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Endophytic fungi from Parijoto (*Medinilla speciosa*) leaves and their potential as biocontrol agents against Corn Pest Larvae

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ABSTRACT
Parijoto (*Medinilla speciosa*) leaves are widely used for their antimicrobial properties. Endophytic fungi from these leaves show potential as entomopathogenic agents against *Helicoverpa armigera* larvae, a moth pest of cotton and corn.The research activities conducted in the laboratory include the isolation and characterization of endophytic fungi, as well as entomopathogenic testing against *H. armigera* larvae using a spraying technique with endophytic fungal extracts. In this study, 5 isolates were obtained from leaves and assessed their entomopathogenic properties. The isolate code is EP1, EP2, EP3, EP4, EP5. Bioassays were conducted to evaluate the effectiveness of these fungi in controlling H. armigera larvae. Among the tested fungi, EP5 demonstrated the highest entomopathogenic activity, significantly reducing the survival rate of the larvae. These findings indicate that EP5 holds substantial promise as a biocontrol agent for managing H. armigera populations with mortality percentage 30,78%, offering a sustainable and environmentally friendly alternative to chemical pesticides. The study underscores the potential of utilizing endophytic fungi from parijoto leaves in integrated pest management strategies.

Keywords:
biocontrol agent; endophytic fungi; *Helicoverpa armigera*; Parijoto; sustainable agriculture

Introduction

Endophytes are microorganisms that reside in plant tissue. Endophytic microorganisms consist of bacteria and fungi that form colonies and live and complete their life cycle in the host tissue (Sari, 2020). Endophytic fungi are found in almost all plants and produce a variety of secondary metabolite compounds including alkaloids, terpenoids, steroids, quinones, isocoumarin derivatives, flavonoids, peptides and phenols obtained from extracts of endophytic fungi. Endophytic fungi found in medicinal plants have been studied to contain cytotoxic, anti-fungal, anti-viral and anti-microbial activities. One of the medicinal plants that contains many bioactive compounds is the parijoto plant (*Medinilla speciosa* Reinwe. Ex Blume.). Parijoto is a plant from the Melastomataceae family that grows wild in rainforests on mountain slopes at an altitude of 800-2,300 m above sea level (asl) (Deshmukh et al., 2022).

Corn (Zea mays) is a crucial crop that is frequently infested by the pest H. armigera, commonly known as the corn borer (Bamisile et al., 2021). *H. armigera* is prioritized because it exploits a broader host range and preferentially damages reproductive structures (flowers, fruits, pods, bolls), producing immediate and often greater yield losses across multiple crops. In contrast, *S. frugiperda* is predominantly associated with maize and primarily causes defoliation/whorl injury its yield impact is more contingent on plant stage and compensatory growth, making its relevance comparatively narrower for cross-commodity protection (Sutiharni & Afifah, 2022). This pest causes huge losses to farmers because its caterpillars damage leaves, flowers and corn cobs. The resulting damage reduces crop yields by 30-80%, reduces corn quality, and causes high control costs. Additionally, attacks by these pests can trigger secondary infections by other pathogens, exacerbating the damage (Altaf et al., 2023). Farmers often rely on

chemical insecticides which are expensive and not always effective, and have negative impacts on the environment and health (Aghdam & Brown, 2021). Therefore, more effective and sustainable pest control solutions are needed to reduce economic losses for farmers (Andreas, 2023). Excessive insecticide use contaminates soils and waters, harms non-target beneficials, drives resistance, and threatens human health (Jha et al., 2023; Mantzoukas et al., 2022; Idrees et al., 2021). As a sustainable alternative, endophytic fungi—symbionts residing in plant tissues—can produce pest-suppressive bioactive compounds, offering environmentally friendlier control options (Kobandaha et al., 2022).

Research has shown that several species of endophytic fungi, such as *Beauveria bassiana* and *Metarhizium anisopliae*, are effective in controlling caterpillar pests on corn plants. This fungus can produce enzymes and toxic compounds that attack the pest's immune and nervous systems, causing death of the caterpillars (Lestari et al., 2022). Additionally, endophytic fungi can enhance plant resistance to environmental stress and other pathogens. Entomopathogenic fungi are fungi that parasitize insects and has the ability to infect or kill insects that become the host (Mantzoukas & Eliopoulos, 2020). Entomopathogenic fungi can infect insects thus reducing the quantity of insect reproduction, disrupting the growth process and development, reduces the level of insect resistance to various environmental factors, as well as the ability to kill insects (Herlinda et al., 2022). Endophytic fungi with Entomopathogens ability are naturally available and can be found from isolation in infected insects, soil, and plant tissue (Amelia et al., 2021).

