole borer, durian seed borer and fruit borer caterpillar—it was found that there were two types of entomopathogenic fungi: *Beauveria* sp. and *Purpureocillium* sp. The *Beauveria* sp. fungus has white, smooth, powdery hyphae on the surface of the culture medium, with growth gradually averaging about 1.5 centimeters per day, taking 14 days to completely cover the entire culture plate. When observed under a microscope, reproductive structures called conidia were found to be round, transparent, and separated individually at the top of the conidiophores (Figure 2. a-c). For *Purpureocillium* sp., the hyphae were reddish-brown, layered in circular rings with a smooth, powdery appearance on the surface of the culture medium, also taking 14 days to fully cover the culture plate. When examined under a microscope, conidia were found to be round, transparent, and separated individually at the top of the conidiophores (Figure 2.d-f). Through molecular analysis using primers in the rDNA region (ITS5 and ITS4), with primer ITS5 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), and ITS comparison with other *Beauveria* sp. and *Purpureocillium* sp. strains identified the fungi as *Beauveria bassiana* (KMB2) and *Purpureocillium lilacinus* (PPO1) (Figure 3).

Life cycle of mealybugs

Mealybugs have a distinct life cycle consisting of several stages: Egg Stage: Female mealybugs lay clusters of eggs mass, often protected by a waxy coating 7-8 day. Nymph Stage: After hatching, the young mealybugs (nymphs) emerge and go through several 1stinstars- 3rd instar 13 days (growth phases) while feeding on plant sap. Adult Stage: Upon maturity, they develop into adults. Adult females are generally larger and more sedentary 16 days, whereas males are smaller and may have wings in 8 days. Reproduction: Adult females can reproduce multiple times, perpetuating total the life cycle 50 days (Figure 4).

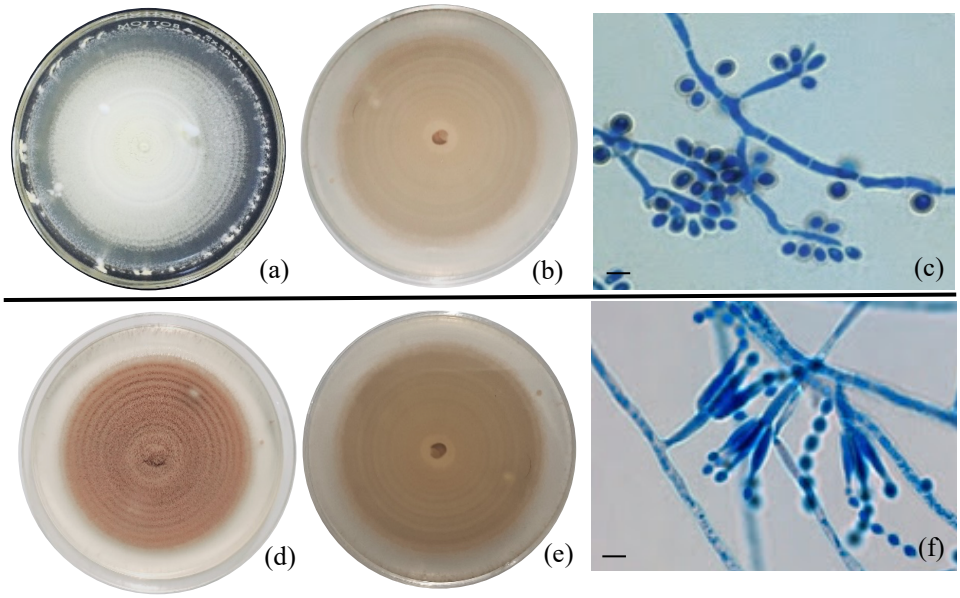

Figure 2. Morphological characteristics of entomopathogenic fungi: (a-b) *Beauveria* sp., showing growth from top-view and slide-view on PDA after 14 days. (c) Conidia and conidiophores of *Beauveria* sp. (d-e) *Purpureocillium* sp., growth from top-view and slide-view on PDA at 14 days. (f) Conidia and conidiophores of *Purpureocillium* sp.

