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Diversity and Entomopathogenic Activity of Pineapple-Associated Endophytic Fungi from Kualu Nenas, Riau, Indonesia, against *Dysmicoccus brevipes* (Hemiptera: Pseudococcidae)

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Abstract

Endophytic fungi hold considerable potential as biological control agents, yet their exploration from pineapple plants cultivated on peatlands remains limited. This study aimed to identify the diversity of endophytic fungi associated with pineapple (*Ananas comosus*) grown in Kualu Nenas, Riau, and to evaluate their entomopathogenic activity against the pineapple mealybug, *Dysmicoccus brevipes*. Endophytic fungi were isolated from leaf sheaths and roots of 1-year-old and 15-year-old pineapple plants. A total of 24 fungal isolates were obtained and morphologically identified into five genera: *Beauveria* sp., *Metarhizium* sp., *Trichoderma* sp., *Fusarium* sp., and *Aspergillus* sp. Preliminary pathogenicity screening against *Tenebrio molitor* larvae showed that 7 of the 24 isolates exhibited entomopathogenic activity, causing 33.33–100% mortality. Five isolates that caused >50% mortality were further evaluated against second-instar *D. brevipes* nymphs using a conidial suspension of 1×10^8 conidia mL^{-1}. Among these isolates, *Beauveria* sp. KN1-D27 showed the highest virulence, causing 82% mortality at 10 days after treatment, with the shortest LT_{50} of 6.4 days. This was followed by *Metarhizium* sp. KN1-A09, which caused 71% mortality with an LT_{50} of 7.2 days, and *Beauveria* sp. KN15-D15, which caused 66% mortality with an LT_{50} of 7.8 days. In contrast, *Trichoderma* sp. and *Fusarium* sp. showed lower efficacy. These findings indicate that *Beauveria* sp. KN1-D27 has potential as a biological control candidate against *D. brevipes* on peatland pineapple.

Keywords: *Ananas comosus*, *Beauveria* sp., biological control, mealybug, *Metarhizium* sp.

Introduction

Pineapple (*Ananas comosus*) cultivated on peatlands in Riau Province faces serious constraints from the pineapple mealybug, *Dysmicoccus brevipes* (Cockerell, 1893), a sap-sucking pest that causes direct damage and transmits pineapple mealybug wilt-associated virus (PMWaV) (Tantawi et al., 2023; Hendra et al., 2026). This pest has been associated with yield losses of 50–70% (Hutahayan et al., 2021). Pest management in peatland ecosystems is particularly challenging because peat soils are characterized by low pH, high organic matter content, and waterlogged conditions, which make them

fragile and limit the suitability of conventional control methods (Bortolozo et al., 2016; Fauzi and Qurani, 2021). Therefore, biological control using local entomopathogenic microorganisms in peatland represents a promising alternative (Safitri, 2018; Supriyanto et al., 2022; Hendra et al., 2026).

Among potential biological control agents, endophytic fungi are of particular interest because they live asymptomatically within plant tissues while providing benefits to the host plant, including enhanced tolerance to biotic and abiotic stresses, growth promotion, and activation of defense mechanisms through secondary metabolites and hydrolytic enzymes (Felber et al., 2015; Ttacca et al., 2022). Several endophytic fungi also exhibit entomopathogenic activity and can suppress insect pests either directly through infection or indirectly by altering host–insect interactions (Gautam and Avasthi, 2019). For example, endophytic colonization by *Beauveria bassiana* has been reported to affect aphid feeding behavior by reducing short probing and increasing the tendency to leave the leaf surface (Manousopoulos et al., 2019). Transcriptomic evidence also shows that *Aphis gossypii* feeding on *B. bassiana*-colonized plant tissues undergoes molecular changes related to immune response, host recognition, energy metabolism, and cellular defense pathways such as apoptosis (Cuenca-Medina et al., 2025b).

B. bassiana and *Metarhizium anisopliae* have shown pathogenic and virulent activity against several insect pests (Reflinaldon and Martinius, 2017; Trizelia et al., 2018; Trizelia et al., 2023). Other studies have reported that *B. bassiana*, *M. anisopliae*, and *Trichoderma* spp. can infect and suppress sap-sucking insects, including aphids and mealybugs, on horticultural crops (Cuenca-Medina et al., 2025a, 2025b). Endophytically colonized leaves of *B. bassiana* and *M. brunneum* caused 37.7–50.0% mortality in *Aphis gossypii* and *Myzus persicae* (González-Mas et al., 2019). In addition, *Cladosporium cladosporioides* showed significant pathogenicity against *M. persicae* (Silva et al., 2025), while *M. flavoviride* has been reported to reduce larval survival (Sabu et al., 2025).

Endophytic fungi from peatland vegetation, such as oil palm, sago, and purun, have been reported to exhibit strong adaptability and biological activity, including the production of bioactive compounds and suppression of soil-borne pathogens (Yurnaliza and Jamilah, 2018; Morrison et al., 2021). Fungal endophytes from these ecosystems are commonly dominated by Ascomycetes and Basidiomycetes, with genera such as *Pestalotiopsis*, *Aspergillus*, *Nigrospora*, *Penicillium*, and *Trichoderma* frequently reported (Ali et al., 2016; Raviraja, 2005; Park and You, 2023). However, most studies on entomopathogenic endophytic fungi have focused on plants grown in mineral soils or under controlled laboratory conditions, while little is known about endophytes from peatland environments. Moreover, no study has specifically isolated and identified endophytic fungi from pineapple plants cultivated in the peatlands of Kualu Nenas, Riau, or evaluated their entomopathogenic activity against *D. brevipes*, the major pest of pineapple.