Thus, the use of endophytic fungi as biological agents is a promising strategy for controlling caterpillar pests on corn plants. This is in line with the principles of sustainable agriculture which prioritizes environmental health and human welfare (Pratiwi & Asri, 2022). However, the application of this technology requires further research and development to ensure its effectiveness, safety and availability for farmers. More in-depth knowledge of the interactions between endophytic fungi, plants and pests is also important to optimize their application in the field (Sukarno et al., 2021). Therefore, the purpose of this study was to isolate, characterize, and evaluate endophytic fungi from parijoto leaves for their entomopathogenic potential against *H. armigera* and to identify the most promising biocontrol candidate.

Methods

Materials

The research uses the design of Factorial Randomized Groups. which consists of one factor, where the treatment carried out is the application of various kinds of isolates of endophytic fungi in the form of spray preparations. Spore density of endophytic fungi sprayed was made the same, 106/ml distilled water, which was applied to 5 larvae per treatment with three repetitions. Materials used in this research: parijoto plant leaves, healthy corn, medium PDA, NaOCl 0.5%, distilled water, alcohol 70% and 95%, tissue, cotton, label paper, and filter paper. The tools that used: petri dishes, test tubes, Erlenmeyer flasks, pipette, aluminum foil, measuring cup, preparation needle, microscope, autoclave, digital scale, spray, ruler, scissors, scalpel

Endophytic fungi were isolated from Parijoto leaves on PDA

The research was carried out in the Microbiology laboratory, Integrated Laboratory, Walisongo State Islamic University Semarang. A total of 5 plants had their tissues taken for exploration. The plant parts taken for the exploration process were in healthy condition and did not show any symptoms of disease infection. Taken are mature leaves. Samples of plant parts were placed in sterile plastic bags and taken to the laboratory for isolation of endophytic fungi. Isolation of endophytic fungi on leaves was carried out based on the method described by (Syaifudin & Kasiamdari, 2022) with several modifications. The leaf surface was cut into small fragments with an area of 1 cm^2 under aseptic conditions using a sterile scalpel. The leaf pieces were washed under running water for 2 minutes, then air-dried. The leaves are soaked in 70% alcohol for 1 minute, 4% NaOCl solution for 1 minute, and sterile distilled water for 2 minutes,

then air-dried on sterile tissue. After sterilization, the sterilized leaf pieces were then placed in a petri dish containing PDA media, incubated at 26 ˚C for 48 hours (Wang et al., 2023).

Endophytic fungi were purified.

Endophytic fungi that have grown on PDA media are then purified by grow and reproduce each fungal colony obtained on new PDA media (Altaf et al., 2023). Based on differences in macroscopic morphology, the isolated fungi are separated from one another and from other colonies that exhibit distinct characteristics, such as colony shape and color. Transfer is done by cutting and take fungal colonies from the previous media then transfer them to the media The new PDA uses a loop needle. Purification is carried out continuously until a pure, non-mutual fungal colony is obtained mixed with other fungal colonies (Maryati, 2016).

Endophytic Endophytic were characterized.

Characterization of endophytic fungi was carried out microscopically and macroscopically. Macroscopic observations were carried out by observing shape and growth, including color, colony surface texture, concentric circles, reverse color of colonies, and diameter of fungal colonies. Direct microscopic observation This is done by taking one dose of endophytic fungal isolates obtained, etched on the glass object that has been sterilized, and then fixed with a spirit burner. Next, it dripped with one drop of methylene blue and was covered with a cover glass. It was observed with a microscope at 40 magnification and 100. Indirect microscopy This is done by sterilizing the object glass and covering the glass in a petri dish. With filter paper. Inoculate the isolated endophytic fungi to the glass object that contains one drop of unused PDA medium solid and cover it with a covered glass to isolate the fungus endophyte (Sofian et al., 2025). Dropped on filter paper with glycerin evenly, then incubated and observed for the next 3-5 days using a microscope. Microscopic characterization was carried out by observing the structure of the hyphae (insulated and non-insulated), fruiting bodies, and spore structure (Demeni et al., 2025).