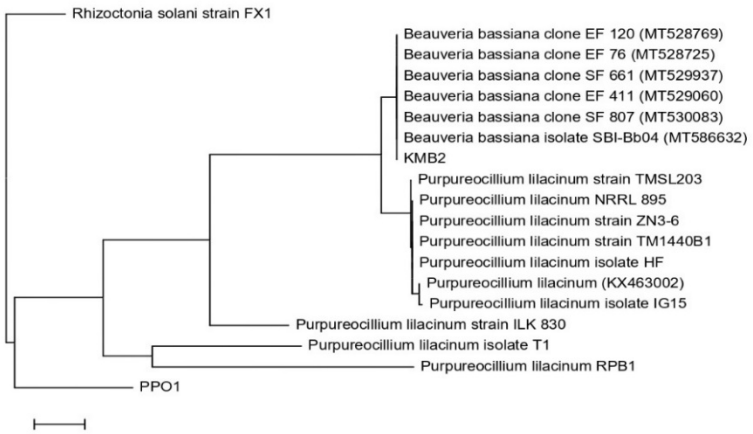

Figure 3. Classification of *Beauveria bassiana* (KMB2) and *Purpureocillium lilacinus* (PPO1) phylogenetic tree obtained from the analysis of the nucleotide sequence in the ITS by maximum likelihood (ML) on MEGA 11.0 configure bootstrap analysis to value equal to 1000

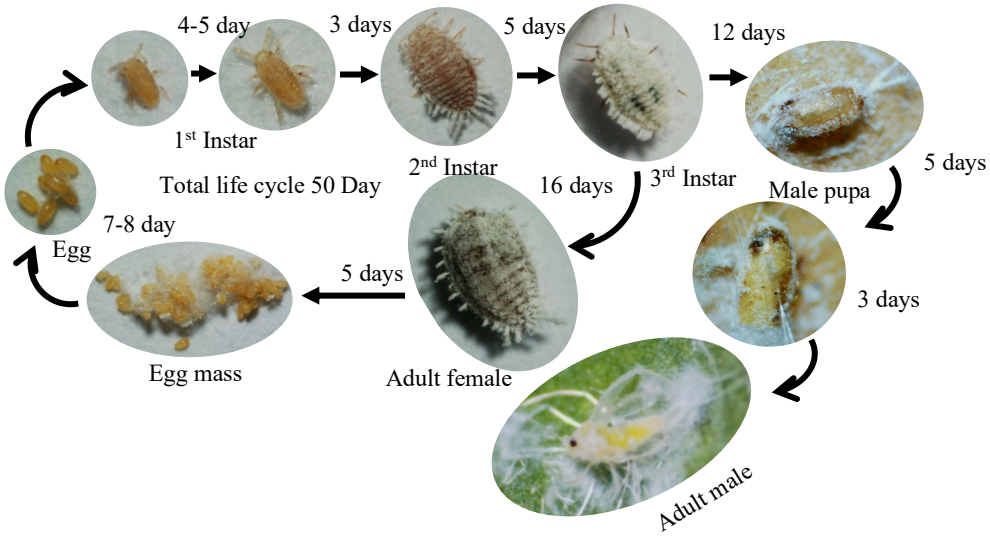

Figure 4. Life cycle of a hemimetabolous insect, based on a mealybug *Planococcus minor* (Maskell)

Effect of Beauveria bassiana and Purpureocillium lilacinus in controlling mealybugs on Durian fruits

