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Exploration of Endophytic Fungi from Corn in North Sumatra Indonesia and Their Potential as Entomopathogens

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Abstract: Entomopathogenic fungi are one of the biological agents that play an important role in controlling insect pests. In addition to being obtained from infected insects and the rhizosphere, this group can also associate as endophytes in plant tissues. This study aims to explore endophytic fungi originating from corn (*Zea mays* L.) in North Sumatra and evaluate their entomopathogenic potential against insects. Plant samples were collected from corn production centers in the lowlands and middlelands of Deli Serdang and Serdang Bedagai Regencies using a purposive sampling method. Isolation was carried out from healthy plant tissues (roots, stems, and leaves), followed by a pathogenicity test using the insect baiting method against *Tenebrio molitor* larvae. A total of 59 endophytic fungal isolates were successfully obtained, of which 19 isolates showed pathogenic activity against *T. molitor*. Isolate EK2_23 (*Beauveria* sp.) showed the highest performance with mortality and mycosis of 85% and 100%, respectively. Morphological identification revealed the presence of four main genera: Beauveria, Metarhizium, Aspergillus, and Trichoderma. This finding confirms that corn endophytic fungi have the potential to be a prospective source of local biological agents for development as mycoinsecticides to control major corn pests.

Keywords: Biological control; Conidial viability; Fungal virulence; Larval mortality; Mycosis

Introduction

Spodoptera frugiperda J. E Smith (Lepidoptera: Noctuidae) is an invasive species that has become a global threat to food crop production, particularly corn (*Zea mays*). Since its spread from the Americas to Africa and Asia, this pest has demonstrated high ecological adaptability, a rapid life cycle, and a broad host spectrum, causing significant yield losses (Day et al., 2017; Goergen et al., 2016; Prasanna et al., 2022). In Southeast Asia, including Indonesia, the intensity of *S. frugiperda* attacks continues to increase and poses a serious challenge to maintaining stable corn production.

Field-level control of *S. frugiperda* still relies heavily on synthetic chemical insecticides. However, intensive use has led to the development of pest resistance to various active ingredients and has negative impacts on the environment and non-target organisms (Sparks et al., 2020; Yu & E, 1991). This situation demands the development of more sustainable control strategies, one of which is through the use of biological agents such as entomopathogenic fungi.

Entomopathogenic fungi (EPF) are a group of microorganisms capable of infecting insects through direct penetration of the cuticle and the production of toxic metabolites that cause host death (Vajri et al., 2024). Several important genera, such as Beauveria and Metarhizium, have been widely reported to be effective in controlling various agricultural pests, including Lepidoptera (Lacey et al., 2015; Vega et al., 2012). Furthermore, the genus Trichoderma, known as an antagonist, has also been reported to possess entomopathogenic potential and the ability to increase plant resistance (Mantzoukas & Eliopoulos, 2020).

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Several species of Aspergillus also exhibit pathogenic activity against insects, although their role is more often associated with saprophytic and endophytic functions (Subbanna et al., 2025).

Recent developments indicate that EPF not only act as insect pathogens but can also live as endophytes within plant tissues without causing disease symptoms. As endophytes, these fungi can provide additional benefits such as increased plant growth, induction of systemic resistance, and indirect protection against herbivores (Mantzoukas & Eliopoulos, 2020; Quesada-Moraga, 2020; Vega et al., 2012). This concept strengthens the plant-mediated pest management approach, which is considered more stable and sustainable than the application of external biological agents.

Although the potential of entomopathogenic fungi as endophytes is increasingly being reported, exploration of local isolates originating from primary host plants remains limited, particularly in tropical regions like Indonesia. The corn agroecosystem in North Sumatra has unique environmental conditions and has the potential to be a source of endophytic microbial diversity with high adaptability. However, information on the diversity and pathogenicity of corn-derived endophytic fungi against *S. frugiperda* in this region remains very limited. This study aims to isolate and identify endophytic fungi from corn plants in North Sumatra and evaluate their potential pathogenicity as entomopathogens. This research is expected to provide a scientific basis for the development of effective, specific, and sustainable local resource-based mycoinsecticides.

