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Exploration and Preliminary Characterisation of Soil Fungi Isolates via the *Tenebrio molitor* Insect-Baiting Method in Agricultural Soils of North Sumatra

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ABSTRACT

Heavy application of chemical pesticides in farming has proven environmentally harmful, has led pests to develop resistance, and has caused health problems. These concerns have prompted a search for environmentally acceptable alternatives, such as soil-borne fungi with potential as biological control agents. This study aimed to isolate and preliminarily characterise soil fungi using an insect-baiting method. Soil samples were collected from farmland and plantations in Deli Serdang and Serdang Bedagai Regencies, North Sumatra, Indonesia, and *Tenebrio molitor* larvae were used as bait. A total of 21 fungal isolates were obtained and characterised morphologically by naked-eye observation and microscopy. The predominant genera were *Aspergillus* sp. (8 isolates), *Trichoderma* sp. (5 isolates), *Fusarium* sp. (4 isolates), and *Penicillium* sp. (2 isolates), followed by *Geotrichum* sp. (1 isolate) and one unidentified fungus. In a pathogenicity test on *Brassica juncea* (mustard) seedlings, the *Trichoderma* sp., *Aspergillus* sp., and *Penicillium* sp. isolates caused no plant mortality, whereas the *Fusarium* sp. and *Geotrichum* sp. isolates caused 20%-30% seedling mortality. Local fungi may therefore contribute to eco-friendly biopesticides suited to regional agricultural conditions.

Keywords: Agricultural soil, fungal isolation, insect-baiting, preliminary characterisation, sustainable agriculture

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INTRODUCTION

Agricultural systems remain central to maintaining the global food supply, especially as population growth continues to drive demand for reliable crop production. Ensuring stable harvests is crucial not only for food availability but also for strengthening national resilience against climate fluctuations and the pressures exerted by pests and diseases (Kementerian Pertanian Indonesia, 2026). One of the most persistent challenges faced by farmers and agronomists is the wide array of soil-borne pests and pathogens that can substantially reduce crop productivity. These organisms, which range from *Ralstonia* bacteria and *Pythium* fungi to nematodes, pose a dual threat: they can cause latent infections and, unless effectively mitigated through biological agents or appropriate biofumigation techniques, lead to long-term degradation of agricultural soil quality (Afriani et al., 2026; Ariani et al., 2026; Sutarman, 2023).

For decades, synthetic pesticides have served as the primary means of suppressing such threats, owing to their rapid action and broad spectrum of targets. However, the persistent and often excessive use of chemical pesticides has created new problems, including resistant pest populations, ecological imbalances, environmental pollution, and potential health risks to humans (Ibrahim, 2026; Kementerian Pertanian Indonesia, 2023). Global statistics indicate that pesticide use continues to rise; in Indonesia, for instance, the total volume of pesticides used in the agricultural sector reached 296 kilotons of active ingredients in 2024 (Food and Agriculture Organization of the United Nations [FAO], 2026).

Growing recognition of these issues has intensified the search for safer and more sustainable pest control alternatives. Soil fungi have the potential to be developed as biological control agents against pests and diseases. Soil microorganisms play ecological roles that include nutrient transformation, organic matter decomposition, interactions among microorganisms, and biological relationships with plants and other organisms (Adal, 2024). Soil fungi also differ widely in morphology, ecological function, and environmental adaptability (Taylor & Sinsabaugh, 2015). Exploring fungi from agricultural soil, specifically in the North Sumatra region, is therefore highly significant because it can provide preliminary information on the diversity of isolates present in cultivation environments. Fungi isolated from soil, such as *Trichoderma*, *Beauveria*, *Metarhizium*, and *Paecilomyces*, can control pests and plant pathogens without causing the accumulation of chemical substances in the environment (da Silva et al., 2025; Solichah et al., 2022). These fungi act through various mechanisms, including competition for nutrients and space, mycoparasitism, infection of insect hosts, production of bioactive secondary metabolites, and induction of systemic resistance in plants (Muliani & Srimurni, 2022; Setiawan et al., 2025; Vajri et al., 2024). They therefore represent a safe and effective alternative to chemical pesticides. However, the diversity and efficacy of soil-borne fungi that function as biological control agents vary across ecosystems, which necessitates further exploration

and identification studies at the regional level. Such research is particularly relevant to North Sumatra, where environmental and climatic conditions can influence the presence and efficacy of beneficial, locally sourced soil-borne fungi.

The exploration of soil fungi serves as an initial step toward obtaining local isolates for further research. Antagonistic and entomopathogenic soil fungi can be studied by various methods, including direct isolation from soil, insect baiting, and collection of naturally infected insects. Using insect baiting as described previously (Humber, 2005; Zimmermann, 1998) this study collected fungal isolates capable of infecting *Tenebrio molitor* larvae.

This method uses live insects as bait to discover fungi capable of infecting them. *T. molitor* larvae are easily reared in the laboratory, making them a common choice for insect-baiting techniques. Furthermore, these mealworms are readily available in markets, inexpensive, and suitable for studying fungus- insect interactions. The procedure has been applied in studies of entomopathogenic fungi in soil from both natural ecosystems and agricultural lands (Ginting et al., 2024; Masoudi et al., 2020). The resulting isolates undergo detailed characterisation and subsequent testing to evaluate their potential for suppressing pests and diseases in food crops and plantation commodities (Sudiarta et al., 2024).

Previous research has emphasised the importance of such exploratory activities. A study by Liu et al. (2021) described a *T. molitor* larva baiting system for isolating and selecting entomopathogenic fungi from soil samples, followed by virulence testing against insect pests and morphological and molecular identification. This approach shows that baiting serves not only as a technique for recovering isolates but also as an initial stage in evaluating their biological potential. Research by Masoudi et al. (2020) explored entomopathogenic fungi in soil using *T. molitor* larvae; their findings indicate that insect baiting can be used to discover fungi that actively interact with insect hosts in the soil. Afiefah et al. (2026) and Ginting et al. (2024) also employed this method to isolate fungi from the rhizosphere of rice and maize plants. The fungi associated with maize included *Aspergillus* and *Fusarium*, whereas those associated with rice included *Aspergillus*, *Trichoderma*, and *Fusarium*. Afifah et al. (2022) identified various fungal species, such as *Trichoderma*, *Aspergillus*, *Penicillium*, *Fusarium*, *Beauveria*, mycorrhizae, and *Metarhizium*, in the rhizosphere and tissues of guava plants during the dry season. In that study, *Trichoderma* was the most dominant genus in the soil, whereas *Metarhizium* was frequently observed on leaf surfaces. Among these fungi, *Metarhizium*, *Penicillium*, *Trichoderma*, *Beauveria*, *Aspergillus*, and mycorrhizal fungi are recognised for their potential as biological control agents, whereas *Fusarium* is a well-known plant pathogen. These results indicate the need to isolate fungi from natural sources to obtain specimens suitable for further testing. However, isolates obtained by insect baiting cannot be considered representative of the entire soil fungal community, because the process tends to select fungi that can infect or associate with the bait insects.