Endophytic fungi originating from peatland may possess adaptive and physiological traits that differ from isolates from mineral soils. Therefore, this study aimed to: (1) explore endophytic fungi from leaf sheath and root tissues of pineapple plants of different ages grown in Riau peatlands; (2) analyze the diversity of endophytic fungal genera based on plant part and plant age; and (3) evaluate the entomopathogenic activity of selected

isolates against *D. brevipes*. The findings are to provide scientific information regarding the potential of local endophytic fungi as biological control agents for the management of pineapple pests in peatland ecosystems.

Methods

Survey and Sample Collection

The study was conducted at the Integrated Pest Management Laboratory, Faculty of Agriculture, Universitas Islam Riau, Indonesia, from August to October 2025. Endophytic fungi were isolated from pineapple leaf sheaths and roots grown on peatlands in Kualu Nenas, Kampar, Riau (0°26'20.97"N 101°15'49.12"E). Samples were collected using purposive sampling from healthy plants located among those infested by mealybugs. One leaf sheath and one root sample were taken from each plant. A total of 10 plants were sampled from two fields (± 15 years old and ± 1 year old). Each sample was labeled with information on location, plant age, and collection date before being transported to the laboratory.

Isolation of Endophytic Fungi

Leaf sheath and root tissues were cut into 0.5 × 0.5 cm pieces and surface-sterilized sequentially in 70% ethanol and 1% NaOCl for 60 seconds each, followed by three rinses with sterile distilled water for 60 seconds each (Elfita et al., 2019). Sterilization effectiveness was assessed by plating 100 µL of the final rinse water on potato dextrose agar (PDA) and checking for microbial growth after incubation at 26°C for 5 days (Silva-Hughes et al., 2015; Pizzi et al., 2025). Tissue fragments were placed on PDA supplemented with 50 mg/L chloramphenicol to inhibit bacterial growth and incubated at 26°C for 4–7 days. Emerging fungal colonies were subcultured onto fresh PDA to obtain pure cultures (Ogofure et al., 2025). Isolates were assigned the prefix “KN,” representing Kualu Nenas, followed by numbers indicating plant age (1 or 15 years). The letters “D” and “A” identified the tissue of origin, referring to the leaf sheath and root, respectively, from which each fungal isolate originated.

Preliminary Pathogenicity Assay

To confirm the entomopathogenic potential of the isolated endophytic fungi, a preliminary assay was conducted against *T. molitor* larvae as a model insect. Larvae were obtained from a commercial bird feed supplier and maintained in plastic boxes (30 × 20 × 15 cm) containing wheat bran as feed and substrate. Fresh carrot slices (10 g per 100 larvae) were provided every 2 days as a moisture source. Environmental conditions were maintained at 27 ± 2°C, 60–70% RH, with a 12:12 L:D photoperiod. Fifth-instar larvae (± 1.5 cm length) were used for the assay. Forty larvae per isolate were placed on PDA plates (9 cm diameter) previously colonized by fungal cultures for 24 hours to allow direct contact between conidia and larvae. Control larvae were placed on PDA plates without fungi. After 24 hours of exposure, larvae were transferred into clean plastic containers (5 cm diameter) containing 10 g of wheat bran. Mortality was recorded daily for 7 days. Dead larvae were surface-sterilized and placed on moist filter paper to confirm fungal sporulation (mycosis). Isolates that caused >50% mortality were selected for further testing against *D. brevipes*.

Morphological Identification

All endophytic fungal isolates were grouped based on macroscopic characteristics (colony color, texture, margin, and growth rate) on PDA. For isolates that showed entomopathogenic activity (causing >50% mortality against *T. molitor* larvae), microscopic identification was performed using a light microscope at 400× magnification to observe conidiophore branching, conidial shape, size, septation, and arrangement

(Barnett and Hunter, 1998). The remaining non-entomopathogenic isolates were identified only to the genus level based on macroscopic characteristics (Abdul et al., 2021; Syahrawati et al., 2025).

Conidial Density and Viability

Conidia from 15-day-old fungal cultures grown on PDA were harvested by adding 5 mL of sterile distilled water containing 0.05% Tween 80, followed by gentle scraping with a sterile loop. Three counts were performed for each isolate, and the average concentration was calculated as conidia mL^{-1} (Oliveira et al., 2024). Viability was assessed by placing 0.1 µl of fungal suspension (1×10^8 conidia mL^{-1}) onto 2% water agar on a sterile glass slide and incubated at 26°C for 24 and 48 hours. Conidial density was determined using a hemocytometer under a light microscope at 40× magnification as well as conidial germination. A conidium was considered germinated if the germ tube length exceeded the conidial diameter. For each isolate, 100 conidia were counted across three replications (Oliveira et al., 2024).

