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RESEARCH PAPER

Synergistic effects of *Lecanicillium lecanii* and botanical extracts on growth and virulence against *Nilaparvata lugens* Stål. (Hemiptera: Delphacidae)

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ABSTRACT

This study evaluated the synergistic potential of the entomopathogenic fungus *Lecanicillium lecanii* combined with plant extracts from *Dioscorea hispida* and *Annona muricata* (soursop) for controlling the brown planthopper (*Nilaparvata lugens*). The research addresses the limited adoption of integrated pest management (IPM) and the continued reliance on chemical pesticides, which contribute to environmental risks and pest resistance. The experiment was conducted in two stages. The first stage examined fungal growth on media supplemented with different concentrations of plant extracts. The addition of plant extracts significantly enhanced colony growth, conidial density, and germination compared with the control. The highest conidial density (1.38×10^9 conidia/mL) and germination rate (91.75%) were obtained with *D. hispida* extract at 5 g/L, indicating superior nutritional support for fungal development. The second stage evaluated the virulence of the optimized fungal culture against *N. lugens* nymphs. Mortality increased in both dose-dependent and time-dependent patterns. The highest concentration (10^9 conidia/mL) resulted in 92.71% mortality within seven days, with a lethal time (LT_{50}) of 3.27 days. Improved conidial density and germination contributed directly to enhanced fungal infectivity and pathogenicity. In conclusion, supplementation of *L. lecanii* with *D. hispida* and *A. muricata* extracts improves fungal growth and significantly increases its effectiveness against *N. lugens*. This synergistic approach provides a promising environmentally friendly alternative to chemical insecticides and supports the development of sustainable IPM strategies for tropical rice agroecosystems.

Keywords: Brown planthopper, entomopathogen, integrated pest management, *Lecanicillium lecanii*, plant extracts

INTRODUCTION

The brown planthopper (*Nilaparvata lugens* Stål) is one of the most destructive pests in rice production, causing significant yield losses and reducing grain quality. In addition to direct feeding damage, this pest also acts as a vector of important viral diseases such as rice grassy stunt virus (RRSV) (Baehaki & Mejaya, 2014; Yuliani & Agustian, 2020). Despite growing awareness of integrated pest management (IPM) in rice cultivation, its implementation remains suboptimal. Many farmers still rely heavily on chemical pesticides for pest control (Pamungkas et al., 2020). Improper application of these chemicals often results in ineffective control and may even increase pest populations (Triwidodo, 2020). Excessive pesticide use can also induce pest resistance, trigger pest resurgence, reduce natural enemy populations, and contribute to environmental pollution (Hadi et al., 2014). These negative impacts highlight the need for more sustainable pest management strategies.

One alternative approach within IPM is the use of biological control agents. These include insects, bacteria, viruses, nematodes, protozoa, and fungi (Khaerati & Indriati, 2015). Among them, entomopathogenic fungi are particularly promising due to their ability to infect insect hosts directly through the cuticle (Trizelia et al., 2015). Several entomopathogenic fungi such as *Metarhizium anisopliae*, *Beauveria bassiana*, and *Lecanicillium lecanii* have been widely studied for pest control (Afifah et al., 2022). *Lecanicillium lecanii* is especially attractive because of its broad host range and cosmopolitan distribution in tropical and subtropical regions (Fadhilah & Asri, 2019). This fungus can infect various insect orders including Hemiptera, Lepidoptera, Coleoptera, Orthoptera, and Thysanoptera (Widan et al., 2022), making it suitable for controlling rice pests such as *N. lugens*.

Previous studies demonstrated the effectiveness of *L. lecanii* in controlling insect pests. Afifah et al.

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(2022) reported that *L. lecanii* caused 74% mortality of *Cylas formicarius* at a concentration of 10^9 conidia/mL (Afifah et al., 2022). Similarly, Khoiroh et al. (2014) found that *L. lecanii* suppressed *N. lugens* populations with mortality reaching 78.33% at 10^{10} conidia/mL. The infection process involves conidial attachment to the insect cuticle, germination, penetration, and subsequent colonization of host tissues leading to insect death (Ortiz-Urquiza & Keyhani, 2016; Shahid et al., 2012). Although effective, the infection process is relatively gradual, and therefore combining fungi with other control agents may enhance their performance.

Plant-based pesticides represent another environmentally friendly control option. Botanical extracts contain bioactive compounds such as alkaloids, flavonoids, and acetogenins that interfere with insect feeding behavior and physiological processes (Asmanizar, 2023; Muhidin et al., 2020). The integration of botanical extracts with entomopathogenic fungi may create complementary effects, where plant extracts provide rapid physiological disruption while fungi contribute to longer-term infection (Joob & Wiwanitkit, 2014; Pinto et al., 2018; Wakil et al., 2017). Such combinations have been reported to enhance pest mortality and reduce the risk of resistance development (Mantzoukas & Eliopoulos, 2020). In addition, plant extracts may improve fungal growth and conidial production, thereby increasing fungal virulence.

Several plant species have potential as botanical pesticides, including soursop (*Annona muricata* L.) and asiatic bitter yam (*Dioscorea hispida* Dennst.). Soursop seeds contain acetogenins that can disrupt insect feeding behavior (Asmanizar et al., 2020), whereas *D. hispida* contains dioscorin, diosgenin, and dioscin that affect insect nervous systems (Muhidin et al., 2020). Despite their potential, information regarding the combined use of these plant extracts with *L. lecanii*, particularly against *N. lugens*, remains limited.