The entomopathogenic activity of endophytic fungi against Helicoverpa larvae was evaluated.

Tests for the ability of endophytic fungi as entomopathogens were carried out to observe the ability and influence of each endophytic fungus on larval development conditions and mortality rate of *H. armigera*. In the test implementation, fourth-instar *H. armigera* larvae were maintained as part of several stages including *H. armigera* larvae maintained by being fed daily corn leaves which is between 10-15 days old. During the rearing process, the larvae placed in a plastic jar measuring 20 cm high and 15 cm in diameter with each jar containing 5 caterpillars. The jar is covered using a rag and tied using a rubber band. If the jar is filled with feces from caterpillars and if it is dirty then it needs to be replaced with transfer of larvae to a new jar. Spore density of endophytic fungi sprayed were made the same, 106/ml aquades

The mortality test for *H. armigera* larvae was carried out by applying fungus endophytes against *H. armigera* larvae. This aims to investigate the effect on larval mortality, thereby identifying which endophytic fungi possess entomopathogenic properties (Andreas, 2023). Entomopathogenic fungal suspension were sprayed onto body of larvae 3 times in day at 07:00, 11:00, and 16:00 from a distance of 30 cm., then transferred into a jar and use a cover that has been ventilated air and lined with gauze, to protect circulate air in the jar and avoid larvae come out of the jar. Observations after application are carried out every day from 0 DSA to 7 DSA or until test larvae die due to fungal treatment endophyte in the larva. Mortality shows the level of ability or number of pest deaths caused by insecticides used. Mortality of *H. armigera* larvae was calculated after 24 hours after application (Boulamtat et al., 2025). Mortality can calculated by formula:

$$\text{Mortality } (\%) = \frac{\text{Number of dead larvae (individuals)}}{\text{Total Total number of larvae}} \text{X } 100$$

Data Analysis

Data analyzed using the analysis of variance test at the error level (α) 5%. If the analysis results show an influence real, further tests were carried out using Duncan's Multiple Range Test (DMRT) at level 5% error.

Results and Discussions

Isolation of Fungal Endophytic

Endophytic fungi that had been inoculated for 7 days began to show hyphal growth on the second day after inoculation. After reaching almost the edge of the petri dish, after that, continue with the purification of the endophytic fungus to obtain a single endophytic fungal isolate. The following are the results of the isolation of endophytic fungi. In isolating parijoto leaves procces, healthy leaves are used, namely mature and old leaves. Mature leaves are dark green, while old leaves are selected for parity, and these are starting to turn yellowish. Apart from variations in leaf age, this study also used variations in leaf direction, namely adaxial and abaxial. Choosing leaf age and leaf parts is an effort to increase the variety of endophytic fungi that grow. This method ensures that the screening process obtains quality endophytic fungal isolates in large quantities.

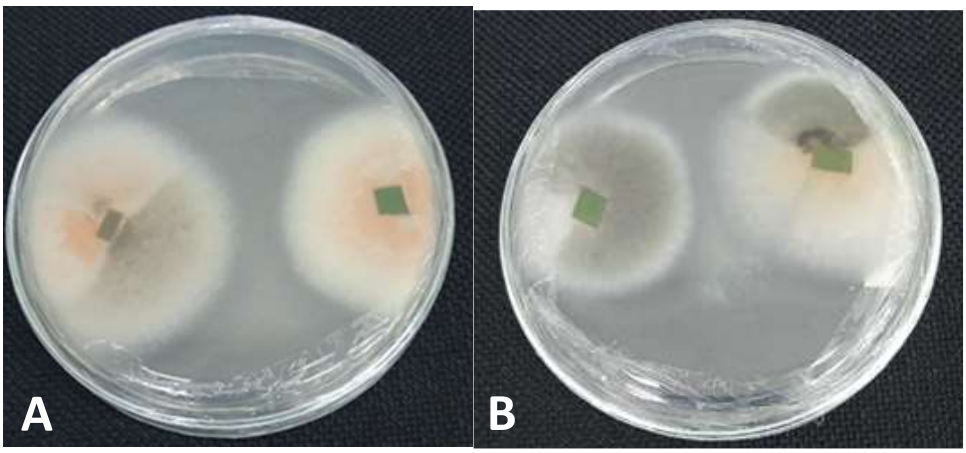

Figure 1. Results of isolation of endophytic fungi on Parijoto leaves. A: Abaxial leaves, B Adaxial leaves