Method

Corn Plant Site Survey and Sampling

The study was conducted at the Plant Protection Laboratory, Medan Area University, from November 2025 to January 2026. Endophytic fungi were explored by collecting roots, stems, and leaves from corn plants. The survey locations for sampling were corn crop centers in North Sumatra Province, namely community plantations in Deli Serdang Regency (lowlands) and Serdang Berdagai Regency (midlands). The selected plants were considered to be the healthiest and least susceptible to pests or diseases (Kasambala Donga et al., 2021). The location code, sampling date, and soil pH were marked on each selected plant sample, which were then transported to the laboratory for further examination.

Isolation of Endophytic Fungi from Plant Tissue

Plant samples (roots, stems, and leaves) were cut into 0.5 cm x 0.5 cm pieces. Surface sterilization was performed on sample pieces by immersion in 70% alcohol (120 seconds), 1% NaOCl (60 seconds), followed by three 60-second rinses in sterile distilled water (Elfita et al., 2019). The final rinse was cultured on Potato Dextrose Agar (PDA) media to ensure effective surface sterilization, as indicated by the absence of microbial growth (Ramirez-Rodriguez & Sánchez-Peña, 2016; Russo et al., 2021). Isolation was performed in Laminar Air Flow. Five sample pieces (roots, stems, and leaves) were grown on Malt Extract Agar (MEA) media and then incubated at 26 °C for four days. Fungal purification was performed on fungi grown from plant tissue (Elfita et al., 2019; Silva et al., 2018). Observations were made on the color and shape of the colonies, as well as the morphology of the fungal hyphae and conidia four days after isolation.

Provision of Tenebrio molitor Test Insects

T. molitor larvae were obtained from a bird food stall located in the fishing area of Medan City. The larvae were kept in plastic boxes and fed rice bran (chicken feed). After molting or reaching a length of approximately 1.5 cm, the larvae were ready for pathogenicity testing.

Pathogenicity testing using the insect baiting method

The pathogenicity test for endophytic fungi on *T. molitor* larvae aims to select fungal isolates with potential entomopathogenic properties. This test was conducted by placing 40 *T. molitor* larvae in MEA media containing isolated fungal cultures. The larvae were left in the culture media for 24 hours to allow contact between the fungal conidia and the insects. As a control, the larvae were placed in media without the endophytic fungal culture. After 24 hours, 10 larvae were transferred to each 5 cm diameter plastic box and fed rice bran. The number of infected and dead larvae was observed for 7 days after treatment. Fungi with entomopathogenic potential and high mortality rates in *T. molitor* larvae were selected for further testing.

Morphological Identification of Entomopathogenic Fungi

Identification of entomopathogenic fungi included macroscopic and microscopic observations using the identification key of Hunter & Barnett (2019). Macroscopic observation involved visual observation of colony color and growth, while microscopic observation involved removing a portion of the fungal colony and placing it on a glass slide, observing the branching of the conidiophores and the shape of the fungal conidia.

Colony Growth Observation

Colony growth was observed on entomopathogenic fungal isolates. Seven-day-old entomopathogenic fungal isolates were transferred to Petri dishes containing MEA medium. The diameter of the transferred entomopathogenic fungal isolates was 0.7 cm. After transfer, the entomopathogenic fungal isolates were incubated at 25ºC. The colony diameter of each isolate was measured every two days until day 6.

Conidial Density and Viability Measurement

Conidial density and viability were tested using a 15-day-old entomopathogenic fungal suspension. Conidial density was determined by counting the number of conidia using a hemocytometer and observed under a light microscope at 400× magnification. Conidial viability was assessed by the percentage of conidial germination by dropping the conidial suspension onto 2% water-agar or thin MEA media placed on a sterile glass slide, then incubating at 25°C for 24 hours. Each treatment was replicated four times, and observations were made using a binocular microscope at 400× magnification on 100 conidia per replicate. Conidia were considered germinated if they formed a germ tube whose length exceeded the diameter of the conidia.