Therefore, this study aimed to evaluate the synergistic interaction between *L. lecanii* and botanical extracts of *A. muricata* and *D. hispida* against *N. lugens*. Specifically, this research examined (i) the effect of plant extracts on fungal growth and conidia production and (ii) the combined efficacy in increasing virulence against *N. lugens* nymphs. The results of this study are expected to contribute to the development of environmentally friendly pest management strategies and strengthen IPM implementation in rice ecosystems.

MATERIALS AND METHODS

Research Site. This research was conducted at the Plant Pest Laboratory, Faculty of Agriculture, Universitas Singaperbangsa Karawang and the Biological Agent Laboratory, Balai Besar Peramalan Organisme Pengganggu Tumbuhan, Jatisari, Karawang. All experiments were carried out under laboratory conditions (25 ± 2 ºC; 70–80% RH; 12:12 h light-dark photoperiod).

Preparation of Plant Extracts. The *A. muricata* seed extract was prepared following Ramadhan & Nurhidayah (2022) with slight modifications. Seeds were cleaned, peeled, dried for one week, and ground into powder. Four hundred grams of powder were macerated in 1 L of 96% acetone for 24 h (Baideng, 2016). The mixture was filtered, and the filtrate was evaporated in a vacuum oven at 50 ºC to obtain a concentrated extract.

The *D. hispida* tuber extract was prepared according to Jannah et al. (2022). Tubers were washed, sliced, dried for one week, and ground into powder. The powder was macerated in 96% ethanol for 24 h, filtered, and evaporated at 50 ºC to obtain a paste-like extract.

Fungal Growth Test. A single-factor completely randomized design with seven treatments and four replications was used (Table 1). Potato Dextrose Agar (PDA) supplemented with *A. muricata* seed extract and *D. hispida* extract was used as growth medium for *L. lecanii*. The extracts were added to PDA, poured into 9 cm Petri dishes, and allowed to solidify. The fungus was inoculated using an N-needle and incubated for 21 days. Colony diameter, conidial density, and germination rate were observed at 3-day intervals. Data were analyzed using ANOVA followed by Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$.

Pathogenicity Test. The treatment showing the highest conidial density and germination rate was selected for bioassay against *N. lugens*. The experiment was arranged in a single-factor completely randomized design with four conidial density treatments and five replications (Table 2). Each experimental unit consisted of 10 third-instar nymphs placed on rice stems and sprayed with *L. lecanii* conidial suspension, as illustrated in Figure 1. Mortality was recorded daily for 7 days. Dead insects were confirmed by absence

of movement and the presence of mycelial growth on cadavers. Parameters observed included nymphal mortality, median lethal time (LT_{50}), median lethal concentration (LC_{50}), and fungal emergence. Data were analyzed using ANOVA followed by Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$. LT_{50} and LC_{50} values were estimated using probit analysis (Finney, 1971) with SPSS v.26.

RESULTS AND DISCUSSION

Growth of *L. lecanii* in Different Media. The colony diameter of *L. lecanii* was significantly influenced by incubation time, with longer incubation resulting in increased colony growth. Figure 2 illustrates the development of *L. lecanii* over a 21-day incubation period in media supplemented with different concentrations of *A. muricata* and *D. hispida* extracts compared to the control. During the early incubation phase (up to 6 days), all treatments exhibited similar growth patterns. However, distinct differences became apparent thereafter. Media supplemented with plant extracts, particularly *A. muricata* at 3 and 5 mL/L and *D. hispida* at 5 g/L, promoted faster radial growth and produced larger colony diameters than the control. By day 12, colonies grown in extract-supplemented media reached approximately 9 cm in diameter and subsequently plateaued, whereas the control treatment reached its maximum diameter (8.85 cm) only by day 18. These results indicate that supplementation with plant extracts significantly accelerated and enhanced the growth of *L. lecanii* (Figure 2).

The enhanced growth observed in extract-supplemented media is likely associated with differences in nutrient availability. The growth of *L. lecanii* is known to vary depending on the nutrient

Table 1. Treatments of *L. lecanii* growth media supplemented with different concentrations of *A. muricata* seed extract and *D. hispida* extract

Code	Treatment
Control	*L. lecanii* (Control)
AM1	*L. lecanii* + *A. muricata* seed extract (1 mL/L)
AM2	*L. lecanii* + *A. muricata* seed extract (3 mL/L)
AM3	*L. lecanii* + *A. muricata* seed extract (5 mL/L)
DH1	*L. lecanii* + *D. hispida* extract (1 g/L)
DH2	*L. lecanii* + *D. hispida* extract (3 g/L)
DH3	*L. lecanii* + *D. hispida* extract (5 g/L)