Preparation of *Dysmicoccus brevipes*

Mealybugs were initially collected from infested pineapple plants in Kualu Nenas, Kampar, Riau, and subsequently reared on potted pineapple plants (2 months old) maintained in insect cages (40 × 40 × 60 cm) at 27 ± 2°C, 70–80% RH, with a 12:12 h light: dark photoperiod. Third-generation, second-instar nymphs of *D. brevipes* obtained from laboratory rearing were used for the bioassay. Selected fungal isolates showing >50% mortality against *T. molitor* were further evaluated against these nymphs (Figure 1).

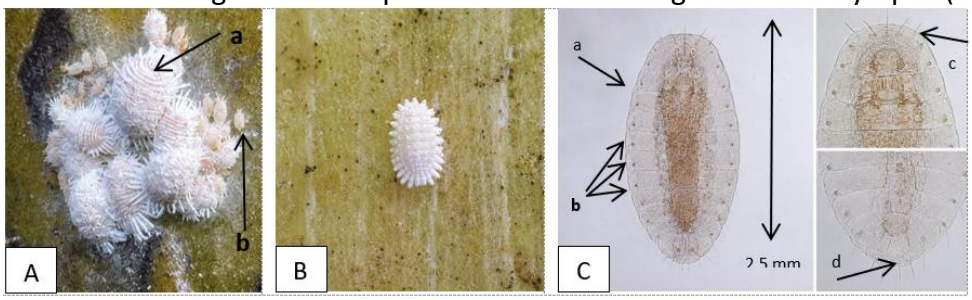


Figure 1. A) *Dysmicoccus brevipes*: a) female, b) first instar nymph. B) Nymph. C) Microscopic characteristics: a) oval and slightly stout body, b) short and uniform filaments consisting of 17 pairs, c) anterior ostiole, d) posterior ostiole not elongated.

Virulence Assay of Selected Fungal Isolates

The experiment used a Completely Randomized Design (CRD) with selected fungal isolates (5 isolates) + control (water + 0.05% Tween 80), each with 4 replications. Each replication consisted of 15 nymphs, totaling 60 nymphs per isolate. A conidial suspension (1×10^8 conidia mL^{-1} in sterile distilled water + 0.05% Tween 80) was prepared from 15-day-old fungal cultures. Using a hand-held sprayer, 1 mL of conidial suspension was sprayed onto each group of 15 nymphs placed on a pineapple leaf disc (2 cm diameter) on moist filter paper in a Petri dish (5 cm diameter). Control nymphs were sprayed with sterile distilled water + 0.05% Tween 80 only. Nymphs were incubated at 26 ± 1°C, 80% RH. Mortality was recorded daily for 10 days. Nymphs were considered dead if no movement was observed after gentle probing with a fine brush. Dead nymphs were surface-sterilized (70% ethanol for 10 seconds, rinsed with sterile water) and placed on moist filter paper to confirm mycosis. Observed variables included mortality (%) at day 10, mycosis (%), and LT50 (lethal time to kill 50% of nymphs).

Data Analysis

Mortality data were analyzed using ANOVA, followed by Tukey's HSD test at a 5% significance level. The LT_{50} was estimated using Probit analysis. All statistical analyses were performed using RStudio version 2026.04.0+526, and the LT50 estimation was conducted using POLO-Plus Version 2.0.