Data analysis

Mortal and sporulation observations were conducted starting one day after treatment by counting the number of dead larvae every 24 hours. Larval sporulation was visible when the larval body was covered with hyphae or conidia. Larval mortality (M) was calculated using the formula of Rustama (2008).

$$M = \frac{n}{N} + x\ 100\% \quad (1)$$

Description:
n = total number of dead larvae
N = total number of treated larvae.

Sporulating fungi were identified to the genus level based on macroscopic characteristics (colony color and growth) and microscopic characteristics (conidiophore branching and conidia shape) using the Hunter & Barnett (2019) identification key.

Colony growth was measured based on fungal diameter every 2 days until day 6 using a ruler. Conidial germination was determined after 24 hours of incubation using a binocular microscope at 400X magnification. The germination percentage was calculated from 100 conidia using the formula 2.

$$D = \frac{\text{Number of germinated conidia}}{\text{number of conidia observed}} x100\% \quad (2)$$

The data are presented in tabular form and analyzed using statistics 8. If there is a significant difference, the LSD test is continued at a significance level of 5%.

Result and Discussion

Endophytic Fungal Colonization of Corn Plant Parts

Observations showed that the level of endophytic fungal colonization of corn plants varied across altitudes. In general, colonization was higher in lowland areas (52.83%) than in midland areas (47.17%) of the total isolates obtained (Table 1).

Table 1. Number of endophytic fungi isolated from corn plant tissue

Plant parts	Location		Total
	Lowlands (Deli Serdang)	Midlands (Serdang Bedagai)	
Roots	13	11	24
Stems	5	6	11
Leaves	10	8	18
Number	28	25	53

A total of 53 endophytic fungal isolates were successfully isolated (Figure 1), with a relatively larger number originating from the lowlands (28 isolates) compared to the midlands (25 isolates). The distribution of isolates showed that roots were the tissue with the highest colonization rate (24 isolates), followed by leaves (18 isolates) and stems (11 isolates). This pattern indicates a preference for endophytic fungal colonization in underground tissues, likely related to intensive interactions in the rhizosphere zone.

Mortality of T. molitor larvae after endophytic fungus application

Pathogenicity testing indicated that some endophytic fungal isolates have potential as entomopathogens. Of the 34 isolates tested, 19 (55.88%) caused mortality of *T. molitor* larvae, with varying degrees of mortality. Several isolates, namely EK2_23, EB4_22, EK4_1, EK2_1, EK3_2, ED5_23, and EB4_23, showed mortality rates exceeding 50%, thus categorizing them as having relatively high virulence .

However, not all dead larvae showed signs of advanced infection, such as sporulation. Of the 19 isolates that caused mortality, only 12 were able to produce sporulation on the larval body (Table 2). This indicates that larval mortality is not always followed by external pathogen development.

Isolate EK2_23 demonstrated the most prominent performance with a sporulation rate of 100%, while the other isolates showed mycosis rates ranging from

22.22% to 56.41%. Mycosis symptoms were characterized by mummification of the larvae followed by hyphal growth on the body surface several days after infection (Figure 2).