Figure 1 shows successful isolation of endophytic fungi from Parijoto leaves, with mycelial colonies emerging from tissue discs placed on PDA from both the abaxial surface (A) and the adaxial surface (B). The clear growth halos surrounding the leaf fragments indicate viable endophytes and distinct colony morphologies, suggesting more than one taxon. These primary isolates are suitable to proceed to the purification stage with subculturing by hyphal tip transfer onto fresh medium to obtain axenic (pure) cultures for downstream identification and bioassays.

Purification of Fungal Endophytic

The fungal isolate obtained was then purified by transferring each different character to a new petri dish. The purification stage begins by selecting isolates with different characteristics, including color, edges, growth rate, elevation, and appearance of spores. With these indicators in one petri dish, it is very likely that more than two endophytic fungal isolates will grow. The following are the results of purifying the parijoto leaf endophytic fungus

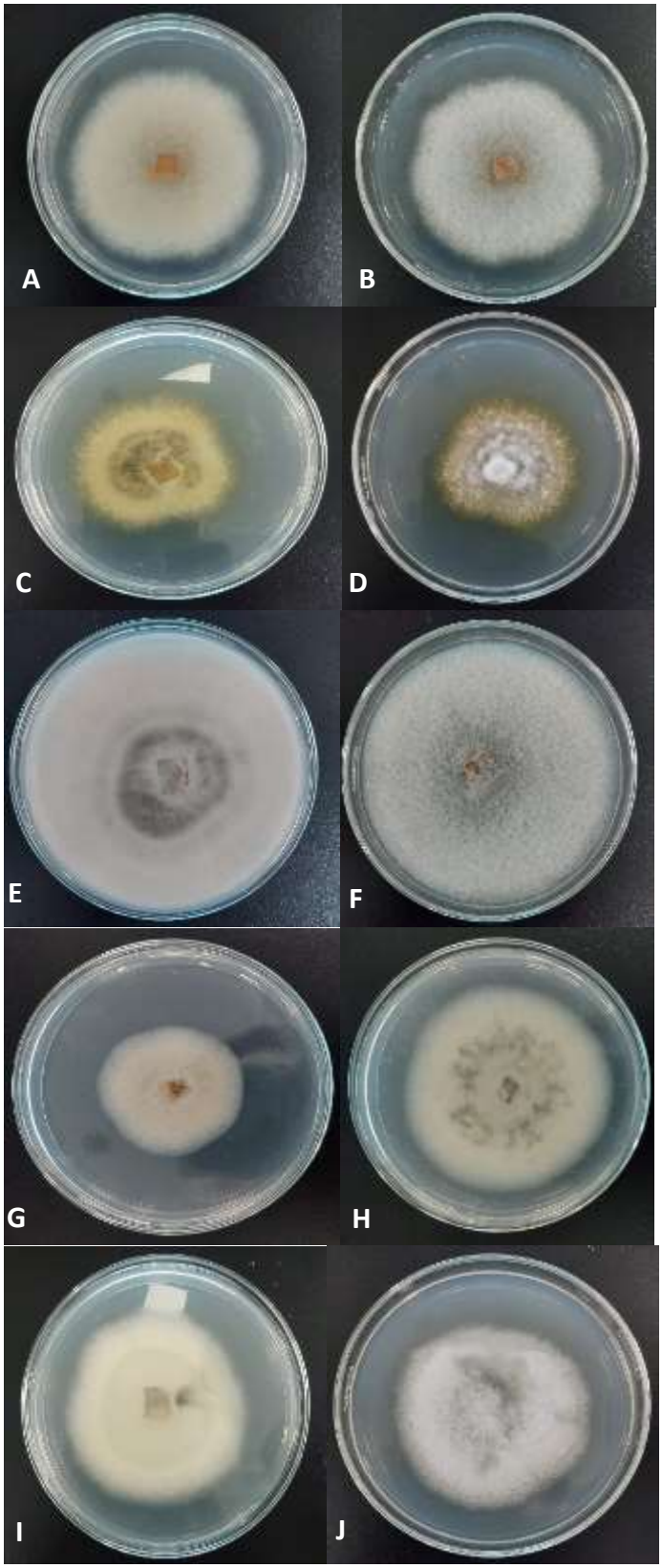

Figure 2. Pure culture of endophytic fungi A, B = Isolate EP1, C, D = Isolate EP2, E, F = Isolate EP3, G, H = Isolate EP4, and I, J = Isolate EP5 on PDA medium 72 hours (left part: back of petri; right part: front of petri)