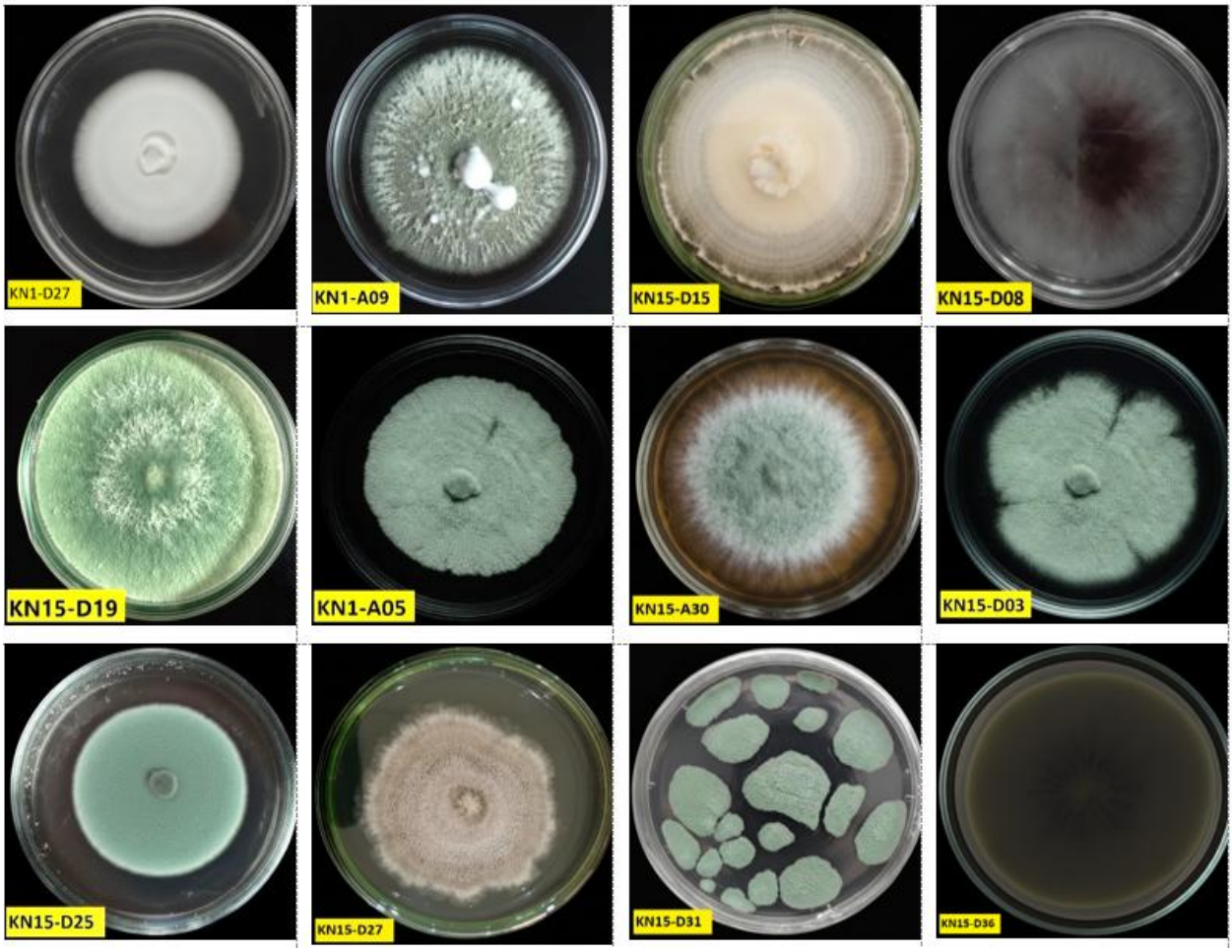
Results

Morphological Characteristics

A total of 24 endophytic fungal isolates were obtained from pineapple plants of two different age groups. The older plants harbored a higher number of endophytic fungi (16 isolates) compared to the younger one (8 isolates). Among the plant parts examined, the leaf sheath showed the highest level of colonization with 17 isolates, while the root yielded 7 isolates. This indicates that the leaf sheath provides a more favorable habitat for endophytic fungal growth than root tissues (Table 1, Figure 2).

Table 1. Number of endophytic fungal isolates obtained from different ages of pineapple

Plant Part	Age (years)		
	15	1	Total
Leaf sheath	12	5	17
Root	4	3	7
Total	16	8	24



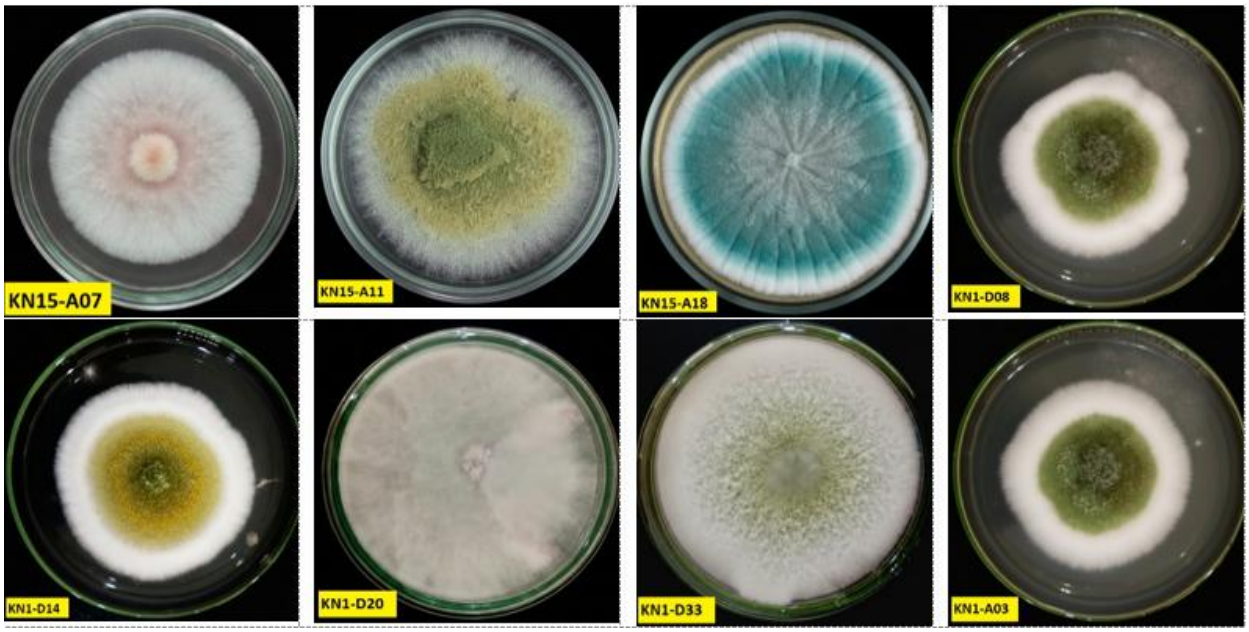


Figure 2. Morphological characteristics of endophytic fungal isolates from pineapple