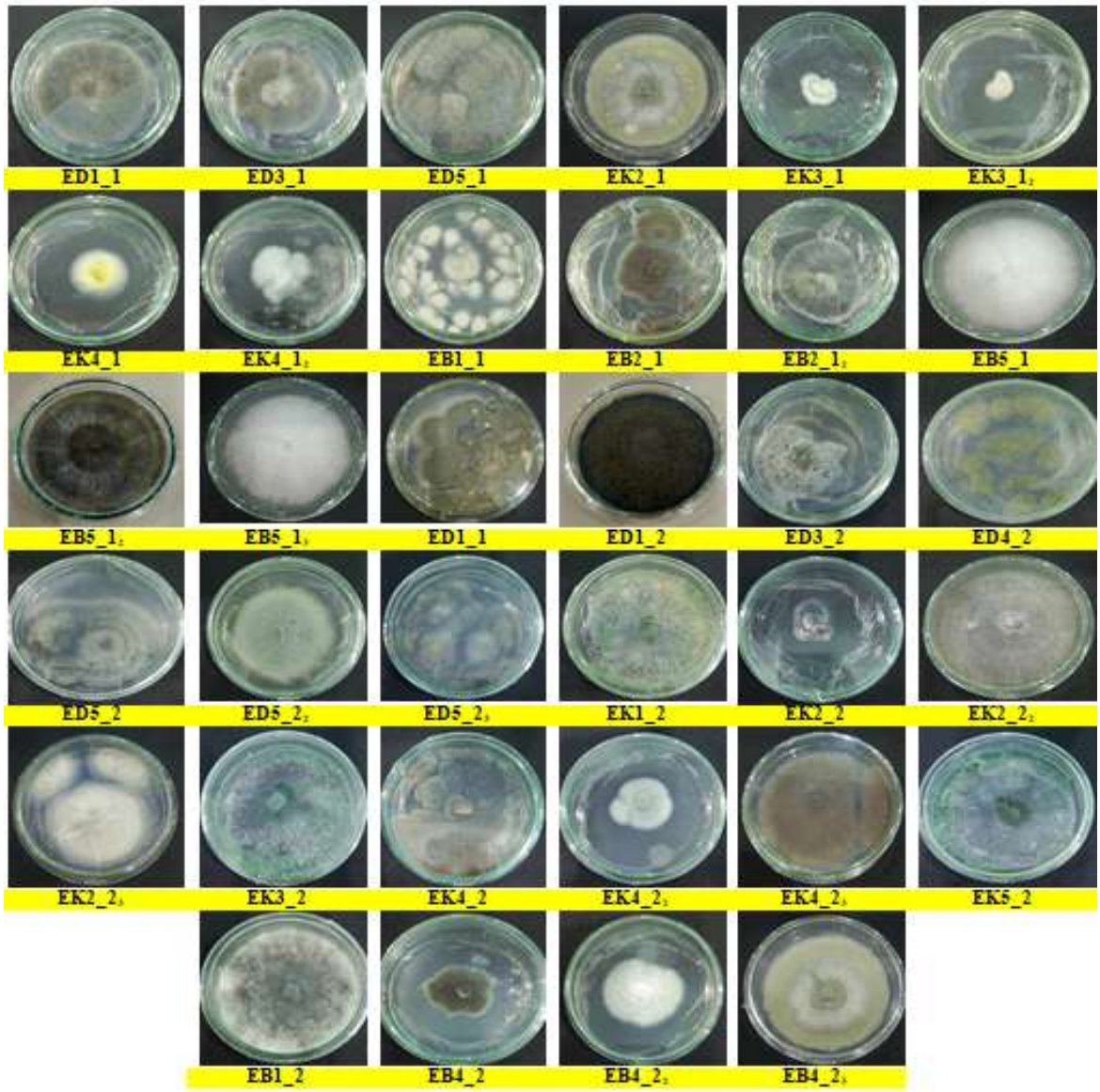

Figure 1. Form of endophytic fungal colonies in corn plants at 15 days after inoculation (dsi) (E: Endophyte; D: Leaf; K: Root; B: Stem; _1: Lowland (Deli Serdang); _2: Midland (Serdang Berdagai).