Although insect baiting has been employed in various studies on entomopathogenic fungi, information on fungal isolates recovered with *T. molitor* from agricultural soils in North Sumatra remains limited. This study is therefore essential for documenting the characteristics of isolates obtained from the research sites, comparing their morphological traits, and identifying which isolates warrant further analysis. It does not aim to encompass the entire diversity of soil fungi but rather to characterise the isolates successfully obtained through insect baiting at the studied locations. Specifically, this research aimed to explore and isolate fungi from selected agricultural soils in North Sumatra using the *T. molitor* insect-baiting method, followed by preliminary characterisation based on macroscopic and microscopic features. It also sought to establish baseline information on the diversity of the isolated fungal groups, providing a foundation for future research, including identity verification through molecular approaches and assessment of the biological functions of selected isolates. The findings are expected to expand knowledge of fungi recoverable by insect baiting from agricultural environments in North Sumatra and to guide subsequent exploratory efforts based on the characterisation evidence obtained.

MATERIALS AND METHOD

Research Location and Design

This research was conducted at the Medan Agricultural Development Polytechnic and the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. The study employed a quantitative descriptive method. Sampling locations were selected purposively, focusing on agricultural and plantation sites. Soil samples were collected from eight sub-districts across Deli Serdang Regency and Serdang Bedagai Regency, which were selected because they serve as agricultural and plantation hubs.

Equipment Sterilisation

All equipment used for culture and medium preparation, including Petri dishes, test tubes, and media bottles, was autoclaved. The equipment was thoroughly washed, soaked for 15 minutes in a Clorox solution (in the event of contamination), rinsed with clean water, and dried. Once dry, the Petri dishes were wrapped in paper and sterilised in an autoclave at 121 °C and 15 psi (pounds per square inch) for approximately 15-20 minutes, as is standard for laboratory glassware.

Preparation of Potato Dextrose Agar Medium

The potato dextrose agar (PDA) medium was prepared from 200 g of potatoes, 20 g of agar, 20 g of dextrose, and 1 L of distilled water. The potatoes were peeled, cut into small pieces, washed thoroughly, and boiled in 600 mL of distilled water until fully cooked.

The potatoes were then removed, and the extract was filtered through gauze. Agar and dextrose were added, and the total volume was brought to 1,000 mL with distilled water. The mixture was heated until the agar dissolved and then sterilised in an autoclave at 121 °C and 15 psi for 15 minutes. The medium was cooled to approximately 45-50 °C before being poured into Petri dishes in a laminar airflow cabinet to prevent airborne contamination.

Soil Sampling

Soil samples were collected from Deli Serdang Regency and Serdang Bedagai Regency, covering agricultural land used for rice, chili, maize, onion, amaranth, and peanut cultivation, as well as plantation areas for coffee, cocoa, and oil palm. At each site, five sampling points were determined diagonally across the cultivation area (Figure 1). During sampling, the soil surface was cleared of plant debris and leaf litter using a hoe sanitised with an alcohol wipe. At each point, approximately 1 kg of soil was collected at a depth of 5-10 cm into a zip-lock plastic bag and transported to the laboratory in a cooler box. The samples were subsequently stored at 10 °C. Sampling at each planting site was conducted in three replicates across eight sub-districts.

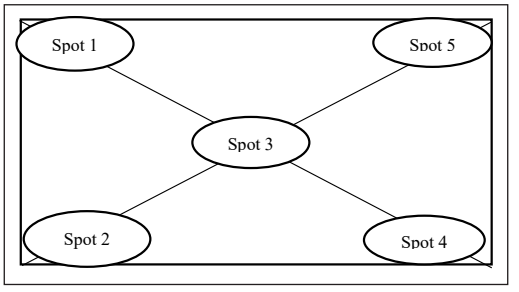


Figure 1. Soil sampling plot

Exploration of Fungal Biological Control Agents

Fungi were explored from soil using the insect-baiting method described by Humber (2005) and Zimmermann (1998). Live insects were placed in Petri dishes (150 × 25 mm) containing soil samples collected from various fields and crops (Anderson, McGee, Nehl, & Mensah, 2007). Mealworms (*T. molitor*) were used as bait. Ten bait insects were added to each Petri dish of soil. The dishes were kept in a moist chamber at 20-25 °C until the mealworms died and were colonised by fungi. Cadavers with fungal growth were then isolated and cultured on PDA medium. After 7 days, all fungal colonies were identified based on their morphological features as potential biological control agents (Lacey, 1997).

Isolation of Fungal Biological Control Agents

Before fungi were isolated from the cadavers, the *T. molitor* cadavers were surface-sterilised by soaking in 70% alcohol for 1 minute, rinsing three times with sterile distilled water, and drying on sterile tissue paper in a laminar airflow cabinet. The fungus-infected larvae were then inoculated into Petri dishes and observed one week later for colony growth. The fungi that grew were separated by colony colour and transferred to fresh medium. This process

was repeated until a single colony was obtained, which was then used for macroscopic and microscopic identification.

Pathogenicity Test

The isolated fungi were cultured on PDA medium, into which 10 germinated mustard (*Brassica juncea*) seedlings were transferred (Figure 2). The experiment consisted of two treatments: P0 (control), mustard seedlings without fungal treatment; and P1 (treatment), mustard seedlings inoculated with the fungal isolate. This test served as a preliminary step to assess the potential of fungi obtained from agricultural and plantation soils prior to more rigorous laboratory evaluations, such as Koch's postulates, antagonism assays, and other relevant tests.

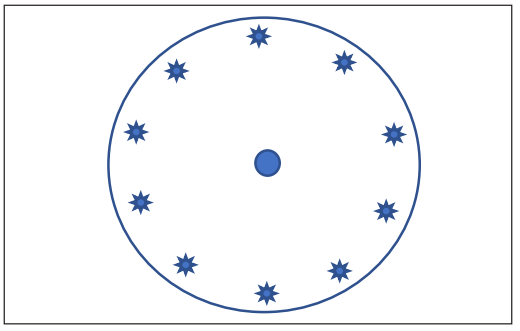


Figure 2. Pathogenicity test in PDA medium

Legend:

Fungal inoculum

Germinated mustard seed

Observations were carried out daily for 7 days to record mustard seedling mortality. In this procedure, mustard seeds were first germinated on moist sterile cotton in Petri dishes under light. After germination, the seedlings were transferred to Petri dishes containing PDA medium, and the fungal isolate derived from the exploration stage was placed at the centre of the same PDA medium (Figure 2). Seedling mortality was calculated using Equation 1.

$$\text{Mortality (\%)} = (\text{dead seedlings} \div \text{initial seedlings}) \times 100\% \quad [1]$$

Identification of Fungal Biological Control Agents

The isolates were grown on PDA medium and identified based on macroscopic and microscopic morphological features. Each fungal isolate was characterised by its morphological traits and compared with standard references such as Barnett & Hunter (1998).