The results showed considerable variation in pathogenic ability among the tested isolates. Several isolates were able to cause high mortality and mycosis, while others showed moderate to no pathogenic effects on the larvae. The mortality percentage indicated the ability of the isolates to kill the larvae, whereas the mycosis percentage confirmed fungal infection through fungal growth on the cadavers. Among the 24 isolates tested, isolate KN1-D27 demonstrated the highest virulence, causing 100% larval mortality and mycosis. Two other isolates, KN1-A09 and KN15-D15, also showed high entomopathogenic activity, with 80% mortality and mycosis. Moderate mortality rates (33.33–53.33%) were observed in isolates KN15-D08, KN15-D19, KN1-A05, and KN15-A30. In contrast, the remaining isolates did not exhibit pathogenic effects on *T. molitor* larvae, showing 0% mortality and mycosis (Table 2).

Table 2. Mortality and mycosis percentage of *Tenebrio molitor* larvae caused by endophytic fungal isolates from pineapple tissues

No	Isolate	Mortality of Larvae (%) ± SD	Mycosis (%)± SD
1	KN1-D27	100.00 ± 0.0	100.00 ± 0.0
2	KN1-A09	80.00 ± 2.4	80.00 ± 2.4
3	KN15-D15	80.00 ± 2.4	80.00 ± 2.4
4	KN15-D08	53.33 ± 2.2	53.33 ± 2.2
5	KN15-D19	53.33 ± 1.3	53.33 ± 1.3
6	KN1-A05	46.67 ± 1.3	46.67 ± 1.3
7	KN15-A30	33.33 ± 5.6	33.33 ± 5.6
8	KN15-D03	0.00 ± 0.00	0.00 ± 0.00
9	KN15-D06	0.00 ± 0.00	0.00 ± 0.00
10	KN15-D09	0.00 ± 0.00	0.00 ± 0.00
11	KN15-D12	0.00 ± 0.00	0.00 ± 0.00
12	KN15-D22	0.00 ± 0.00	0.00 ± 0.00
13	KN15-D25	0.00 ± 0.00	0.00 ± 0.00

No	Isolate	Mortality of Larvae (%) ± SD	Mycosis (%)± SD
14	KN15-D27	0.00 ± 0.00	0.00 ± 0.00
15	KN15-D31	0.00 ± 0.00	0.00 ± 0.00
16	KN15-D36	0.00 ± 0.00	0.00 ± 0.00
17	KN15-A07	0.00 ± 0.00	0.00 ± 0.00
18	KN15-A11	0.00 ± 0.00	0.00 ± 0.00
19	KN15-A18	0.00 ± 0.00	0.00 ± 0.00
20	KN1-D08	0.00 ± 0.00	0.00 ± 0.00
21	KN1-D14	0.00 ± 0.00	0.00 ± 0.00
22	KN1-D20	0.00 ± 0.00	0.00 ± 0.00
23	KN1-D33	0.00 ± 0.00	0.00 ± 0.00
24	KN1-A03	0.00 ± 0.00	0.00 ± 0.00

Microscopic characteristics

Among the 24 endophytic fungal isolates obtained from pineapple tissues, seven isolates showed varying degrees of mortality against *T. molitor* larvae (33.33 - 100%). These 7 isolates were selected for detailed macro- and microscopic characteristics (Figure 3), along with their corresponding mortality and mycosis percentages.

All isolates showed high conidial densities ranging from 4.3 ×10^8 to 7.1 ×10^8 conidia mL^{-1}, indicating good sporulation ability under laboratory conditions. Among them, *Trichoderma* sp. (KN15-D19) exhibited the highest conidial density (7.1 ×10^8 conidia mL^{-1}) and germination rate (92.6% at 24 h and 99.1% at 48 h), suggesting superior spore production and viability compared to other genera. *Metarhizium* sp. (KN1-A09) also demonstrated strong performance, with over 90% germination within 24 hours. In contrast, *Fusarium* sp. (KN15-D08) recorded the lowest spore density and germination percentage, indicating lower viability and sporulation efficiency. Interestingly, although *Beauveria* sp. showed a relatively lower conidial density compared to *Trichoderma* and *Metarhizium*, its virulence against *T. molitor* was higher, indicating that pathogenicity does not necessarily correlate with spore density but may depend on specific virulence factors and secondary metabolites produced by the isolate (Table 3).

Table 3. Conidial density and germination rate of endophytic fungal isolates

Isolate	**Genus**	Conidial density (1x108 conidia mL^{-1})	Germination (%) ± SD	
			24 h	48 h
KN1-D27	*Beauveria* sp.	5.6 ± 0.30 c	88.3 ± 1.9 c	99.0 ± 1.1 a
KN1-A09	*Metarhizium* sp.	6.2 ± 0.25 b	90.7 ± 1.7 b	98.5 ± 0.8 a
KN15-D15	*Beauveria* sp.	5.0 ± 0.22 d	86.1 ± 2.2 d	95.8 ± 1.3 b
KN15-D08	*Fusarium* sp.	4.3 ± 0.28 e	82.4 ± 2.5 e	93.2 ± 1.6 c
KN15-D19	*Trichoderma* sp.	7.1 ± 0.35 a	92.6 ± 1.5 a	99.1 ± 0.6 a
KN1-A05	*Aspergillus* sp.	6.0 ± 0.27 b	89.4 ± 2.0 bc	97.5 ± 0.9 ab
KN15-A30	*Trichoderma* sp.	6.8 ± 0.31 a	91.2 ± 1.8 b	98.8 ± 0.7 a

Values followed by the same letter within a column are not significantly different according to Tukey's HSD test ($p < 0.05$).