Testing of *Beauveria bassiana* and *Purpureocillium lilacinus* in controlling mealybugs, it was found that using both fungi at all three concentrations (10^4, 10^6, and 10^8 spores per milliliter) effectively controlled the growth of the mealybugs. Specifically, 1–3 days after spraying *B. bassiana* and *P. lilacinus*, the mortality rate ranged from 18% to 79%. Observing the mealybugs revealed that the white fungal hyphae of *B. bassiana* grew over and covered the mealybug nymph (Figure 5). In the case of *P. lilacinus*, the mealybugs showed abnormalities, becoming swollen, ruptured, and leaking internal fluids, with reddish-brown hyphae covering their bodies. (Figure 5) As time progressed until the end of the experiment (7 days after spraying), the results were consistent with the initial period. The treatment with *P. lilacinus* showed a 100% mortality rate of mealybugs, while *B. bassiana* resulted in a 97% mortality rate (Table 2). The concentrations of 10^6 and 10^4 spores per milliliter followed, respectively, with statistically significant differences (Figure 6).

The experimental results can be summarized as follows: A statistical comparison of the daily mortality rates of mealybugs and the different concentrations of *B. bassiana* and *P. lilacinus* at all 3 concentration levels showed significant differences. It was observed that higher fungal concentrations resulted in a higher daily mortality rate of mealybugs. Furthermore, the wax-

coated stage of the mealybug larvae also influenced the experiment, as the fungi might not be effective if the spray did not directly contact the insects.

Table 2. Mortality of *Planococcus minor* (Maskell) Nymphs Infected by *Beauveria bassiana* and *Paecilomyces lilacinus*

Treatment	**Mortality (%)[1]**			
Nymph of mealybugs on durian fruits	1 DAI[2]	3 DAI	5 DAI	7 DAI
Tr1 Healthy control	0c[3]	0c	0c	0c
Tr2 Spray of *B. bassina* (10^4 spore/ml)	18b	39b	68b	75b
Tr3 Spray of *B. bassina* (10^6 spore/ml)	32b	65b	76b	83a
Tr4 Spray of *B. bassina* (10^8 spore/ml)	58a	76a	89a	97a
Tr5 Spray of *P. lilacinus* (10^4 spore/ml)	24c	42c	56b	65b
Tr6 Spray of *P. lilacinus* (10^6 spore/ml)	37b	57b	83a	91a
Tr7 Spray of *P. lilacinus* (10^8 spore/ml)	68a	79a	92a	100a
C.V. (%)	18.9	15.8	12.9	8.1
F-value	*	*	*	*

[1] Percent of Mortality = (total number of insects – number of insect dead) x 100
[2] DAI = Day after inoculation
[3] Average followed by lowercase English letters in column rows have no significant statistical differences P= 0.05 by Duncan's Multiple Range Test (DMRT).

Figure 5. Nymph of *Planococcus minor* (Maskell) infected by *Beauveria bassiana* and *Paecilomyces lilacinus* (10^8 spore/ml) at 1,3 and 7 days after inoculation

