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Identification and Characterisation of Entomopathogenic Microbes: An Innovative Science Learning Resource to Improve Student Science Literacy on the Topic of Agricultural Biotechnology

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Abstract: The challenge in science education is to connect biotechnology concepts with real-world contexts and local potential. This study aims to explore and identify local entomopathogenic microbes as innovative learning resources on the topic of agricultural biotechnology, specifically biological control of onion pests. This exploratory study includes field surveys in the onion centre of Guntarano Village and laboratory analysis. Isolation of entomopathogenic fungi from infected Liriomyza spp. larvae was carried out using the insect bait method. The isolates obtained were purified, identified based on morphological characteristics (macroscopic and microscopic), and their spore density was calculated. The exploration successfully screened six potential fungal isolates. Morphological identification revealed the presence of Aspergillus flavus, Aspergillus niger, Trichoderma sp., and one isolate suspected to be Metarhizium sp. All isolates had high spore densities (> 10^7 conidia/mL), with the Metarhizium sp. (Larva 4) and A. niger (Larva 2) isolates showing the highest densities. The diversity of entomopathogenic microbes found has the potential to be a source of biopesticide learning in science education. This material can be utilised to develop modules, worksheets, or learning media based on local potential to enhance science literacy and students' understanding of agricultural biotechnology.

Keywords: Agricultural biotechnology; Entomopathogens; Liriomyza; Science learning resources; Science literacy

Introduction

Contextualising learning materials with local potential is an effective strategy for improving students' science literacy (Januarti et al., 2024; Susilawati et al., 2025; Verawati et al., 2024). Guntarano Village, located in the Palu Valley, Central Sulawesi Province, Indonesia, is one of the main centres for local red onion cultivation (Allium cepa L.) with a planting area of 83 hectares (Arfan et al., 2018). Although this region has high production potential, the average productivity achieved by farmers is only around 4.5 tonnes per hectare, far below its genetic potential of 11.10 tonnes/ha for dry bulbs and 14.85 tonnes/ha for wet bulbs (Lapanjang et al., 2023). This phenomenon of productivity gap can be used as a case study in learning to train students' critical thinking skills in identifying problems in the field of agriculture (Cahyaningtyas et al., 2017; Hastuti et al., 2022).

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One of the main factors limiting productivity that can be raised as learning material is attacks by plant pests, particularly leaf miner flies of the genus Liriomyza (Arfan et al., 2020; Supartha et al., 2023). This pest was first reported in the Palu Valley in 2005 and can cause damage of up to 60-80% with characteristic symptoms of small spots (Weintraub et al., 2017), linear-spiral larval gnawing, and brown, dried leaves (Chang et al., 2020). Three species of Liriomyza (L. chinensis, L. sativae, and L. huidobrensis) have been identified, with L. chinensis as the most dominant species (59%) (Hamid et al., 2018). This condition provides an opportunity to develop learning materials on the classification and identification of plant pests (Morris et al., 2021).

Pest control has thus far relied heavily on the use of synthetic pesticides. However, long-term use has caused various negative impacts, including pest resistance, population resurgence (Azhari et al., 2021), decline in natural enemy populations (Sarah et al., 2023), environmental pollution, and threats to human health (Yuliandri et al., 2023). This issue can be used as a starting point in learning to introduce the concept of Integrated Pest Management (IPM) and the importance of sustainable agriculture, which is in line with the basic competencies in the science curriculum.

One potential alternative approach to be raised as learning material is the use of microorganisms as biological agents, particularly entomopathogenic microbes such as Metarhizium anisopliae, Beauveria bassiana, and Trichoderma spp (da Silva et al., 2025; McGuire et al., 2020; Woo et al., 2023). These microbes effectively infect insect pests without harming non-target organisms (Fathy et al., 2025), making them a good example of biotechnology applications in agriculture (Saif et al., 2024). However, information regarding the potential of local microbes as entomopathogens in the red onion fields of Guntarano Village is still very limited and has not been extensively explored, either for research or educational purposes.

This research focuses on the exploration, identification and characterisation of location-specific microbes that not only have the potential to act as biological control agents against onion leaf miners, but also as an innovative learning resource in science education. Through this approach, students can learn the concepts of agricultural biotechnology directly through the exploration of local microbes, while developing their scientific literacy through activities involving the exploration, isolation, and initial identification of microorganisms with entomopathogenic potential. The results of this study are expected to form an important basis for the development of learning modules or other educational media that contribute to improving students' understanding of the use of microbes as local biopesticides that support sustainable agricultural systems.

Method

This exploratory study was conducted in Guntarano Village, Donggala Regency, Central Sulawesi, and laboratory analysis was carried out at the Microbiology Laboratory. The study consisted of two main stages.

Stage I – Field Survey and Sampling

The survey was conducted purposively at several shallot cultivation sites. Samples collected included soil and Liriomyza larvae showing signs of fungal infection. Soil samples were taken diagonally from a depth of 5–7 cm. Each point yielded 0.5 kg of soil. Samples were stored in sterile plastic bags for further analysis.