Table 2. Mortality and mycosis of *T. molitor* larvae 7 days after endophytic fungus application (DAA)

Code of isolat	Mortalitas larva (%) ± SD		Mikosis (%) ± SD	
EK2_2$_3$	84.00 ±	0.00a	100.00 ±	0.00a
EB4_2$_2$	70.00 ±	0.00a	50.00 ±	5.00a
EK4_1	60.00 ±	0.26a	56.41 ±	5.20ab
EK2_1	60.00 ±	0.28a	26.32 ±	5.00b
EK3_2	56.70 ±	0.34a	22.22 ±	5.00b
ED5_2$_3$	53.33 ±	1.24a	38.89 ±	5.30b
EB4_2$_3$	53.33 ±	2.90a	50.14 ±	5.00ab
EK4_2	50.00 ±	1.58a	52.94 ±	5.46ab
ED5_2	46.67 ±	1.43a	30.30 ±	5.30b
ED3_2	46.67 ±	0.75a	24.15 ±	5.00b
EK5_2	43.33 ±	2.20b	30.30 ±	5.30b
ED5_1	40.00 ±	3.22b	28.57 ±	5.00b

Code of isolat	Mortalitas larva (%) ± SD		Mikosis (%) ± SD	
ED4_2	36.67 ±	2.14bc	0.00 ±	0.00c
EK1_2	36.67 ±	1.78bc	0.00 ±	0.00c
ED1_1	33.33 ±	0.00c	0.00 ±	0.00c
ED3_1	33.33 ±	0.00c	0.00 ±	0.00c
EB2_1	30.00 ±	0.00c	0.00 ±	0.00c
EK4_2_3	30.00 ±	0.00c	0.00 ±	0.00c
EB1_1	23.33 ±	0.00 c	0.00 ±	0.00c
EB1_2	0.00 ±	0.00d	0.00 ±	0.00c
EB5_1	0.00 ±	0.00d	0.00 ±	0.00c
EB5_1_2	0.00 ±	0.00d	0.00 ±	0.00c
ED1_2	0.00 ±	0.00d	0.00 ±	0.00c
EB4_2	0.00 ±	0.00d	0.00 ±	0.00c
ED5_2_2	0.00 ±	0.00d	0.00 ±	0.00c
EK2_2	0.00 ±	0.00d	0.00 ±	0.00c
EK2_2_2	0.00 ±	0.00d	0.00 ±	0.00c
EK4_2_2	0.00 ±	0.00d	0.00 ±	0.00c
ED1_1	0.00 ±	0.00d	0.00 ±	0.00c
EB5_1_3	0.00 ±	0.00d	0.00 ±	0.00c
EB2_1_2	0.00 ±	0.00d	0.00 ±	0.00c
EK4_1_2	0.00 ±	0.00d	0.00 ±	0.00c
EK3_1_2	0.00 ±	0.00d	0.00 ±	0.00c
EK3_1	0.00 ±	0.00d	0.00 ±	0.00c
Control	0.00 ±	0.00d	0.00 ±	0.00c

Note: According to LSD at the 5% level, numbers followed by the same lowercase letter in the same column are not significantly different.

(a) (b) (c)

Figure 2. Normal and CEP-infected *T. molitor* larvae: (a) Normal larvae; (b) CEP-infected larvae; and (c) CEP sporulation in infected larvae.

Identification of entomopathogenic fungi originating from corn endophytes

Identification of isolates with mortality rates above 50% revealed the presence of four genera of entomopathogenic fungi: Beauveria, Metarhizium, Aspergillus, and Trichoderma (Table 3). Morphological characteristics indicate that Beauveria has white colonies with a cottony texture, round conidia, and septate hyphae. Metarhizium is characterized by brownish-green colonies, relatively slow growth, and cylindrical to round conidia. Meanwhile, Aspergillus exhibits dark-colored colonies with a fast growth rate, while Trichoderma has dark green colonies with round conidia and septate hyphae. This variation in characteristics reflects both the taxonomic diversity and the functional potential of the isolates obtained.

Table 3. Macroscopic and microscopic characterization of entomopathogenic fungi originating from corn endophytes

Isolat cendawan	Colony color	Colony growth	Hifa	Konidia	Species
EK2_2_3	White cotton	Very slow	Partitioned	Round	*Beauveria* sp.
EB4_2_2	White cotton	Very slow	Partitioned	Round	*Beauveria* sp.
EK4_1	Olive green	Slow	Partitioned	Round cylinder	*Metarhizium* sp.
EK2_1	Olive green	Slow	Partitioned	Round cylinder	*Metarhizium* sp.
EK3_2	Dark green	Fast	Partitioned	Round	*Trichoderma* sp.
ED5_2_3	Yellowish green	Fast	Partitioned	Round	*Aspergillus* sp.
EB4_2_3	Yellowish green	Fast	Partitioned	Round	*Aspergillus* sp.