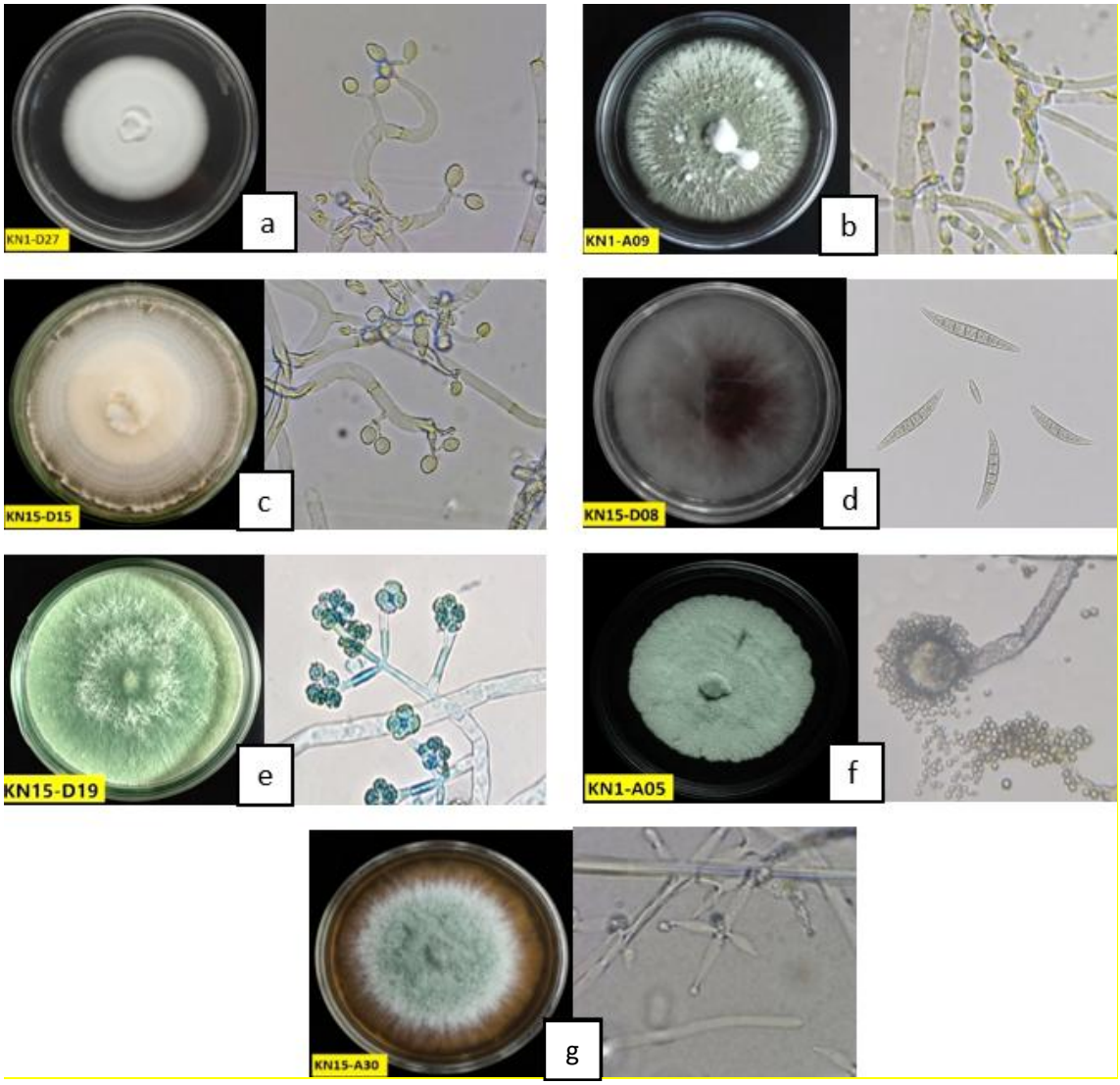


Figure 3. Macroscopic and microscopic characteristics of pineapple-associated endophytic fungal isolates from Kualu Nenas, Riau, Indonesia: (a) *Beauveria* sp. KN1-D27; (b) *Metarhizium* sp. KN1-A09; (c) *Beauveria* sp. KN15-D15; (d) *Fusarium* sp. KN15-D08; (e) *Trichoderma* sp. KN15-D19; (f) *Aspergillus* sp. KN1-A05; and (g) *Trichoderma* sp. KN15-A30.