Before microscopic observation, fungal specimens were prepared using the wet mount method. A drop of lactophenol cotton blue (LPCB) solution was placed in the centre of a clean microscope slide. LPCB highlights hyphal and spore structures with a blue stain, making them easier to observe. A small sample of the fungus was collected with a sterile inoculating loop, placed in the drop of solution, and gently teased apart to prevent the structures from clumping. Finally, the specimen was carefully covered with a coverslip, lowered at a 45-degree angle to avoid trapping air bubbles, and excess liquid was absorbed from the edges with tissue before the slide was examined under the microscope.

Macroscopic observations included colony diameter measurements and visual assessment of colony colour, shape, texture, and edge characteristics on Petri dishes.

Microscopic observations were made using a compound microscope with a scale of 10 μm. For this purpose, fungal preparations were made from the isolates. The microscopic characteristics observed included the structure of hyphae, phialides, conidia, and conidiophores.

Spatial Distribution of Soil Sampling Locations

This mapping aimed to identify the locations where soil samples were collected. The map was developed from data gathered during soil sampling, which served as the basis for fungal exploration. Geographic coordinates were obtained using the Open Camera application, which is integrated with the Global Positioning System (GPS) and automatically records location coordinates that are displayed on each image; in addition to coordinates, it captures elevation, the angle at which the photograph was taken, and the time. These data were subsequently used to create a map showing the origins of the soil samples in ArcGIS 10.3.

RESULTS

Exploration of Fungal Biological Control Agents

Soil samples were obtained from eight sub-districts in Deli Serdang and Serdang Bedagai Regencies where agricultural and plantation activities are intensive (Table 1). Sampling sites included rice, maize, chili, and peanut fields as well as oil palm, coffee, and cocoa plantations. Fungi were isolated by the insect-baiting method, using *T. molitor* larvae to recover potentially useful fungi from the soil.

The isolation results (Table 2) showed that *Aspergillus* sp. was the most common genus, with eight isolates obtained from rice, maize, and oil palm soils in six sub-districts: Sei Rampah, Kutalimbaru, Hamparan Perak, Sunggal, Pagar Merbau, and Tanjung Morawa. *Trichoderma* sp. followed with five isolates collected from coffee, cocoa, oil palm, and rice fields in Sunggal, Tanjung Morawa, and Hamparan Perak. Two *Penicillium* sp. isolates were obtained from rice and cocoa plants in the Perbaungan and Sunggal areas, while one isolate remained unclassified at the genus level because, under the microscope, it did not form conidia or conidiophores and exhibited only very thin, hyaline hyphae lacking the distinctive characteristics of the other isolates. Further identification using the polymerase chain reaction (PCR) technique with internal transcribed spacer (ITS) markers is therefore required, and the unidentified fungus should also be cultured on specific media designed to stimulate growth and spore formation. Based on this study, macroscopic and microscopic identification can reach only the genus level and is insufficient to establish the role of each fungus definitively as a biological control agent.

The study also found four *Fusarium* sp. isolates, collected from amaranth, chili, peanut, and oil palm fields in the sub-districts of Tanjung Morawa, Sunggal, Percut Sei Tuan, and Kutalimbaru. One *Geotrichum* sp. isolate was found in a chili plantation area in Sunggal.

Location Mapping

Soil samples were collected from different agricultural areas; the sampling locations are shown in Table 1.

Table 1
Origin of soil samples

Sample Number	North Latitude (N)	East Longitude (E)	Village	Sub-District	Regency
1	3.50476	98.82508	Naga Timbul	Tanjung Morawa	Deli Serdang
2	3.53479	98.89713	Tanjung Mulia	Pagar Merbau	Deli Serdang
3	3.654388	98.575508	Paya Bakung	Hamparan Perak	Deli Serdang
4	3.603915	98.595292	Paya Geli	Sunggal	Deli Serdang
5	3.604452	98.59569	Paya Geli	Sunggal	Deli Serdang
6	3.532182	98.88664	Tanjung Garbus	Pagar Merbau	Deli Serdang
7	3.57337	98.75073	Amplas	Percut Sei Tuan	Deli Serdang
8	3.55696	98.53063	Mencirim	Kutalimbaru	Deli Serdang
9	3.569166667	98.95916667	-	Perbaungan	Serdang Bedagai
10	3.513055556	99.12805556	-	Sei Rampah	Serdang Bedagai
11	3.60269	98.59999	Paya Geli	Sunggal	Deli Serdang
12	3.592735	98.554007	Sei Semayang	Sunggal	Deli Serdang
13	3.522972	98.75281	Ujung Serdang	Tanjung Morawa	Deli Serdang

Based on the data in Table 1, soil samples were collected from eight sub-districts (Perbaungan, Sei Rampah, Tanjung Morawa, Kutalimbaru, Hamparan Perak, Pagar Merbau, Sunggal, and Percut Sei Tuan) at a total of 13 sampling points. These data were then used to construct a map of the origins of the soil samples in ArcGIS 10.3, as presented in Figure 3.

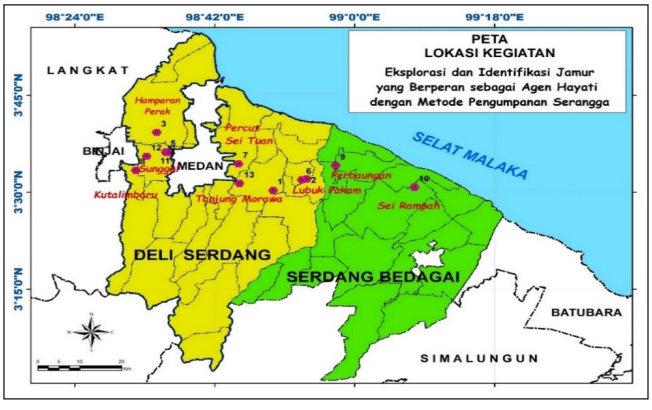


Figure 3. Map showing agricultural soil sampling sites

A total of 21 fungal isolates were obtained from 13 sampling points. These comprised five *Trichoderma* sp. isolates (three from Sunggal and one each from Tanjung Morawa and Hamparan Perak); eight *Aspergillus* sp. isolates (two each from Kutalimbaru and Pagar Merbau and one each from Sei Rampah, Hamparan Perak, Sunggal, and Tanjung Morawa);

two *Penicillium* sp. isolates (from Sunggal and Perbaungan); four *Fusarium* sp. isolates (one each from Percut Sei Tuan, Sunggal, Kutalimbaru, and Tanjung Morawa); and one *Geotrichum* sp. isolate (from Sunggal). Additionally, one unidentified isolate was obtained from Sunggal. Based on this, the highest number of isolates, eight, was obtained from the Sunggal sub-district. Kutalimbaru and Tanjung Morawa each yielded three isolates, while Hamparan Perak and Pagar Merbau each provided two; the remaining sub-districts contributed one isolate each.