Colony growth of entomopathogenic fungi

Observations of colony growth on days 2 and 4 after incubation revealed variations in the rate of colony expansion among entomopathogenic fungal isolates. Isolate EK3_2 (*Trichoderma* sp.) exhibited the fastest growth rate compared to other isolates, with an average colony area of 2.74 cm² on day 2 and increasing to 6.63 cm² on day 4.

Conversely, isolate EB4_22 (*Beauveria* sp.) exhibited the slowest growth rate, with colony areas of 0.89 cm² on day 2 and 3.67 cm² on day 4, respectively. These differences in growth rates reflect the diversity of physiological characteristics among isolates, which potentially impact the competitiveness and adaptability of fungi to specific environmental conditions.

Density and viability of conidia of entomopathogenic fungi

The analysis results showed that conidial density among entomopathogenic fungal isolates did not differ significantly (Table 4). However, conidial viability showed significant variation between isolates, particularly with differences in incubation duration. In general, conidial viability increased after 2 x 24 hours of incubation compared to 1 x 24 hours, indicating a gradual process of conidial activation and germination.

Isolate EK3_2 (*Trichoderma* sp.) demonstrated the highest viability, while isolate EK2_23 (*Beauveria* sp.) had the lowest. This variation indicates that successful conidial germination is not solely determined by the number of conidia but is also influenced by the physiological characteristics and adaptive capabilities of each isolate.

Table 4. Density and germination of conidial entomopathogenic fungi originating from corn endophytes

Species cendawan	Code of isolat	Conidia density ($1x10^8$ konidia/ml)	Germination (%) ± SD	
			24 h	48 h
Trichoderma sp.	EK3_2	5.42 ± 0.20a	87.22 ± 0.04a	88.00 ± 0.03a
Aspergillus sp.	$EB4\_2_3$	6.78 ± 0.55a	62.41 ± 0.12a	78.01 ± 0.04a
Metarhizium sp.	EK2_1	6.00 ± 0.84a	47.56 ± 6.21b	56.23 ± 5.33b
Metarhizium sp.	EK4_1	3.57 ± 0.21a	46.65 ± 4.21bc	53.33 ± 5.79bc
Aspergillus sp.	$ED5\_2_3$	2.25 ± 0.20a	42.56 ± 4.22bc	51.56 ± 7.00bc
Beauveria sp.	$EK2\_2_3$	6.52 ± 0.12a	42.22 ± 1.51bc	51.50 ± 4.79bc
Beauveria sp.	$EB4\_2_2$	5.58 ± 0.91a	50.37 ± 3.31bc	54.50 ± 5.79bc

Note: According to LSD at the 5% level, numbers followed by the same lowercase letter in the same column are not significantly different.

Discussion

The results of this study indicate that endophytic fungi isolated from corn plants have significant potential as entomopathogenic agents. The higher colonization rates in lowlands compared to midlands indicate that environmental factors, such as temperature and humidity, play a significant role in determining the diversity of endophytic microbes. This finding aligns with reports that the distribution and colonization success of endophytic fungi are strongly influenced by ecological conditions and microbial interactions within the soil and plant tissues (Ahsan et al., 2024). Previous research also reported that the abundance of endophytic fungi in chili and oil palm (Kuswardani et al., 2025) is higher in lowlands than in midlands. According to Irmawan (2007), rainfall, crop cultivation methods, and plant sampling locations all influence the abundance of endophytic fungi.