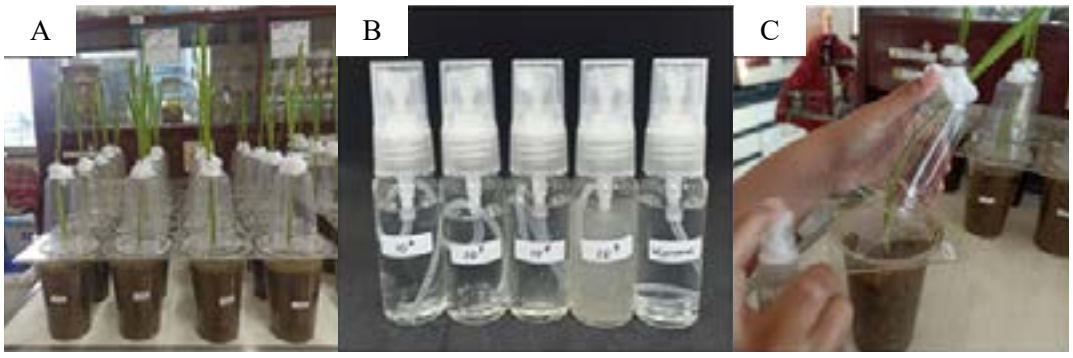

Figure 1. Mortality bioassay of *N. lugens*. A. Testing arena; B. *L. lecanii* conidial suspension; C. Application to nymphs.

Table 2. Conidia density treatments of *L. lecanii* against *N. lugens*

Code	Treatment
Control	Distilled water
A	10^6 conidia/mL
B	10^7 conidia/mL
C	10^8 conidia/mL
D	10^9 conidia/mL

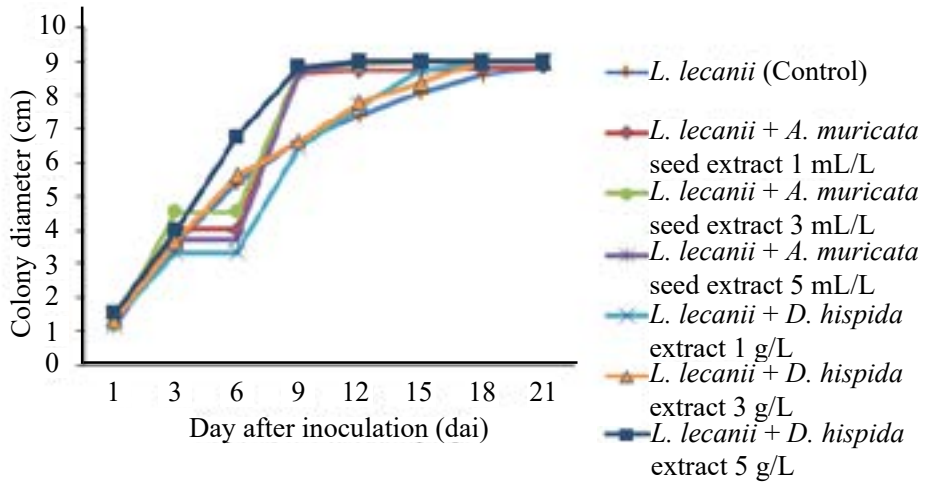

Figure 2. Average colony diameter of *L. lecanii* over time on growth media supplemented with *A. muricata* and *D. hispida* extracts.

composition of the culture medium, which plays a crucial role in fungal development. According to Afifah et al. (2022), nutrient availability is a key factor affecting the growth of entomopathogenic fungi. Essential nutrients required for fungal development include carbon, nitrogen, and mineral elements such as zinc (Zn), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), and manganese (Mn), as well as vitamins and water (Senja et al., 2023). Plant extracts such as *A. muricata* seeds and *D. hispida* tubers may provide additional nutrients and organic compounds that stimulate fungal metabolism and growth (Baideng, 2016; Jannah et al., 2022). Furthermore, Safavi et al. (2007) reported that the nutritional composition of the culture medium directly affects both the growth rate and colony diameter of entomopathogenic fungi, highlighting the importance of appropriate media selection for optimizing fungal development in biocontrol applications.

The nutritional content of *D. hispida* tubers (per 100 g) includes 23.2 g carbohydrates, 2.1 g protein, 0.2 g fat, 20.0 mg calcium, 69.0 mg phosphorus, and 0.6 mg iron (Siqhny & Putri, 2022). In comparison, *A. muricata* extract dried at 50 ºC contains 19.33 g carbohydrates, 17.85 g protein, and 18.18 g fiber (Olabinjo & Odunayo, 2020). Additionally, *A. muricata* seeds contain 22–24% oil, consisting of 28.07% saturated fatty acids and 71.93% unsaturated fatty acids (Rahmani, 2008). Unsaturated fatty acids are particularly beneficial because they are readily hydrolyzed by lipase enzymes produced by entomopathogenic fungi and can serve as carbon and energy sources for growth (Cliquet & Jackson, 2005). Therefore, the nutritional profile of these plant extracts likely enriched the culture media and supported the rapid development of *L. lecanii* colonies observed in this study.