Identification of Fungal

Macroscopic Observation

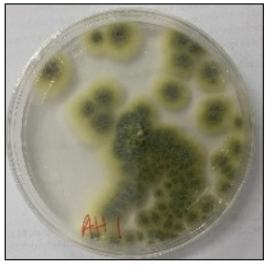
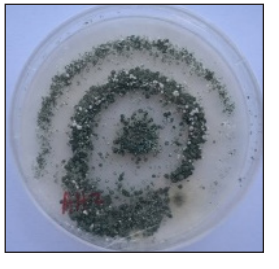
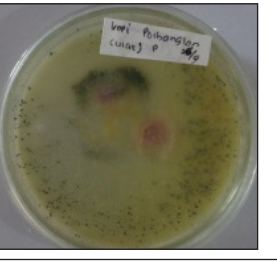
Macroscopic observation provides a general understanding of fungal morphology to assist identification. The characteristics observed included size, shape, colour, surface, and texture; however, accurate identification requires microscopic examination. Fungi isolated from *T. molitor* and grown on PDA showed various forms and colours, and some were brightly coloured. The macroscopic observation of the 21 isolates revealed bright colours such as white, green, yellowish green/brownish green, sage green, light yellow, and pink (Table 2).

Aspergillus sp. showed a range of green hues, from yellowish green to brownish green, with a small, bumpy texture on the PDA surface. The colonies appeared white within the first 2 days after inoculation and then turned yellowish green as many conidia formed. In most *Aspergillus* sp. strains, fresh colonies or conidia are yellowish green, but older cultures may turn brown or green with brown patches, often forming circular patterns on PDA. Colony diameters ranged from 3 to 8 cm at 4- 7 days of age.

Trichoderma sp. colonies were dark green with a bumpy surface because of the high spore density. The reverse side of the colonies was initially white, and early growth on PDA was white and thin. Some *Trichoderma* species also produced substances that turned the PDA yellowish. Colony diameter reached 3 cm on day 4 and 9 cm on day 7, and dark green mycelia were visible by day 10. *Penicillium* sp. displayed grayish-green colonies with light yellow edges, thick mycelia, and a soft, cotton-like surface. In this study, only two *Penicillium* sp. isolates were successfully examined.

Fusarium sp. colonies exhibited pink to reddish hues, a distinctive feature of some *Fusarium* species, which often produce pink colonies on growth media. These colonies usually had a denser or darker centre, which served as another distinguishing trait. The texture appeared somewhat smooth and spread across the medium, typical of *Fusarium* sp. at certain growth stages. *Geotrichum* sp. showed white, cottony, moderately thick mycelia on PDA with irregular light-yellow shades. Colony diameters ranged from 3 to 9 cm after 3-7 days of incubation.

Table 2
Macroscopic characterisation of fungi

Isolate	Sampling Location and Host Plant	Macroscopic Characterisation	Figure
AH1	Perbaungan (Rice)	The fungal colony first showed a white colour when grown on PDA medium. As the incubation continued, the centre of the colony changed to a grayish-green colour, while the outer areas turned light green. The colony spread irregularly and unevenly across the surface of the medium. The diameter of the colony varied from 3 to 5 cm during the incubation period of 3 to 7 days.	*Penicillium* sp. (Biverticillate) 
AH2	Sunggal (Coffee)	The first colony on PDA medium was white. It became dark green over time. The mycelium is circular in shape with a white base. The upper surface is rough and granular. The colony reaches 3 cm on day 4 and 9 cm on day 7.	*Trichoderma* sp. 
AH3	Sunggal (Coffee)	The mycelium that grows is initially thin and white. Colony growth turns the medium yellowish. Thin green mycelium grows on PDA media, reaching 3 cm on day 4 and 9 cm on day 7	*Trichoderma* sp. 

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Table 2 (*continued*)

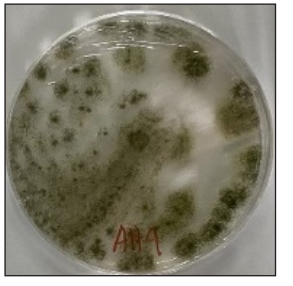
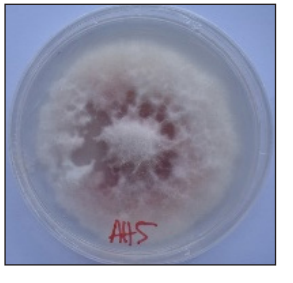
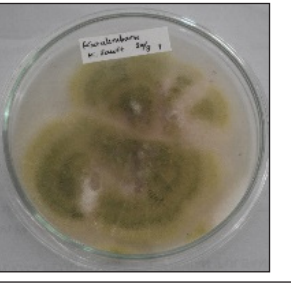
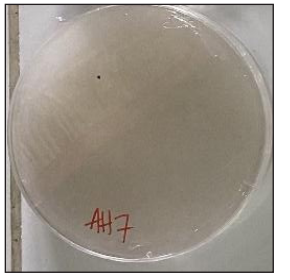
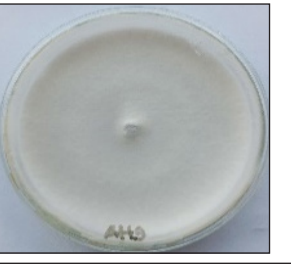
Isolate	Sampling Location and Host Plant	Macroscopic Characterisation	Figure
AH4	Sei Rampah (Corn)	Observations in PDA medium revealed that the mycelium was initially white. The centre was yellowish-green. The growth was 3-5 cm in size and lasted for 3-7 days. The mycelium spread irregularly.	*Aspergillus* sp. 
AH5	Tanjung Morawa (Spinach)	Fungal colonies on PDA medium have cotton-like mycelium. The mycelium is white with a pink, purple, or yellow tinge. It measures 3-7.5 cm at 3-7 days.	*Fusarium* sp. 
AH6	Kutalimbaru (Palm oil)	Observations of mycelium in PDA medium revealed an initial white colour. Over time, it changed colour to yellowish-green, forming an irregular circular pattern. The growth was 3-5 cm in size and lasted 3-7 days.	*Aspergillus* sp. 