Characterization of endophytic fungi was performed

Each purified fungus was then subjected to microscopic characterization in the form of observing hyphae and reproductive structures. Based on the identification results of the macroscopic and microscopic characteristics of endophytic fungi isolated from Parijoto leaves, pure isolates of 5 types of endophytic fungi were obtained. Endophytic fungi isolated from Parijoto plant organs in the UIN Walisongo integrated laboratory area were also found in other plant organs. Below is a tabulation table of the morphological characters of endophytic fungi.

Table 1. Results of macroscopic observations of endophytic fungal isolates

Isolate	Colony surface color	Colony Elevation	Margin colony	Radial Line	Reverse Color	Hyphae characterictic
EP1	White	convex	Filiform	No	brownish	The hyphae are insular and branched
EP2	Brownish	convex	Undulate	No	brown	The hyphae are insular and branched
EP3	White	convex	Entire	Yes	grey	The hyphae are insular and branched
EP4	Cream	flat	Entire	Yes	yellowish	The hyphae are insular and branched
EP5	White	convex	Filiform	Yes	brownish	The hyphae are insular and branched

From Figure 2 the macromorphological characters recorded for isolates EP1–EP5 surface colour (white/brownish/cream), colony elevation (convex vs. flat), margin architecture (filiform, undulate, entire), presence of radial lines, and reverse pigmentation (brownish, brown, grey, yellowish). Provide diagnostically useful cues for preliminary grouping of endophytic entomopathogenic fungi. White convex colonies with pale-to-brownish reverses (EP1, EP5) fall within the well-documented range for *Beauveria* spp., whose cultures typically present white, cottony surfaces and yellowish–brown reverses as colonies mature (Akrich et al., 2023). By contrast, the combination of flat growth, conspicuous radial zonation, and a yellowish reverse observed in EP4, as well as the appearance of radial lines in EP3, is congruent with developmental trajectories reported for *Metarhizium* spp., which often begin white on PDA and transition to yellow-green/olive-green with ochreous to pale-yellow reverses as sporulation proceeds (Ma et al., 2024). The uniform note of "branched hyphae" across isolates fits both genera. It underscores that colony-level traits (e.g., radial zoning and reverse pigmentation often linked with sporulation fronts and pigment deposition) are more discriminating at this screening stage than gross hyphal branching alone (Geremew et al., 2024). Taken together, the table supports a working hypothesis that EP1/EP5 are Beauveria-like, while EP3/EP4 show *Metarhizium*-like macromorphology, pending finer-scale confirmation.

To preliminarily differentiate the endophytic fungi recovered from Parijoto leaves, we recorded key macromorphological traits on PDA, a rapid and inexpensive approach that complements later microscopic and molecular identification. As summarized in Table 1, the five isolates (EP1–EP5) show consistent yet distinct phenotypes: surface colors span white/cream to brownish; colony elevation is mostly convex (EP1, EP2, EP3, EP5) with one flat form (EP4); margins range from entire to filiform or undulate; radial lines are absent in EP1–EP2 but present in EP3–EP5; and reverse pigmentation varies from brownish to grey or yellowish. All isolates display insular, branched hyphae.

These observations align with contemporary guidance that macromorphology (surface/reverse colour, elevation, margin, radial zonation) remains informative for initial

classification but is inherently plastic shaped by medium, temperature, and time must be integrated with micromorphology (e.g., phialide arrangement, conidial shape/size) and multilocus phylogenetics (e.g., ITS, TEF1-α, RPB1/2) to achieve species-level resolution suitable for publication and regulatory use (Olumuyiwa et al., 2025).Recent taxonomic and ecological treatments of entomopathogenic/endophytic Hypocreales explicitly demonstrate these principles. *Metarhizium* lineages exhibit consistent progressions in colony pigmentation and reverse colours that correlate with sporulation timing, while *Beauveria* retains white surfaces with pale yellow-to-brown reverses across media—patterns mirrored by EP3–EP5 versus EP1/EP5, respectively (Akrich et al., 2023).