These findings are consistent with previous studies. Dewi et al. (2022) reported that supplementation with *A. muricata* seed extract at 5 mL/L increased the colony diameter of *L. lecanii* to 8.82 cm, which is comparable to the results obtained in the present study. Similarly, Prayogo (2011) demonstrated that the addition of peanut, soybean, and coconut oil extracts at 10 mL/L enhanced the growth and development of *L. lecanii.* In contrast, Susanti et al. (2016) reported that the growth of *B. bassiana* on PDA control medium (3.20 cm) was higher than that on media supplemented with *Ageratum conyzoides* leaf extract, indicating the presence of inhibitory compounds in the extract. This difference suggests that plant extracts may either stimulate or inhibit fungal growth depending on their phytochemical composition. The superior growth observed in *A. muricata* and *D. hispida* treatments in this study indicates that these extracts provide favorable nutritional conditions for *L. lecanii*. Such species-specific interactions between plant-derived substrates and entomopathogenic fungi highlight the importance of selecting suitable additives to optimize fungal cultivation. The morphological development of *L. lecanii* at 21 days after inoculation is presented in Figure 3.

Impact of Plant Extracts on *Lecanicillium lecanii* Conidia Density. The effect of plant extract supplementation on the growth of *L. lecanii* was evaluated based on conidial density (Table 3). The control treatment produced a conidial density of 0.91×10^9 conidia/mL. The addition of *A. muricata* seed extract at concentrations of 1 mL/L and 3 mL/L

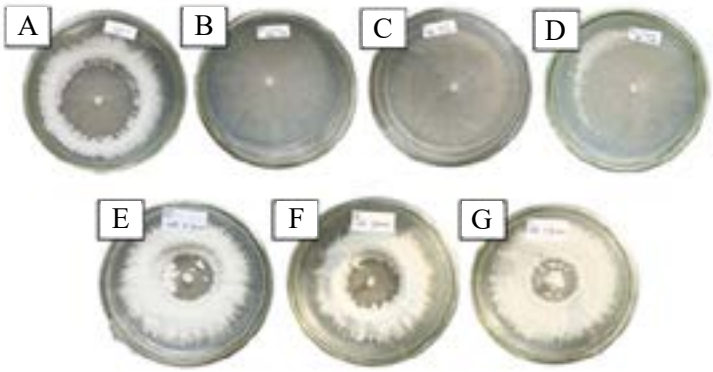

Figure 3. Colony morphology of *L. lecanii* at 21 days after inoculation (dai). A. Control; B. *A. muricata* seed extract (1 mL/L); C. *A. muricata* seed extract (3 mL/L); D. *A. muricata* seed extract (5 mL/L); E. *D. hispida* extract (1 g/L); F. *D. hispida* extract (3 g/L); G. *D. hispida* extract (5 g/L).

Table 3. Average conidial density of *L. lecanii* on growth media supplemented with different concentrations of plant extracts

Treatment	Conidia density (conidia/mL)
L. lecanii (Control)	0.91×10^9 d
L. lecanii + *A. muricata* seed extract (1 mL/L)	0.84×10^9 cd
L. lecanii + *A. muricata* seed extract (3 mL/L)	0.93×10^9 cd
L. lecanii + *A. muricata* seed extract (5 mL/L)	1.07×10^9 bc
L. lecanii + *D. hispida* extract (1 g/L)	1.15×10^9 ab
L. lecanii + *D. hispida* extract (3 g/L)	1.28×10^9 a
L. lecanii + *D. hispida* extract (5 g/L)	1.38×10^9 a

Means followed by the same letter are not significantly different (DMRT, 5%) after $\sqrt{(x+0.5)}$ transformation.

resulted in conidial densities of 0.84×10^9 and 0.93×10^9 conidia/mL, respectively, which were not significantly different from the control. However, supplementation with *A. muricata* extract at 5 mL/L significantly increased conidial density to 1.07×10^9 conidia/mL. A more pronounced enhancement was observed with *D. hispida* extract. The lowest concentration (1 g/L) produced 1.15×10^9 conidia/mL, while the highest concentration (5 g/L) resulted in the maximum conidial density of 1.38×10^9 conidia/mL. These findings indicate that *D. hispida* extract, particularly at higher concentrations, substantially promotes conidia production, suggesting its potential as an effective supplement for improving fungal growth and biocontrol performance of *L. lecanii*.

Conidial density is an important parameter in evaluating the effectiveness of entomopathogenic fungi, as higher spore densities generally lead to increased infection efficiency and faster host mortality. The variation in conidial production among treatments is likely related to differences in nutrient availability provided by each plant extract. Fungi require carbohydrates as carbon sources for metabolic processes and proteins as nitrogen sources, which are essential for vegetative growth and sporulation. The tubers of *D. hispida* contain relatively high carbohydrate levels (23.2 g/100 g), whereas *A. muricata* seeds contain approximately 19.33 g/100 g carbohydrates. The higher carbohydrate content in *D. hispida* may provide greater energy for fungal metabolism and conidia formation, explaining the consistently higher conidial densities observed in *D. hispida* treatments. These results support the concept that nutrient composition of the culture medium strongly influences colony development and conidia production, which ultimately determines fungal effectiveness as a biological control agent.

The conidial densities obtained in this study were higher than those reported in previous research. The highest density observed with *D. hispida* extract at 5 g/L (1.38×10^9 conidia/mL) exceeded the maximum value of 2.41×10^8 conidia/mL reported by Dewi (2022) using *A. muricata* seed extract. Similarly, the present results were higher than the conidial density of 1.06×10^8 conidia/mL obtained by Prayogo (2011) with peanut oil extract. These comparisons highlight the superior performance of *D. hispida* tuber extract as a growth-medium additive for enhancing conidia

production of *L. lecanii*. The increased conidial density observed in this study suggests improved fungal vigor, which is expected to enhance infectivity and support the development of more effective and sustainable biological control strategies.