Table 2 (*continued*)

Isolate	Sampling Location and Host Plant	Macroscopic Characterisation	Figure
AH7	Kutalimbaru (Palm oil)	Hints of pink, purple, and yellow on PDA medium. The white colonies grow and spread across the medium. The fungus appears smooth and spreads across the media surface. *Fusarium* often has cottony or velvety colonies when it first begins to grow.	*Fusarium* sp. 
AH8	Kutalimbaru (Palm oil)	Colony growth 2 days after inoculation is white, which then changes to yellowish-green due to the formation of a large number of conidia. In most strains of *Aspergillus*, the colour of fresh cultures or conidia is yellowish-green, but in older cultures it is brown, sometimes green with brown markings in a circular pattern on PDA medium. The diameter is 3-8 cm at a colony age of 4-7 days.	*Aspergillus* sp. 
AH9	Hamparan Perak (Rice)	On PDA medium, a white, cotton-like mycelium is visible. It grows 3-6 cm in diameter and remains white for 3-7 days	*unidentified* 

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Table 2 (*continued*)

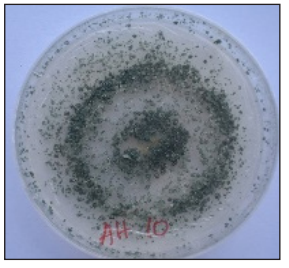
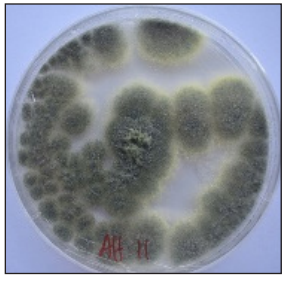
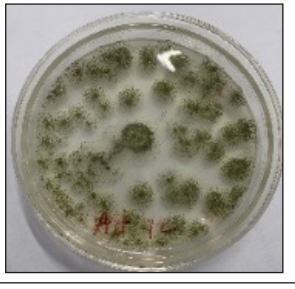
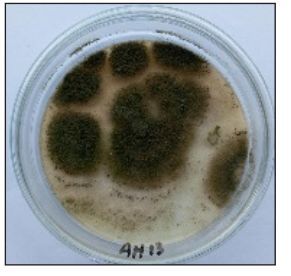
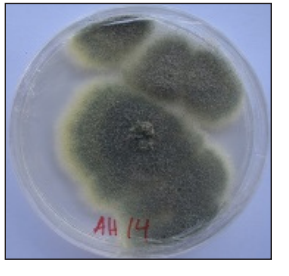
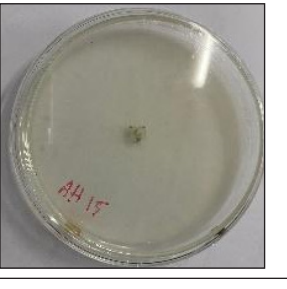
Isolate	Sampling Location and Host Plant	Macroscopic Characterisation	Figure
AH10	Hamparan Perak (Rice)	The fungal mycelium on PDA medium initially grows thin white, then dark green, growing in a circular, spreading, thin dark green pattern. The colony reaches a diameter of 3 cm on day 4 and 9 cm on day 7.	*Trichoderma* sp. 
AH11	Hamparan Perak (Rice)	The colour of the colony on PDA medium is white, which then changes to grayish green, reaching a diameter of 3 cm on day 4, 4.7 cm on day 5, 6.2 cm on day 7, and 9 cm on day 9, and forming a circular asymmetrical pattern surrounded by faded yellow mycelium.	*Aspergillus* sp. 
AH12	Sunggal (Palm oil)	Colonies in PDA medium are initially white, then turn yellowish-green in the centre . They grow to a diameter of 3-5 cm over 3-7 days.	*Aspergillus* sp. 

Table 2 (*continued*)

Isolate	Sampling Location and Host Plant	Macroscopic Characterisation	Figure
AH13	Pagar Merbau (Rice)	Colonies on PDA medium are initially white, then turn yellowish-green in the centre. They measure 3-5 cm in diameter over 3-7 days and form an irregular circular pattern.	*Aspergillus* sp. 
AH14	Sunggal (Cacao)	The colour of the colony on PDA medium is white, which then changes to grayish green, reaching a diameter of 2.5 cm on day 4, 3.5 cm on day 5, and 7.8 cm on day 9, and forming a circular asymmetrical pattern surrounded by faded yellow mycelium.	*Penicillium* sp. (Monoverticillate) 
AH15	Sunggal (Cacao)	The initial colonies on PDA medium are thin and white. This fungus releases a compound that causes the PDA medium to turn yellow. The colony reaches 3 cm on day 4 and 9 cm on day 7. On the 10th day, dark green mycelium appears.	*Trichoderma* sp. 

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Table 2 (*continued*)

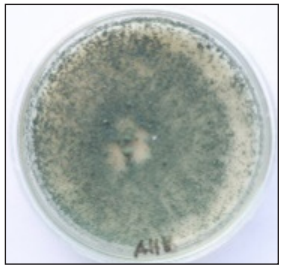
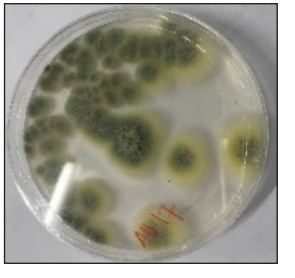
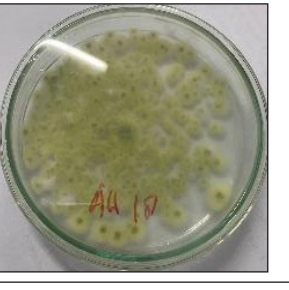
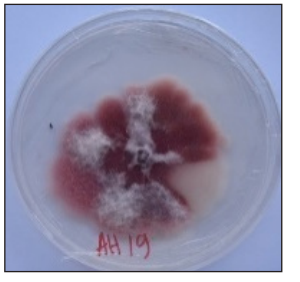
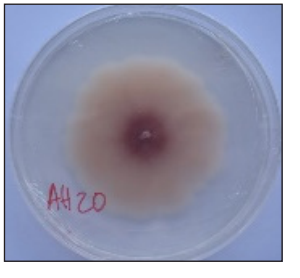
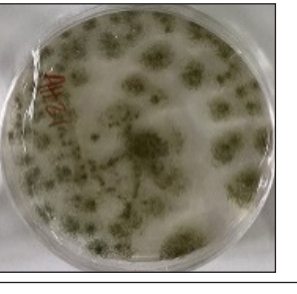
Isolate	Sampling Location and Host Plant	Macroscopic Characterisation	Figure
AH16	Tanjung Morawa (Oil palm)	Initial colonies on PDA medium are white. Colonies gradually turn dark green. They reach 3 cm on day 4 and 9 cm on day 7.	*Trichoderma* sp. 
AH17	Tanjung Morawa (Oil palm)	The mycelium on PDA medium is thick, cottony white with a faint greenish tinge. It measures 3-9 cm in diameter after 3-7 days.	*Aspergillus* sp. 
AH18	Sunggal (Chili)	The mycelium on PDA medium is thick, cottony white with irregular, faded yellow tinges. The diameter is 3-9 cm after 3-7 days.	*Geotrichum* sp. 

Table 2 (*continued*)

Isolate	Sampling Location and Host Plant	Macroscopic Characterisation	Figure
AH19	Sunggal (Chili)	Pink colonies, spreading growth with darker centres. Fungal colonies on PDA medium have cotton-like mycelium. The mycelium is white with a pink, purple, or yellow tinge. It measures 3-7.5 cm at 3-7 days.	*Fusarium* sp. 
AH20	Percut Sei Tuan (Peanut)	The fungal colony on PDA medium has cottony mycelium. The mycelium is white with tinges of pink, purple, or yellow. The size ranges from 3 to 7.5 cm within 3 to 7 days of incubation.	*Fusarium* sp. 
AH21	Pagar Merbau (Onion)	The observation on PDA medium initially shows a white colour. The centre part turns yellowish-green. The size ranges from 3 to 5 cm within 3 to 7 days.	*Aspergillus* sp. 