Entomopathogenic Ability of Endophytic Fungi against H. Larvae.

H. armigera larvae found to be infected with endophytic fungi in testing have the characteristics of the larval body being covered by mycelium and endophytic fungal conidium. The results of analysis of variance on the percentage of mortality of *H. armigera* larvae showed that the entomopathogenic fungus isolate EP5 had a significant effect with the test results indicated by a sig one way anova result of less than 0.05, resulting in an increase in the percentage of larval mortality followed by a single treatment of EP5. and then EP4.

Table 2. Table of mortality test results for endophytic fungal entomopathogenicity

Isolate endophytic fungal	Mortality (%)			
	1	2	3	Mean Mortality ±SD
EP1	8.46	8.03	9.69	8.73^{bc} ± 0.8615
EP2	9.68	7.27	6.34	7.76^{b} ± 1.7237
EP3	11.63	10.09	9.32	10.35^{cd} ± 1.1761
EP4	12.83	10.88	12.82	12.18^{d} ± 1.1229
EP5	30.12	31.67	30.54	30.78^{e} ± 0.8016
Control -	1.21	1.33	0.89	1.14^{a}± 0.2274

Note: a) Numbers followed by letters in the same column indicate that the results are not significantly different in the DMRT test with an error rate of 5%

Table 2 is the result of the endophytic fungi Entomopathogen application technique by spraying on the bodies of larvae, which can be seen across three replicates, larval mortality varied markedly among endophytic fungal isolates, with EP5 consistently producing the highest effect (30.12%, 31.67%, 30.54%; 30.78 ± 0.80%) and forming its own DMRT group (e, α = 0.05), indicating significantly greater virulence than all other isolates. EP4 ranked second (12.18 ± 1.12%, group d), followed by EP3 (10.35 ± 1.18%, group cd) and EP1 (8.73 ± 0.86%, group bc), while EP2 showed the lowest effect among treated groups (7.76 ± 1.72%, group b). The control exhibited only 1.14 ± 0.23% mortality (group a). Thus, the performance order was EP5 > EP4 > EP3/EP1 > EP2 > Control, with overlapping letters indicating no clear separation between EP3 and EP1 and between EP1 and EP2. All isolates outperformed the control, and because larvae (not diet) were sprayed under identical conditions, these differences reflect isolate-intrinsic virulence rather than procedural artifacts. The application of endophytic fungi was characterized by the presence of white mycelial growth on the surface of larval bodies, which served as a clear indicator of mortality. Among the tested isolates, EP5 demonstrated the highest percentage of larval death, suggesting its superior entomopathogenic potential. The ability of fungal isolates to infect insect hosts is strongly influenced by the structural properties of the host cuticle, particularly cuticle thickness, which can act as a barrier to penetration. During infection, EP5 initiates physiological disruption in the insect beginning with the integument, where fungal spores adhere, germinate, and mechanically or enzymatically breach the cuticle. Once penetration is achieved, fungal hyphae and secreted metabolites interact with the larval immune system, suppressing host defenses and facilitating systemic colonization.

Current evidence suggests that spore density plays a crucial role in determining the success of infections and mortality rates. A higher concentration of spores increases the likelihood of adhesion to the larval body, thereby raising the cumulative enzymatic activity (e.g., proteases, chitinases, lipases) and toxin release associated with fungal invasion. This enhanced enzymatic arsenal accelerates cuticle degradation and overwhelms larval immune responses, leading to rapid physiological breakdown and death. Similar findings have been reported in recent studies where high inoculum levels of *Metarhizium anisopliae* and *Beauveria bassiana* were positively correlated with faster mortality and more extensive mycosis in target pests (Geremew et al., 2024). EP5 showed the highest and most consistent mortality among isolates (replicates: 30.12%, 31.67%, 30.54%; mean 30.78% ± 0.80 SD), and it was statistically higher than the others according to DMRT. Consequently, the superior performance of EP5 in this study may be attributed not only to its inherent virulence factors but also to its efficiency in spore adhesion, germination, and enzyme production, making it a promising candidate for integration into sustainable pest management strategies.