Mortality and lethal time of *D. brevipes*

Ten days after treatment, *Beauveria* sp. KN1-D27 resulted in the highest mortality rate for *D. brevipes* nymphs (82%) compared to the other treatments, but this was not significantly different from the result for *Metarhizium* sp. KN1-A09 (71%). Conversely, *Fusarium* sp. KN15-D08 caused the lowest mortality (6%), which did not differ significantly from the control. The LT_{50} values were 6.4, 7.2, and 7.8 days for KN1-D27, KN1-A09, and KN15-D15, respectively. LT_{50} values were not reported for KN15-D19 or KN15-D08 because mortality remained below 50% during the 10-day observation period (Table 4). Figure 4 illustrates the condition of *D. brevipes* nymphs before and after fungal treatment. Healthy nymphs are shown alongside dead nymphs exhibiting discoloration and fungal growth on the body surface. Fungal cultures recovered from the cadavers are also presented.

Table 4. Mortality of *Dysmicoccus brevipes* nymphs at 10 days post-inoculation with selected endophytic fungal isolates

Isolate	Genus	Mortality (%) ± SD	LT50 (days)
KN1-D27	*Beauveria* sp.	82.00 ± 4.47 a	6.4
KN1-A09	*Metarhizium* sp.	71.00 ± 4.18 ab	7.2
KN15-D15	*Beauveria* sp.	66.00 ± 5.48 b	7.8
KN15-D19	*Trichoderma* sp.	46.00 ± 4.18 c	-
KN15-D08	*Fusarium* sp.	6.00 ± 4.47 d	-
Control	-	3.00 ± 2.74 d	-

*Values followed by different letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$). "–" indicates that LT_{50} was not reported because mortality remained below 50% during the observation period

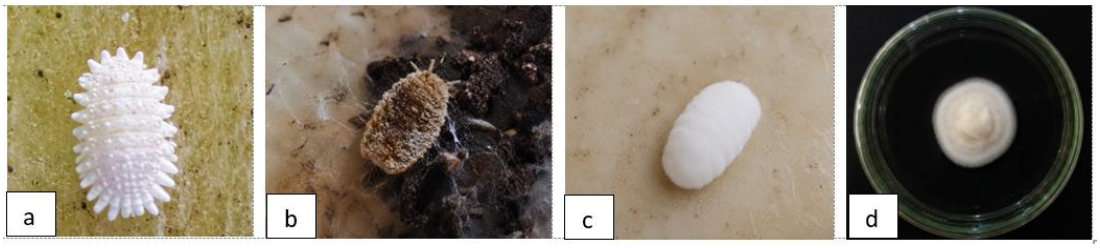


Figure 4. Morphological changes of *Dysmicoccus brevipes* nymphs after treatment. (a) Healthy second-instar nymph; (b) Dead nymph showing no movement and discoloration; (c) Nymph infected by fungus; (d) Re-isolation of entomopathogenic fungus.

Discussion

This study isolated 24 endophytic fungal isolates from root and leaf sheath tissues of pineapple grown in Riau peatlands (Table 1). Older plants, approximately 15 years old, yielded more isolates than younger plants, with 16 and 8 isolates, respectively. This indicates that plant age may influence the abundance and diversity of endophytic fungi, possibly because older plants have longer exposure to environmental microbial communities and undergo physiological and biochemical changes that create more diverse niches for endophyte colonization. Mature plants may also accumulate more complex secondary metabolites that support specific endophytic associations (Hu et al., 2024; Liao et al., 2025). In terms of tissue origin, leaf sheaths yielded more isolates than roots, with 17 and 7 isolates, respectively (Table 1). This pattern suggests that leaf sheaths may provide a more favorable microenvironment for fungal colonization, possibly due to direct exposure to airborne spores, relatively high moisture, and nutrient availability. In contrast, lower colonization in roots may be related to the hypoxic conditions commonly found in water-saturated peat soils, which can limit aerobic fungal growth and reduce root endophyte diversity (Morrison et al., 2021).

Five fungal genera were found: *Beauveria* sp., *Metarhizium* sp., *Trichoderma* sp., *Fusarium* sp., and *Aspergillus* sp. (Table 3; Figure 2). The presence of *Aspergillus* sp. reflects its high adaptability as a saprophytic endophyte commonly found in tropical ecosystems. This genus is known to produce hydrolytic enzymes and secondary metabolites that support tissue colonization and nutrient acquisition (Rodriguez et al., 2009). More importantly, the isolation of *Beauveria* and *Metarhizium* from

asymptomatic pineapple tissues confirms that these well-known entomopathogenic fungi can establish endophytic associations with plants. This dual lifestyle is increasingly recognized as an ecological strategy that allows fungi to persist within plant tissues while retaining pathogenicity toward insect hosts (Bamisile et al., 2021; Ahsan et al., 2024). Their occurrence in pineapple from Kualu Nenas peatland suggests that this ecosystem may harbor entomopathogenic fungi with endophytic capability and possible adaptation to peatland conditions.