Conidia Germination of *L. lecanii* as Influenced by Plant Extracts. The germination of *L. lecanii* conidia was significantly affected by supplementation with plant extracts (Table 4). The control treatment, without plant extract addition, showed a germination rate of 78.56% after 16 hours of incubation. The addition of *A. muricata* seed extract at 1 mL/L produced a comparable germination rate (79.54%) and did not differ significantly from the control. However, increasing the concentration to 3 mL/L and 5 mL/L significantly enhanced germination to 86.37% and 82.07%, respectively. A more pronounced effect was observed with *D. hispida* extract, which exhibited a clear dose-dependent response. The highest germination rate (91.75%) was obtained at 5 g/L of *D. hispida*, representing a substantial improvement compared with the control. These results demonstrate that both plant extracts promoted conidial germination, with *D. hispida* showing the greatest stimulatory effect.

The enhanced germination observed in *D. hispida*-supplemented media may be attributed to bioactive compounds present in the tuber, such as dioscorin and dioscin, which are known to stimulate metabolic activity (Joob & Wiwanitkit, 2014; Siqhny & Putri, 2022). These compounds may facilitate enzymatic processes required for spore activation and germ tube formation. In contrast, *A. muricata* seeds contain acetogenins and alkaloids (Pinto et al., 2018; Tando, 2018), which may stimulate enzymatic pathways, including amylase and protease activity, thereby accelerating germ tube emergence. Such biochemical stimulation likely improves conidial vigor and supports early fungal development.

Conidial germination is a critical indicator of fungal viability and effectiveness as a biological control agent. According to Pertiwi et al. (2016), germination capacity is influenced by several factors, including nutrient composition of the growth medium, temperature, and humidity. The present results suggest that nutrient availability, particularly balanced carbohydrates and proteins, plays a major role in promoting germination. Carbohydrates provide energy for metabolic activation, while proteins supply nitrogen for cellular development. However, as noted by Afifah et al. (2022), high protein content alone does not guarantee increased germination, indicating that a balanced nutrient composition is essential. The superior performance of *D. hispida* extract in this study may therefore result from its balanced nutritional profile that supports both energy supply and metabolic activation during germination.

The germination rates obtained in this study were higher than those reported in previous research. The highest germination rate (91.75%) from *D. hispida* extract at 5 g/L exceeded the 76.11% reported by Dewi (2022) and the 74.31% obtained using maize-based media by Afifah et al. (2022). However, these values were slightly lower than the germination rates of 97.3% and 97.2% reported by Prayogo (2011) when peanut and soybean oil extracts were used. This comparison indicates that while *D. hispida* is highly effective in promoting conidial germination, lipid-rich media may provide additional advantages due to the availability of fatty acids that support fungal metabolism.

Microscopic observations confirmed the germination process of *L. lecanii* conidia. Conidia appeared as asexual spores, and after 16 hours of incubation, germ tube formation was observed, marking the initiation of fungal growth (Figure 4). The micrograph shows individual conidia and emerging germ tubes, indicating active metabolic processes. The enhancement of conidial germination through supplementation with *D. hispida* and *A. muricata* extracts supports the utilization of locally available plant resources to improve the biological performance of entomopathogenic fungi. This approach highlights the potential integration of tropical botanical materials into microbial production systems, contributing to eco-friendly and sustainable pest management strategies.

Mortality of *N. lugens*. The mortality of *N. lugens* treated with different concentrations of *L. lecanii* conidia is presented in Table 5. The untreated control showed no mortality throughout the 7-day observation period. In contrast, all treatments receiving *L. lecanii* conidia exhibited progressively increasing mortality, which varied according to both conidial concentration and exposure time. At the lowest concentration (10^6 conidia/mL), mortality began at 7.5% on day 1 and increased to 38.54% by day 7. At 10^7 conidia/mL, mortality rose from 10% to 53.13%. A higher concentration of 10^8 conidia/mL produced 64.43% mortality, whereas the highest concentration (10^9 conidia/mL) resulted in the greatest effect, with mortality increasing from 17.5% on day 1 to 92.71% by day 7. These results indicate a clear dose-dependent and time-dependent pattern, where higher conidial densities and longer exposure periods lead to increased

mortality. This pattern confirms the effectiveness of *L. lecanii* as a biological control agent against *N. lugens*.

The observed dose–response relationship is consistent with previous studies. Khoiroh et al. (2014) reported that mortality of *N. lugens* increased proportionally with *L. lecanii* conidia density, with the highest mortality (78.33%) obtained at 10^{10} conidia/mL. Similarly, Suhairiyah et al. (2013) found that concentrations of 10^9 and 10^8 conidia/mL caused mortality rates of 83.00% and 80.00%, respectively, in *Spodoptera litura*. Gunawan et al. (2023) further demonstrated that 10^{10} conidia/mL resulted in 100% mortality of *N. lugens*, highlighting the strong virulence associated with high conidial densities. Additional studies also support these findings. Widariyanto et al. (2017) reported that *L. lecanii* at 10^7 conidia/mL caused 90.00% mortality in fourth-instar *Aphis glycines,* while Indriati et al. (2015) observed 65.00% mortality of *Helopeltis antonii* at 10^9 conidia/mL after 7 days. These consistent patterns reflect the classical dose–response relationship described by Finney (1971), in which mortality increases logarithmically with pathogen concentration. The agreement between the present results and previous studies confirms the predictable pathogenic behavior of *L. lecanii* as an entomopathogenic fungus (Bayu et al., 2021; Safavi et al., 2007).