The dominance of isolates in root tissue reinforces the role of the rhizosphere as a hub for interactions between plants and microorganisms. Entomopathogenic fungi such as Metarhizium and Beauveria are known to colonize root tissue endophytically and persist within the plant system through complex interactions with the rhizosphere (Rivas-Franco et al., 2020). Furthermore, systemic colonization of roots, stems, and leaves has also been reported in corn plants inoculated with entomopathogenic fungi (Liu et al., 2022).

Pathogenicity tests showed that more than 50% of isolates were able to cause mortality of *Tenebrio molitor* larvae, indicating strong biocontrol potential. The infection mechanism of entomopathogenic fungi is known to involve the production of extracellular enzymes (such as chitinases and proteases) and secondary metabolites that play a role in cuticle penetration and host insect mortality (Karthi et al., 2024). The differences between mortality and mycosis observed in this study indicate variations in pathogenicity strategies among isolates. Some fungi can cause insect death without obvious external sporulation, possibly related to toxin production or internal infection that does not progress to the sporulation stage (Karthi et al., 2024).

The presence of the Beauveria and Metarhizium genera as endophytes in this study is also consistent with various previous reports showing that these two genera not only function as insect pathogens but also as endophytes that can increase plant resistance to pests such as *Spodoptera frugiperda* (Altaf et al., 2023). In fact,

inoculation of endophytes from these two genera was reported to be able to significantly suppress the development and fitness of these pests (Altaf et al., 2023). Furthermore, the dual function of entomopathogenic fungi as endophytes has also been associated with increased plant growth and resistance to biotic and abiotic stress. Endophytic colonization by *Beauveria bassiana* and *Metarhizium anisopliae* has been shown to increase maize growth parameters, such as plant height and biomass (Liu et al., 2022; Vajri, Trizelia, RA, et al., 2024). The presence of other genera such as Trichoderma and Aspergillus in this study indicates the functional diversity of the endophyte community. Trichoderma is widely known as an antagonist and plant growth stimulant, and under certain conditions, it can also exhibit activity against insects (Ahsan et al., 2024).

Variation in colony growth rates between isolates indicates differences in metabolic capacity and physiological adaptation. This is important in the context of field applications, as fast-growing isolates tend to be more competitive in substrate colonization. However, growth capacity does not always correlate directly with virulence, as demonstrated in this study and supported by previous studies on entomopathogenic fungi (Karthi et al., 2024). Furthermore, varying conidial viability suggests that propagule quality is a key factor in successful infection. Conidial germination is strongly influenced by physiological and environmental conditions and is a critical stage in the life cycle of entomopathogenic fungi (Karthi et al., 2024). Interactions between endophytic fungi and plants can also trigger plant defense responses. Rivas-Franco et al. (2020) reported that colonization by Metarhizium can modulate plant defense hormones such as jasmonic acid and salicylic acid, which play a role in increasing resistance to pathogens and herbivores.

Overall, the results of this study reinforce the concept that endophytic fungi are a source of adaptive and multifunctional biological agents. Local isolates obtained from corn agroecosystems in North Sumatra have the potential to exhibit a higher level of adaptation to local environmental conditions, making them more effective for development as sustainable, locally resource-based mycoinsecticides (Vajri et al., 2026).

Conclusion

This study demonstrates that endophytic fungi originating from corn in North Sumatra are a potential source of entomopathogenic agents with diverse infection capacities. Isolate EK2_23 (*Beauveria* sp.) was identified as a superior candidate based on its high mortality and mycosis rates, confirming the strategic role of this genus as an endophytic entomopathogen. Variations in tissue colonization, growth rate, and conidial viability indicate the differentiation of biological functions among isolates relevant to infection effectiveness. These findings strengthen the concept of the dual role of fungi as endophytes and biological control agents. Practically, the obtained local isolates offer strong prospects for development as adaptive mycoinsecticides in corn pest control. Further validation on primary target pests and at the field scale is crucial to ensure their sustainability and effectiveness.

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Author Contributions

All authors contributed together for each stage of research.

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Conflicts of Interest

The authors declare no conflict of interest.

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