The mortality assay shows a clear, monotonic rank of endophytic fungal isolates, with EP5 producing the highest larval mortality (30.78 ± 0.80%) followed by EP4 (12.18 ± 1.12%), EP3 (10.35 ± 1.18%), EP1 (8.73 ± 0.86%), and EP2 (7.76 ± 1.72%), while the negative control remained low (1.14 ± 0.23%). Different superscript letters indicate these means are significantly separated by DMRT at $\alpha = 0.05$, confirming that each step up the ranking reflects a statistically meaningful increase. Contemporary studies show that endophytic entomopathogenic fungi can suppress *S. frugiperda* through a combination of direct mycosis and plant-mediated antibiosis. For example, in maize colonized by *Beauveria bassiana* or *Metarhizium anisopliae*, larval mortality frequently rises well above untreated controls, though typically below the maxima achieved by direct conidial sprays. Mechanistically, endophytes can produce insecticidal metabolites (e.g., beauvericin, destruxins) and hydrolytic enzymes, and may prime host tissues, thereby impairing insect digestion and immunity—routes compatible with the observed graded mortality among isolates (EP5 > EP4 > EP3 > EP1 > EP2) (Aravinthraju et al., 2024). The magnitude and consistency of EP5 indicate strong candidacy for integrated pest management.

The compound that functions as a stomach poison is tannin. Spraying each larva with a fixed volume/concentration ensures a consistent delivered dose. When mixed into diet or applied to leaves, tannins can bind proteins and vary across surfaces, creating uneven ingestion and higher variance. Tannin will prevent insects from digesting food and can reduce their digestive ability food by reducing the activity of digestive enzymes (protease and amylase) which play a role in catalyzing proteins into amino acids needed for larval growth, it binds enzymes by tannin, causing the enzyme's work to be hampered, resulting in cell metabolic processes can be disturbed and the larvae will lack nutrition, if this process continues, it will impact on larval death. Insect death is a manifestation of various physiological disorders of living creatures. Research conducted by Suroto et al (2023) shows that Interaction between the entomopathogenic fungus *Metarhizium* sp. and *Penicillium* sp. with the insect pest larva *S. frugiperda* shows the relationship that occurs between biotic factors and insect pests. Analysis show that one of these types of biotic factors can resulting in increased mortality. Although the research is different, the mechanism of action of endophytes in killing caterpillar pests is believed to be the same (Rindiani et al., 2024).

Tannins, as one of the secondary plant metabolites, play a significant role in natural defense mechanisms against insect pests. Their mode of action is not only through inhibiting digestive enzyme activity such as proteases and amylases but also through forming complexes with proteins, which causes insects to suffer nutritional deficiencies for growth (War et al., 2012). This effect is consistent with the findings of Suroto et al. (2023), who reported that the interaction between entomopathogenic fungi *Metarhizium* sp. and *Penicillium* sp. with *S. frugiperda* larvae disrupted larval metabolism, thereby increasing mortality. Thus, both tannins and insect pathogens target similar vulnerabilities in insect physiology, particularly related to digestion and energy metabolism.

Furthermore, Rojas et al. (2017) explained that the role of endophytes in pest control is not limited to colonizing host tissues but also involves producing toxic metabolites that suppress insect immune systems and exacerbate digestive organ damage. This is reinforced by Rindiani et

al. (2024), who suggested that larval mortality caused by endophyte infection likely shares a similar mechanism with tannins, namely through inhibition of digestive enzymes and disruption of cellular metabolism. By comparing these studies, it becomes evident that biocontrol strategies using secondary metabolites and entomopathogenic fungi have strong potential for sustainable agriculture, particularly in suppressing invasive pest populations such as *S. frugiperda.*

Conclusion

Five endophytic fungi were successfully isolated from Medinilla speciosa leaves, resulting in five fungal isolates. The isolate code is EP1, EP2, EP3, EP4, EP5. All isolates exhibit entomopathogenicity, as they affect the normal formation of *H. armigera* larvae and are capable of causing larval mortality larvae. The application of endophytic fungi had a significant effect on larval mortality as determined by the DMRT test. Mortality resulting from 8,73 – 30,78% with the treatment with the highest percentage is EP5.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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