The high mortality observed in this study was also associated with the use of *D. hispida* extract as a growth-medium supplement, which enhanced

Table 4. Germination percentage of *L. lecanii* conidia in response to different concentrations of *A. muricata* and *D. hispida* extracts

Treatment	Conidial germination (%)
L. lecanii (Control)	78.56 c
L. lecanii + *A. muricata* seed extract 1 mL/L	79.54 c
L. lecanii + *A. muricata* seed extract 3 mL/L	86.37 ab
L. lecanii + *A. muricata* seed extract 5 mL/L	82.07 bc
L. lecanii + *D. hispida* extract 1 g/L	86.34 ab
L. lecanii + *D. hispida* extract 3 g/L	87.94 a
L. lecanii + *D. hispida* extract 5 g/L	91.75 a

Means followed by the same letter are not significantly different (DMRT, 5%).

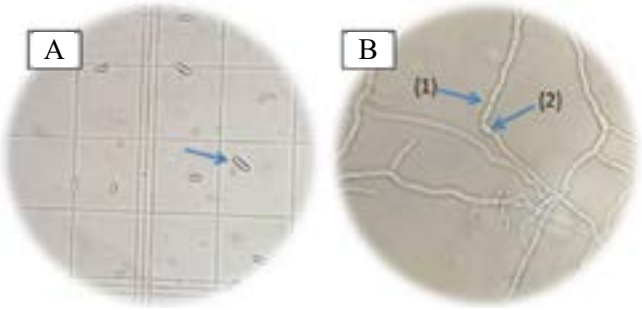

Figure 4. Microscopic observation of *L. lecanii*. A. Conidial morphology at 40 × 10 magnification; B. Germination of *L. lecanii* 16 h after incubation at 40 × 10 magnification: (1) Conidia; (2) Germ tubes.

Table 5. Mortality of *N. lugens* at different concentrations of *L. lecanii* conidia over 7 days after application (DAA)

Conidia density of *L. lecanii* (conidia/mL)	Mortality of *N. lugens* (%)						
	Observation (days)						
	1	2	3	4	5	6	7
Control	0.00 b	0.00 c	0.00 c	0.00 c	0.00 d	0.00 d	0.00 d
10^6	7.50 a	15.00 b	16.11 b	17.01 b	23.96 c	26.34 c	38.54 c
10^7	10.00 a	17.50 b	18.89 b	25.35 b	36.46 bc	43.30 b	53.13 b
10^8	15.00 a	22.50 ab	24.44 b	36.46 b	48.26 b	53.57 b	64.43 b
10^9	17.50 a	32.50 a	48.06 a	64.93 a	78.13 a	83.04 a	92.71 a

Means within a column followed by the same letter are not significantly different (DMRT, 5%).

fungal virulence. Bioactive compounds present in plant extracts, including alkaloids, acetogenins, and flavonoids, may exert an initial toxic effect and weaken insect physiological defenses (Joob & Wiwanitkit, 2014; Pinto et al., 2018; Ramadan & Yuliani, 2025). This initial stress likely facilitates infection by *L. lecanii*, which proceeds through conidial germination, cuticle penetration, and systemic proliferation within the host body (Minarni et al., 2021; Valero-Jiménez et al., 2016). In addition, plant extracts improve fungal growth, conidial density, and germination rate, thereby increasing infectivity and accelerating mortality (Safavi et al., 2007; Xie et al., 2022). The combined effects of plant-derived compounds and fungal infection create synergistic pressure on pest populations, reducing the likelihood of resistance development and enhancing biological control efficacy (Kleespies & Zimmermann, 1992; Liu et al., 2023; Wu et al., 2021; Zimmermann, 2007).

The infection process of entomopathogenic fungi occurs through four stages: inoculation, germination, penetration, and host destruction (Pranata et al., 2014). After conidia adhere to the insect cuticle, germination occurs on the integument surface. Enzymes such as proteases, chitinases, lipases, and amylases degrade the cuticle, enabling fungal penetration into the hemocoel. Once inside, the fungus proliferates, produces blastospores, and spreads through the hemolymph, damaging vital tissues and ultimately causing paralysis and death (Ginting et al., 2020; Masyitah et al., 2017; Widariyanto et al., 2017).

Regression analysis further demonstrated a strong relationship between conidial density and *N. lugens* mortality ($R^2 = 0.84$; $P = 0.07$) (Figure 5). Environmental factors also influence infection success. High humidity (>90%) and temperatures around 25 ºC favor conidial germination, fungal growth, and pathogenicity (Senja et al., 2023; Muhtady & Fitri, 2021). Additionally, fungal isolate origin (Bayu et al., 2021), culture age (Mishra et al., 2013; Chairudin et al., 2016), and host stage play important roles in determining pathogenic efficacy. Nymphal stages, which possess thinner cuticles, are generally more susceptible to infection than adults because reduced cuticle thickness facilitates fungal penetration (Chairudin et al., 2016).