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Microscopic Observation

Microscopic observation aimed to explore the finer structural details of the fungi. After the isolates were grown on PDA, preparations were made for microscopic examination. This observation supports the identification of fungal isolates based on microscopic structures, namely hyphae, conidia, phialides, and conidiophores.

This microscopic analysis is crucial for confirming the genus-level identification of the isolates. The morphological observations were consistent with the descriptions in fungal identification references, especially Barnett & Hunter (1998) and other reliable sources. The microscopic features are presented in Table 3.

Pathogenicity Test on Mustard Seeds

Pathogenicity tests were conducted on mustard seedlings grown in Petri dishes under aseptic conditions. The observations indicated that 16 isolates were non-pathogenic, as the mustard seedlings remained viable for 7 days, suggesting that most of the isolates obtained can be studied further. In contrast, five isolates showed the potential to cause wilting and, in some cases, death of the plants compared with the control (Figure 4).

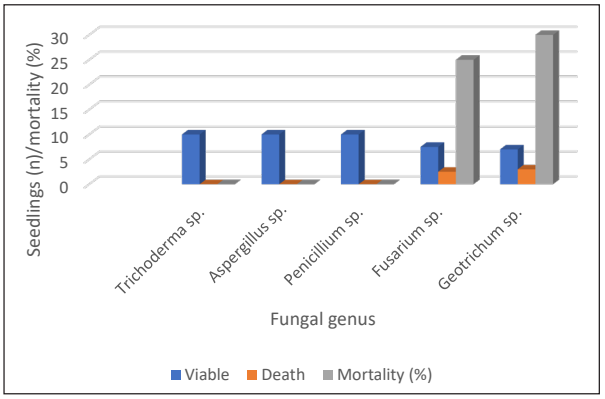
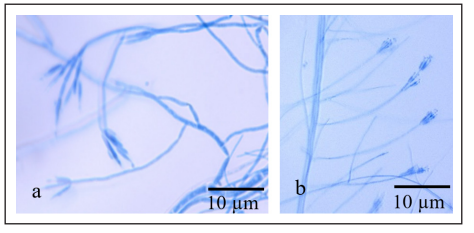
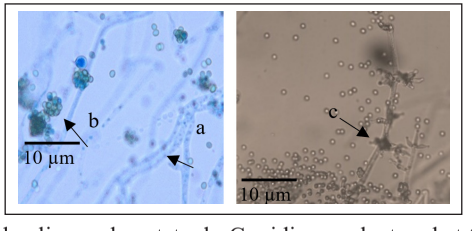
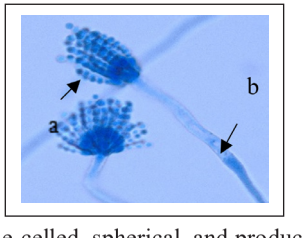


Figure 4. Pathogenicity test on mustard seedlings

Based on the observational data in Figure 4, the fungi *Penicillium* sp., *Aspergillus* sp., and *Trichoderma* sp. did not exhibit toxicity or pathogenicity toward mustard seedlings 7 days after inoculation. In contrast, *Fusarium* sp. caused pathogenicity, resulting in 20%-30% mortality among the mustard seedlings over the 7-day observation period, while *Geotrichum* sp. caused 30% mortality (Figure 4). These findings suggest that *Penicillium* sp., *Aspergillus* sp., and *Trichoderma* sp. are suitable for further research, including molecular identification to the species level and antagonism assays against plant diseases and pests in both laboratory and field settings. Furthermore, this study serves as an initial screening to identify soil-borne fungi with potential for development into biological-based biopesticides, which are products characterised by a long shelf life and effective pest and disease control.

Table 3
Microscopic characteristics of fungi

Fungi	Microscopic characteristics	Figure
Penicillium sp.	Characterised by septate hyphae (1.5-5 µm) and branched conidiophores that are monoverticillate and biverticillate, with phialides shaped like bottles or ampoules (2.5- 5 µm). Conidia are unicellular, smooth or rough, and form chains resembling a brush. The shapes of conidia can vary from round to fusiform.	 a. Monoverticillate (Phialides extend directly from the hyphae); b. Biverticillate (Phialides extend from branches arising from the hyphae)
Trichoderma sp.	Exhibits septate, hyaline hyphae. The conidiophores are relatively short, septate, and branch at wide angles (close to 90°). Phialides are typically flask-shaped and produce unicellular, round to ellipsoidal conidia that cluster at the tips in small sticky groups. Some species may produce secondary metabolites that turn the medium yellow.	 a. Hyphae are hyaline and septate; b. Conidia are clustered at the hyphal tips in small, mucilaginous groups; c. The conidiophores are relatively short, septate, and branch at wide angles (close to 90°)
Aspergillus spp.	Shows septate hyphae and erect conidiophores that end in a vesicle. Phialides are arranged either singly or in pairs, depending on the species. The conidia are unicellular, spherical, yellow-green, forming chains that appear clustered like a flower.	 a. The conidia are single-celled, spherical, and produced in basipetal chains; b. The conidiophores were hyaline, erect, and simple in structure, terminating in a swollen apex that appeared either globose or club-shaped.

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Table 3 (*continued*)

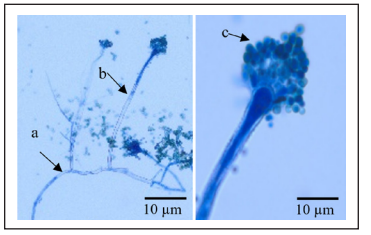
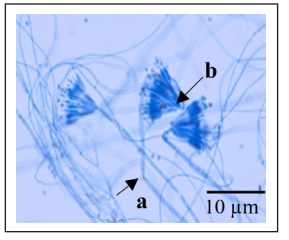
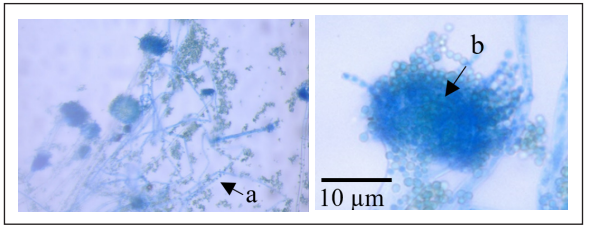
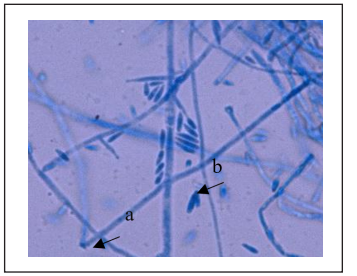
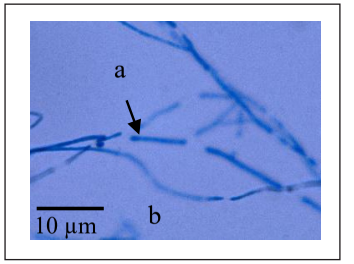
Fungi	Microscopic characteristics	Figure
Aspergillus sp.	Displays hyaline, septate hyphae with smooth-walled conidiophores (typically under 300 µm in length and 5- 10 µm in width), ending in a subclavate vesicle (20-30 µm). Uniseriate phialides densely cover the upper two-thirds of the vesicle. Conidia are verrucose, spherical, 2- 3.5 µm in diameter, and develop in chains.	 a. Septate hyphae and hyaline; b. The conidiophores (phialides); c. Conidia are verrucose and spherical
Aspergillus sp.	Hyphae are hyaline to light brown. Vesicles (7- 17 µm) are subglobose to pyriform. Structures are biseriate, with metulae (2-3.5 µm × 4-6 µm) and phialides (2-3 µm × 5-7 µm). The conidia are small (2.5- 4.0 µm), echinulate or spiny, resembling *Penicillium* heads.	 a. The hyphae are elongated, hyaline to light brown, and give rise to conidiophores; b. The conidia are globose in shape, with echinulate or spinose surfaces.
Aspergillus sp.	Septate hyphae with fairly long conidiophores (8-17 µm). Vesicles are globose to subglobose (20- 45 µm). Most strains are biseriate, with metulae (8-10 × 5-7 µm) supporting flask-shaped phialides (7-12 × 3-4 µm). Conidia are round to ellipsoidal (3- 6 µm), with smooth or rough walls.	 a. Septate hyphae and rough-textured conidiophores; b. Conidia are round to ellipsoidal