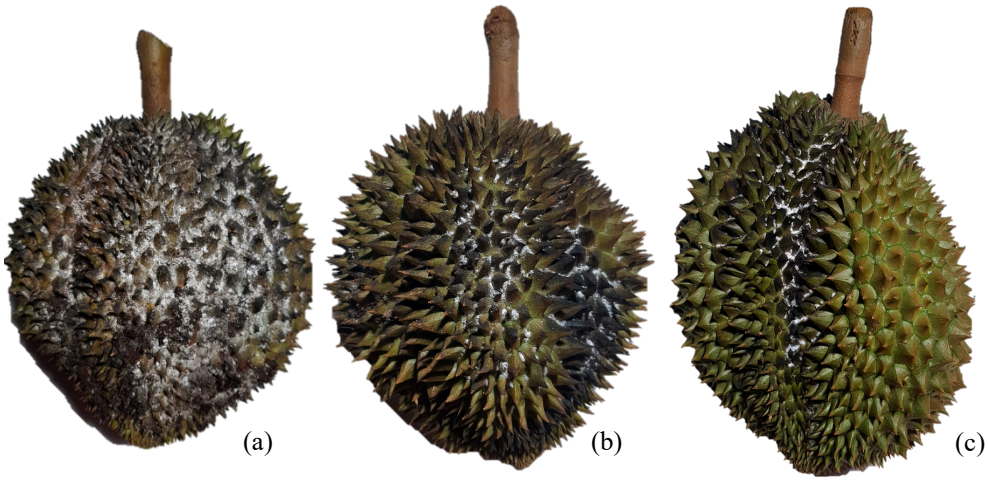

Figure 6. Efficacy of Entomopathogenic fungi for controlling *Planococcus minor* (Maskell) in durian fruits: (a) Healthy control. (b) *Beauveria bassiana*, (c) *Purpureocillium lilacinum* at 10^8 spore/ml. (7 Days after inoculation)

Discussion

From isolating entomopathogenic fungi from nine species of insect pests—namely, *Curculionidae*, *Scarabaeidae*, *Cerambycidae*, *Psyllidae*, mealybugs, thrips, shot hole borers, durian seed borers, and fruit borers—it was found that there were two types of entomopathogenic fungi: *Beauveria* sp. and *Purpureocillium* sp. Molecular analysis using primers in the rDNA region (ITS5 and ITS4) identified these fungi as *Beauveria bassiana* (KMB2) and *Purpureocillium lilacinus* (PPO1). This aligns with studies by Dowd and Vega (2003), Muerrle *et al.* (2006), and Mascarin and Jaronski (2016), which isolated entomopathogenic fungi from insect pests and found that both *B. bassiana* and *P. lilacinus* can infect a wide range of insects, including weevils, fruit beetles, scarab beetles, fruit flies, brown planthoppers, and mosquitoes (Luangsa-ard *et al.,* 2011; Moreno-Gavira *et al.,* 2020). Additionally, *B. bassiana* was also found to infect durian shot hole borers (Anutrakunchai *et al.,* 2019). In testing the effectiveness of the fungi *B. bassiana* and *P. lilacinus* in controlling mealybugs, it was found that using both fungi at all three concentrations (10^4, 10^6, and 10^8 spores per milliliter) was effective in controlling the growth of mealybugs. The concentration of 10^8 spores/ml resulted in the highest percentage of mortality for *P. minor* nymphs, achieving rates of 97% and 100%, respectively. This aligns with research by Boston *et al.* (2020), which tested the fungus *B. bassiana* on fruit beetles (*Carpophilus* spp.) and found a larval mortality rate of approximately 73-80%. Similarly, the study by Mascarin and Jaronski (2016)

tested *B. bassiana* at concentrations ranging from 10^3 to 10^7 spores per milliliter, effectively inhibiting the growth of various insect species, such as scarab beetles, weevils, planthoppers, and fruit flies (Burckhardt *et al.,* 2014). Additionally, *B. bassiana* and *P. lilacinus* have also been used against non-pest insects, such as mosquitoes (Zapata *et al.,* 2020; Fabrice *et al.,* 2020; Ullah *et al.,* 2018; Bukhari *et al.,* 2011). Currently, aside from using the fungus *P. lilacinus* for controlling insect pests, there have also been reports of its use in controlling root-knot nematodes in eggplant and chickpea. *P. lilacinum* effectively reduced the nematode population and the number of galls in plant roots. Interestingly, the application of *P. lilacinum* resulted in significant enhancements in plant growth and biomass, even under nematode infection, while it improved plant photosynthetic pigments, such as chlorophyll and carotenoids (Khan and Tanaka, 2023; El-Marzoky *et al.,* 2023; Rajendran *et al.,* 2024).

In summary, the results of this experiment represent only a preliminary test conducted in a controlled laboratory environment, where factors such as temperature and humidity can be regulated. To obtain conclusive results, further studies need to be conducted in actual field conditions to assess the effectiveness and survival of *B. bassiana* and *P. lilacinus* in nature. Additionally, increasing the fungal population to sufficient levels for spraying in large durian orchards is necessary. The information gathered can then be disseminated to interested farmers, providing them with guidelines to help address mealybug infestations in durian orchards in the future.

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