Morphological observations of infected *N. lugens* nymphs revealed typical symptoms of entomopathogenic fungal infection (Figure 6). Infected insects exhibited body stiffening, likely caused by depletion of internal fluids during fungal colonization. Several days after inoculation, dense white mycelia developed on the insect surface, particularly between the legs, wings, and thorax, consistent with previous descriptions (Abadi et al., 2019). In addition, infected nymphs showed progressive darkening of the body surface due to melanization, a defense response triggered by phenoloxidase activity (Khoiroh et al., 2014). Although melanization functions as a protective mechanism, high spore densities accelerate infection and overwhelm host defenses. The persistence of melanization and post-mortem mycosis serves as a diagnostic indicator of successful fungal infection (Fadhilah & Asri, 2019; Widariyanto et al., 2017). The extensive mycelial growth observed in this study indicates systemic colonization and confirms the high virulence of *L. lecanii* cultured in *D. hispida*-supplemented media.

The increased conidial density and germination observed in extract-supplemented media translated directly into higher virulence against *N. lugens*. This relationship highlights the complementary interaction

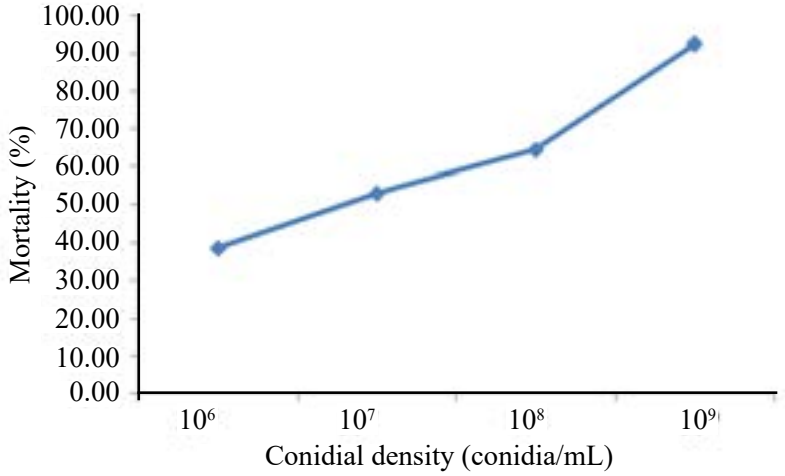

Figure 5. Regression analysis of the relationship between conidial density and mortality *N. lugens*.

between improved fungal quality and enhanced infection efficiency. The integration of plant-derived substrates with entomopathogenic fungi therefore represents a promising approach for improving biological control performance. These findings support the development of eco-friendly pest management strategies that reduce reliance on chemical insecticides.

Overall, the combination of *L. lecanii* and *D. hispida* extract demonstrates strong potential as a sustainable biocontrol agent. This approach utilizes locally available plant resources to enhance fungal performance and supports the development of environmentally sound integrated pest management (IPM) strategies in rice agroecosystems (Hadi et al., 2014; Mantzoukas & Eliopoulos, 2020).

Lethal Time (LT_{50}) and Lethal Concentration (LC_{50}). Table 6 presents the probit analysis of LT_{50} of *L. lecanii* against *N. lugens*. The LT_{50} values represent the time required for 50.00% mortality of the *N. lugens* population after exposure to different conidial concentrations. The untreated control exhibited an LT_{50} of 9.90 days. At the lowest conidial density (10^6 conidia/mL), the LT_{50} decreased to 8.26 days. Increasing concentrations further reduced the LT_{50} values, with 10^7, 10^8, and 10^9 conidia/mL producing LT_{50} values of 5.72, 5.10, and 3.27 days, respectively. These results indicate that higher conidial densities accelerate mortality and shorten the time required to reach 50.00% death in *N. lugens*. The negative correlation between conidial concentration and LT_{50} aligns with the probit mortality model proposed by Finney (1971), where higher pathogen doses increase infection probability and reduce lethal time (Abdel-Raheem et al., 2021; Safavi et al., 2007).

These findings are consistent with previous studies. Ramli & Kusnara (2019) reported that insecticides with lower LT_{50} values are more effective because they induce faster mortality. Anggarawati et al. (2017) demonstrated that *L. lecanii* at 10^9 conidia/mL achieved an LT_{50} of 4.2 days and caused 100% mortality in *Helopeltis antonii*. Similarly, Dewi et al. (2022) found that *L. lecanii* at a density of 10^6 conidia/mL infected *Cylas formicarius*, with the lowest LT_{50} observed at 10^9 conidia/mL. Abdel-Raheem et al. (2021) also recorded an LT_{50} of 4.2 days for *Aphis craccivora* exposed *L. lecanii* at a density of 10^9 conidia/mL, confirming that higher conidial concentrations reduce lethal time.