Table 3 (*continued*)

Fungi	Microscopic characteristics	Figure
Fusarium sp.	Its hyphae are hyaline and septate and grow well on PDA. It produces two main spore types. Macroconidia are sickle-shaped, multi-septate (typically 3- 5 cells), large, and curved at one end. Microconidia are smaller, oval to elliptical, aseptate or with a single septum, and often borne in clusters on short conidiophores or directly from hyphae. Chlamydospores, which are thick-walled survival structures, also form in intercalary or terminal positions.	 a. Hyphae are hyaline and septate; b. The fungus produces both macroconidia and microconidia.
Geotrichum sp.	Microscopic examination shows hyphae breaking into arthroconidia, asexual spores formed by hyphal fragmentation. It lacks complex conidiophore structures. The segmented hyphae indicate this reproductive method is typical of *Geotrichum* species.	 a. Hyphae breaking into arthroconidia; b. asexual spores formed by hyphal fragmentation

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DISCUSSION

Exploration across 13 sampling points in eight sub-districts of North Sumatra yielded 21 fungal isolates using the *T. molitor* insect-baiting method. These results demonstrate that this approach is effective for obtaining fungal isolates from the soil samples examined. However, the number of isolates obtained does not necessarily reflect the full diversity of soil fungi, because isolation outcomes are also influenced by sample condition, bait characteristics, testing procedures, and the ability of the microorganisms to grow on the media employed.

T. molitor has been used primarily as a bait organism for the detection and isolation of entomopathogenic fungi from soil. Liu et al. (2021) explained that the *T. molitor* baiting system can be used to isolate entomopathogenic fungi, which must subsequently be selected through virulence testing, morphological and molecular characterisation, and evaluation of physiological traits. Based on this work, successful isolation can be regarded as only the initial stage of the selection process. This evidence is insufficient to establish the biological function of the isolates obtained.

The baiting method was used in this study to clarify the objectives and the procedures applied. The fungal isolates obtained cannot be definitively classified as biological control agents because no further testing has demonstrated that they cause pest mortality or act as antagonists against diseases. Furthermore, the success of this method in isolating fungi does not imply that it is more effective than other techniques. This limitation arises from the selective nature of the isolation method: certain fungi may interact with the bait and grow on the culture medium, whereas others remain undetected because of differences in growth requirements, physiological conditions, infection capabilities, and other specific biological traits. A review of the diversity and detection of soil fungi, by Bollmann-Giolai et al. (2022) likewise emphasises the importance of appropriate detection approaches for understanding fungal communities and their relationship to plant health.

Thus, the 21 isolates obtained represent microbial resources detectable through an insect-baiting exploration procedure. These findings provide a foundation for further characterisation, specifically of the taxonomic identity and biological function of each isolate. The primary contribution of this research lies in the exploration of soil fungi from agricultural land in North Sumatra. However, conclusions about the dominance of specific genera, community diversity, and the potential of these isolates as biological control agents require further evidence.

Exploration using insect baiting yielded eight *Aspergillus* sp. isolates from the soil, the highest number of isolates among the groups reported. These results demonstrate that the method employed was successful in isolating *Aspergillus* sp. However, recovering eight isolates is insufficient to conclude that this genus dominates the soil fungal community at the study sites. Rather, the findings suggest that the isolated taxa primarily reflect the group captured by the method applied.

Aspergillus sp. was the most common fungal genus identified in agricultural areas. This fungus is a known saprophyte that interacts with plants. Afifah et al. (2022) noted that *Aspergillus* sp. is often found in soil and that its conidia disperse easily into the air, predominantly on twigs and leaves. During the dry season, when temperatures can reach 34 °C, this fungus can survive and adapt to these conditions. The genus *Aspergillus* is known for producing secondary metabolites that effectively combat bacterial pathogens (Carroll et al., 2020; Sumilat & Lintang, 2021). Ecologically, it performs diverse functions, such as decomposing organic matter and producing secondary metabolites. Certain species generate compounds capable of influencing the growth of other microorganisms, which makes the genus significant in microbiological research and in the exploration of biological activities. *Aspergillus* species can tolerate high temperatures and remain enzymatically active, producing alkalophilic proteolytic enzymes at 40 °C. These fungi also produce several enzymes that inhibit the growth of other microbes, including amyloglucosidase, protease, cellulase, lactase, invertase, and pectinase (Izzatinnisa et al., 2020; Rukmi & Lunggani, 2019). However, the characteristics of *Aspergillus* sp. observed in this study do not apply uniformly across all species and strains. The potential of a given isolate must therefore be determined through testing that specifically evaluates the traits intended for use.

Additionally, research by Firhalzar & Wisdawati, (2022) showed that *Aspergillus* sp. isolated from the rhizosphere could inhibit the growth of *Ganoderma boninense* by up to 80%. These results provide a basis for evaluating the potential antagonistic activity of certain *Aspergillus* sp. isolates against plant pathogens. However, the reported inhibition figures were derived from different isolates and cannot be directly applied to the isolates in this study. Consequently, although the *Aspergillus* sp. isolates obtained here may be considered for further research, their biological control effectiveness still requires verification through direct testing.

The exploration also yielded five *Trichoderma* sp. isolates. The presence of this genus is significant because many *Trichoderma* species have been extensively studied for their ecological roles, including biological control of pathogens, association with plant growth, and interactions within the rhizosphere. Rahhutami et al. (2021) reported that *Trichoderma* sp. functions as a decomposer of organic matter, maintains soil health, stimulates plant root systems, and naturally improves the physical structure of the soil, thereby suppressing the growth of harmful microbes. However, it cannot be assumed that these benefits apply to every isolate obtained.