Pathogenicity assays against *Tenebrio molitor* larvae showed variable virulence among isolates. *Beauveria* sp. KN1-D27 caused the highest mortality, reaching 100%, followed by *Metarhizium* sp. KN1-A09 and *Beauveria* sp. KN15-D15, each causing 80% mortality (Table 2). These results support the view that entomopathogenic activity is strongly influenced by isolate-specific traits rather than genus identity alone (Nicoletti et al., 2024). When tested against the target pest, *D. brevipes*, the same three isolates remained the most virulent, causing 82%, 71%, and 66% mortality, respectively (Table 4). However, mortality against *D. brevipes* was generally lower than against *T. molitor* (Tables 2 and 4). This difference may be associated with host-related factors, particularly the waxy and powdery secretion covering mealybug nymphs, which can interfere with conidial attachment, germination, and cuticular penetration. In addition, honeydew and waxy filaments produced by mealybugs may further reduce fungal infection efficiency.

The lower activity of *Fusarium* sp. KN15-D08 and *Trichoderma* sp. KN15-D19 against *D. brevipes* suggests that activity against *T. molitor* does not necessarily translate into efficacy against the target pest. *Fusarium* sp. KN15-D08 caused 53.33% mortality in *T. molitor* but only 6% in *D. brevipes*, with the latter not differing significantly from the control (Tables 2 and 4). *Trichoderma* sp. KN15-D19 caused 53.33% and 46% mortality in the two insects, respectively. These differences should be interpreted cautiously because the bioassay conditions differed between insect species. The results do not establish whether mortality involved direct fungal infection or other mechanisms. Although *Trichoderma* spp. are widely recognized as antagonists of plant pathogens (Ferreyra-Suarez, 2024), the mechanism underlying the activity of KN15-D19 was not examined in this study.

All selected isolates showed high conidial densities, ranging from 4.3×10^8 to 7.1×10^8 conidia mL^{-1}, with viability exceeding 90% after 48 hours (Table 3), indicating good physiological quality. However, high conidial production and viability did not always correspond to high virulence. For example, *Trichoderma* sp. KN15-D19 had the highest conidial density, 7.1×10^8 conidia mL^{-1}, and viability, 99.1%, but showed lower virulence than *Beauveria* and *Metarhizium* isolates (Tables 2–4). Conversely, *Beauveria* sp. KN1-D27 produced a lower conidial density, 5.6×10^8 conidia mL^{-1}, but caused the highest mortality against both test insects. These findings indicate that virulence is determined not only by conidial quantity or germination rate, but also by qualitative virulence traits, including cuticle-degrading enzymes such as proteases, chitinases, and lipases, as well as insecticidal secondary metabolites such as beauvericin, bassianolide, and destruxins (Pedrini, 2022; Wang et al., 2022).

The high sporulation and viability observed in all isolates may reflect their adaptation to peatland conditions. Peat soils are typically characterized by high organic matter content, low pH, waterlogged conditions, and limited nutrient availability

(Imran et al., 2022; Aravinthraju et al., 2024). Although such conditions are stressful for many microorganisms, the isolates obtained in this study maintained high physiological quality, suggesting possible adaptation to the peatland environment. This local adaptation may be advantageous for biocontrol applications because these isolates are already associated with the target agroecosystem and may be more compatible with low pH, high moisture, and native microbial competition in peat soils (Valente et al., 2025).

From an ecological and applied perspective, the presence of endophytic entomopathogenic fungi such as *Beauveria* and *Metarhizium* in pineapple tissues highlights their potential for sustainable pest management in peatland agriculture. The most promising isolates, particularly *Beauveria* sp. KN1-D27, *Metarhizium* sp. KN1-A09, and *Beauveria* sp. KN15-D15, showed strong pathogenicity against *D. brevipes* and may serve as candidate biological control agents. In addition to pest suppression, endophytic fungi such as *Trichoderma* and *Aspergillus* may contribute to plant tolerance against biotic and abiotic stresses, including drought, salinity, and soil-borne pathogens (Ferreyra-Suarez, 2024). Therefore, the integration of locally adapted endophytic fungi into pineapple pest management could reduce reliance on synthetic pesticides, which are often less suitable for fragile peatland ecosystems.

This study was limited by morphology-based fungal identification, laboratory bioassays, and sampling restricted to Kualu Nenas. Molecular identification, field validation, broader sampling across Riau peatlands, and investigation of KN1-D27's virulence mechanisms are needed to strengthen the findings.

Conclusion

A total of 24 fungal isolates were obtained and morphologically identified as belonging to five genera: *Beauveria*, *Metarhizium*, *Trichoderma*, *Fusarium*, and *Aspergillus*. Five isolates that caused >50% mortality in *Tenebrio molitor* were further evaluated against second-instar nymphs of *D. brevipes* using a conidial suspension at a concentration of 1×10^8 conidia mL^{-1}. Among these isolates, *Beauveria* sp. KN1-D27 exhibited the highest virulence (82% mortality; LT_{50} of 6.4 days). These findings indicate that *Beauveria* sp. KN1-D27 has the greatest potential as a candidate biological control agent against *D. brevipes* on pineapple plants cultivated in peatlands.

Declaration

Author contribution

Yolma Hendra designed and supervised the study, analyzed the data, and drafted the manuscript. Saripah Ulpah collected samples, conducted experiments, and curated data. Sulhaswardi contributed to methodology, morphological identification, and manuscript revision. Tondi Halomoan assisted with data analysis, validation, and laboratory work. All authors read and approved the final manuscript.

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Competing interest

The authors declare that they have **n**o known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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