The reduction in LT_{50} at higher concentrations

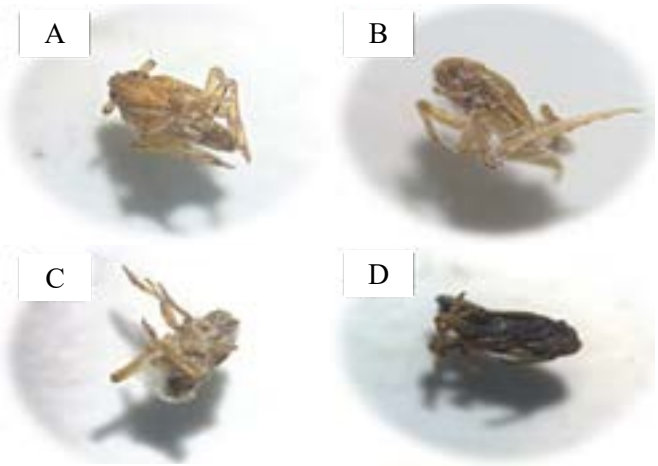

Figure 6. *N. lugens* nymph 7 days after application of *L. lecanii.* A. Healthy, uninfected nymph; B. Initial appearance of mycelium on *N. lugens* nymph; C. Nymph fully infected and covered with *L. lecanii* mycelium; D. Nymph undergoing melanization with black discoloration.

Table 6. Probit analysis of the LT_{50} concentration of *L. lecanii* on *N. lugens* mortality

Conidial density (conidia/mL)	Lethal time (LT_{50})
Control	9,90
10^6	8,26
10^7	5,72
10^8	5,10
10^9	3,27

can be explained by increased conidial adhesion and cuticular penetration (Ortiz-Urquiza & Keyhani, 2016). At elevated densities, enzymatic activity, particularly proteases and chitinases, enhances cuticle degradation and accelerates hemocoel colonization (Masyitah et al., 2017; Valero-Jiménez et al., 2016). Consequently, fungal infection progresses more rapidly, limiting insect immune responses and leading to faster mortality (Shahid et al., 2012).

LC_{50} denotes the concentration required to cause 50.00% mortality in a test population (Hasyim et al., 2016). The probit analysis revealed an LC_{50} value of 5.78×10^8 conidia/mL with a confidence range of 1.38×10^6 to 2.62×10^9 conidia/mL. This result indicates high susceptibility of *N. lugens* nymphs to *L. lecanii.* The relatively thin cuticle of third-instar nymphs likely facilitates fungal penetration, contributing to the observed virulence (Afifah et al., 2022). Comparable studies reported higher LC_{50} values, such as 1.9×10^9 conidia/mL for *B. bassiana* against *Riptortus linearis* (Nandita & Santoso, 2017) and 7.58×10^9 conidia/mL for *L. lecanii* against *Menochilus sexmaculatus* Hadi et al. (2020). The lower LC_{50} observed in this study suggests enhanced virulence of *L. lecanii* cultured with *D. hispida* extract.

This improved virulence may be attributed to the nutritional and biochemical support provided by the plant extract, which enhances sporulation, conidial viability, and pathogenic enzyme production (Safavi et al., 2007; Senja et al., 2023). Environmental conditions also play an important role. Optimal temperature (around 25 ºC) and high relative humidity (>90%) favor germination, enzyme activity, and infection efficiency (Mishra et al., 2013; Muhtady & Fitri, 2021). In addition, host factors such as cuticle thickness and physiological condition influence susceptibility, further determining infection success.

Overall, the LT_{50} and LC_{50} results confirm that supplementation with *D. hispida* extract enhances the pathogenic performance of *L. lecanii*. The improved conidial quality increases virulence while maintaining consistency across concentrations. This synergistic interaction provides a promising, eco-friendly strategy for brown planthopper management and supports the development of sustainable integrated pest management systems in rice agroecosystems (Mantzoukas & Eliopoulos, 2020; Wu et al., 2021; Xie et al., 2022).

CONCLUSION

The provided research uniquely demonstrates a synergistic effect between the entomopathogenic fungus *L. lecanii* and extracts from *D. hispida* and *A. muricat*a. A key and novel finding is that the addition of these plant extracts to the fungal growth medium significantly enhances its biological efficacy against *N. lugens*. Specifically, treatments using *D. hispida* extract resulted in a peak conidia density of 1.38×10^9 conidia/mL, a germination rate of 91.75%, and a rapid LT_{50} of 3.27 days, all of which surpass values reported in most previous studies. These results highlight that this combination is not merely a viable alternative to chemical pesticides, but a promising and potentially effective biocontrol agen, offering enhanced virulence and faster insect mortality that is directly attributed to the improved fungal growth and viability induced by the plant extracts. These findings underscore the importance of integrating entomopathogenic fungi with botanical extracts as part of sustainable pest management strategies, offering a viable alternative to chemical pesticides while supporting sustainable rice pest management. Further research should examine field-scale applications, formulation stability, and integration within IPM frameworks.

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AUTHORS' CONTRIBUTIONS

LA conducted the laboratory research, including determination of conidial density, and drafted the initial manuscript. MRF coordinated the study design, supervised the assessment of brown planthopper mortality, and performed statistical analyses. NWS supervised the research process and ensured the accuracy of the results. FMB contributed to manuscript revision and coordinated communication among

authors. AK provided laboratory assistance during the research. All authors interpreted the results, critically reviewed the manuscript, and approved the final version for publication.

COMPETING INTEREST

The authors declare that they have no financial or non-financial competing interests that could have influenced this research. All aspects of the study, including data collection and analysis, were conducted transparently and without bias.

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