Trichoderma sp. is a cosmopolitan soil fungus that can survive at high temperatures. It is saprophytic and antagonistic to several plant pathogens, especially soil-borne pathogens and some airborne pathogens, and it can also produce secondary metabolites that are widely used in agriculture. Jumadi et al. (2021) stated that *Trichoderma* sp. can be applied to three types of plants: nursery plants, horticultural plants, and annual plants, which shows that it

can be used on various plant species. This fungus produces antibiotics, toxins, and enzymes that effectively inhibit pathogen growth and break down organic matter (Contreras-Cornejo et al., 2024). It also increases plant resistance to abiotic stress and releases P ions that can increase phosphorus availability in the soil. As a plant growth-promoting fungus (PGPF), *Trichoderma* sp. contributes to an environment that supports plant growth and produces significant amounts of secondary metabolites (Elita et al., 2021; Shang et al., 2020; Sutarman et al., 2021; Zin & Badaluddin, 2020). These findings indicate that the function of *Trichoderma* sp. is not limited to pathogen inhibition but also extends to processes that support plant growth.

Firhalzar & Wisdawati (2022) reported that the rhizosphere fungus *Trichoderma* sp. inhibited the growth of *Ganoderma boninense* by 56.86% under laboratory conditions. This report indicates that certain *Trichoderma* isolates warrant further investigation, particularly for controlling soil-borne pathogens. However, the inhibition percentages reported in that study cannot be assumed to correspond to the characteristics of the isolates obtained in the current research, because the experimental conditions and strain identities may differ.

Further testing can be conducted using the *Trichoderma* sp. isolates obtained in this study. Such follow-up assessments may include molecular identification, evaluation of antagonistic activity against diseases, determination of the required dosage, and assessment of the fungus's shelf life. If these laboratory tests demonstrate promising activity, subsequent evaluation on host plants will be necessary to assess the consistency of the response under actual field application conditions. Until such evidence is available, these isolates are better viewed as candidates for biological evaluation than as established biological control agents.

The *Penicillium* sp. isolates obtained in this study add to the group of fungi successfully isolated from agricultural soil. This genus comprises filamentous fungi found in soil and on various organic substrates. It thrives at temperatures between 25 °C and 30 °C, which makes it well suited to life in soil, but it probably struggles to survive on twigs and leaves exposed to strong sunlight. Some species produce enzymes involved in the decomposition of organic matter, while others are known to produce metabolites that can influence the growth of other microorganisms. *Penicillium* sp. is a filamentous cellulolytic fungus that produces cellulase enzymes. Additionally, the presence of penicillin allows *Penicillium* sp. to use antibiosis to suppress pathogenic fungi without harming the host (Putra et al., 2018). These traits vary between species and strains; therefore, the ecological function of an isolate cannot be determined from genus-level identification alone.

According to Ayaz et al. (2023), *Trichoderma* sp.*, Penicillium* sp.*, and Aspergillus* sp. are among the most commonly used fungal genera as biological control agents, with antagonistic properties against many plant pathogens. A review by Sun & Shahrajabian (2025) discusses the potential of both *Trichoderma* and *Penicillium* in supporting plant

growth and biological control. Both fungi can facilitate nutrient solubilisation, produce bioactive compounds, and interact with plant pathogens; the review also emphasises the need to select appropriate species and strains because their biological characteristics are not uniform. Based on the literature, the *Penicillium* sp. isolates obtained could be considered for further research on their physiological characteristics, enzymatic activity, or antagonistic potential. This study cannot yet determine whether the *Penicillium* sp. isolates can control plant pathogens, because specific testing has not been conducted. Safety evaluations are also necessary before the isolates can be considered for agricultural use, given that the nature of their metabolites and biological interactions may vary among members of this genus.

In addition to groups of fungi that may have beneficial functions, this exploration also yielded isolates of *Fusarium* sp. and *Geotrichum* sp. The presence of these two genera shows that isolation from agricultural soil can yield fungi with diverse biological characteristics. However, genus-level identification is insufficient to determine whether a specific isolate is pathogenic to plants. The genus *Fusarium* encompasses numerous species and species complexes that differ in host range and ability to cause disease. Nikitin et al. (2023) discuss the ecological diversity of *Fusarium* in soil and the challenges of identifying groups associated with plant diseases. Although some members are known to cause wilt, root rot, or stem rot, such pathogenic traits do not apply uniformly across the genus.

Research by Solihin et al. (2021) studied the pathogenicity of *Geotrichum* sp. in dragon fruit and found that early symptoms included dry black spots. By the fourth day, the spots turned yellowish-black, began to rot, and became soft. By the seventh day, symptoms of soft rot appeared on the surface of the plant's stem. The disease spread rapidly, infecting almost the entire stem and causing yellow stem disease. Re-isolation from the pathogenicity test confirmed the presence of *Geotrichum* sp. at a 100% occurrence rate. This report does not directly prove that the *Geotrichum* sp. isolates obtained from agricultural soil in this study have the same pathogenic capability. Within the scope of the current research, *Fusarium* sp. and *Geotrichum* sp. are better viewed as isolates requiring further characterisation, including inoculation onto relevant host plants, observation of symptoms, assessment of disease progression, and re-isolation of the microorganisms from symptomatic tissue.

The contribution of this research lies in the initial exploration of local soil fungal resources from several agricultural locations in North Sumatra using the *T. molitor* insect-baiting method. The results provide a basis for further research on the identity, physiological characteristics, and possible biological functions of the isolates obtained. This research has not proven the effectiveness of the isolates as biological control agents,

nor has it determined whether all isolates are pathogenic or nonpathogenic. More specific conclusions about these functions must be based on further testing in accordance with the intended use.

CONCLUSION

Fungal isolates were successfully obtained from agricultural and plantation soils using an insect-baiting method. A total of 21 isolates were collected from 13 sampling points in eight sub-districts across Deli Serdang and Serdang Bedagai Regencies in North Sumatra, Indonesia. Preliminary characterisation indicated that 16 isolates showed no pathogenicity toward mustard seedlings in the preliminary test and merit further study, whereas the remaining five showed pathogenic potential toward mustard seedlings.

Macroscopic and microscopic observations revealed several fungal genera: *Penicillium* sp. (2 isolates), *Trichoderma* sp. (5 isolates), and *Aspergillus* sp. (8 isolates), along with one unidentified isolate. The other five isolates comprised *Fusarium* sp. (4 isolates) and *Geotrichum* sp. (1 isolate). These findings demonstrate that the *T. molitor* insect-baiting method is an effective approach for recovering a diverse range of fungal isolates from agricultural soil and provides initial insights into their morphological characteristics. Further molecular identification and functional assays are required to confirm the taxonomic identity of these isolates and to evaluate their potential as biological control agents or plant pathogens.

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