Stage II – Exploration and Identification of Microbes

The second stage consisted of three main sub-stages focused on the exploration, isolation, and identification of local microbes with potential as biological control agents.

Isolation of Entomopathogenic Microbes

Isolation was carried out using the insect bait method (Zimmermann, 1986). Soil samples were placed in sterile containers, then 20 Liriomyza instar 2–3 larvae were added (Senthilkumar et al., 2021). The containers were incubated in dark conditions for two weeks. Dead larvae showing fungal growth were sterilised on the surface with a 1% sodium hypochlorite solution, rinsed with sterile water, and placed on moist filter paper in Petri dishes to stimulate fungal growth. The growing fungi were inoculated onto Potato Dextrose Agar (PDA) medium supplemented with lactic acid (Arsi et al., 2020).

Purification and Morphological Identification

Isolates were purified by repeated subculturing on PDA medium. Morphological identification was performed macroscopically (shape, colour, and texture of colonies) and microscopically (shape of conidia and conidiophores) with reference to the identification key by Samson et al. (Samson et al., 1988).

Spore Density Calculation

Spore density was calculated using a haemocytometer with three dilution levels (10^4, 10^6, and 10^8). Spore concentration was calculated using a standard formula. The data obtained were analysed descriptively to describe the characteristics and abundance of the isolates that were successfully identified.

Result and Discussion

Result

The exploration of entomopathogenic microbes was conducted in four stages with the aim of screening potential isolates from Liriomyza spp. larvae showing symptoms of infection. In the initial exploration, five replicates were conducted with three dishes each, resulting in a total of 15 dishes. Each dish was incubated with three infected larval isolates, resulting in a total of 45 larvae used for exploration. The exploration stage was continued through four further screening stages to screen the most potential isolates based on their morphological characteristics. The number of isolates obtained from each exploration stage is presented in Table 1.

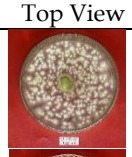
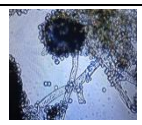
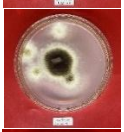
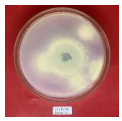
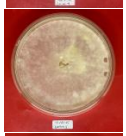
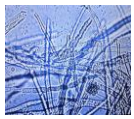
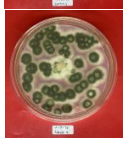
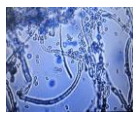
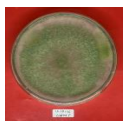
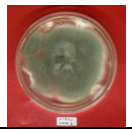
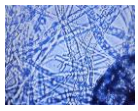
Table 1. Number of Isolates Obtained at Each Stage of Exploration and Screening

Exploration Stage	Number of Cups	Number of Isolates
Preliminary Exploration	15	45
Exploration 2 (screening 1)	31	31
Exploration 3 (screening 2)	21	21
Exploration 4 (screening 3)	6	6

Morphological Characteristics and Identification of Isolates

The third stage of screening yielded six fungal isolates that showed stable growth and distinctive morphological characteristics. Two isolates have been identified as belonging to the genus Aspergillus, one isolate has been identified as belonging to the genus Trichoderma, and the remaining three isolates are awaiting molecular confirmation via PCR.

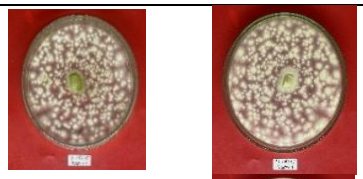
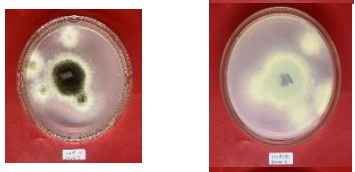
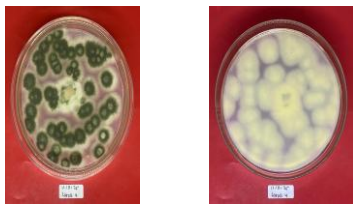
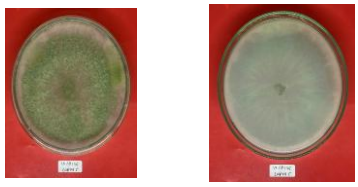
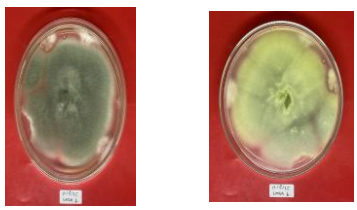
Table 2. Results of Morphological Identification of Six Potential Isolates

No	Isolate code	Macroscopis		Microscopis	Description
		Top View	Bottom view		
1.	Larvae 1				*Asperigilus flavus*
2.	Lavae 2				*Asperigilus niger*
	Larvae 3				Cannot be identified (awaiting PCR results)
4	Larvae 4				Dugaan *Metarhizium* sp. (menunggu hasil PCR)
5	Larvae 5				*Trichoderma* sp.
6	Larvae 6				Cannot be identified (awaiting PCR results)

Microbial Morphological Characteristics

Each isolate was characterised based on its macroscopic appearance (colony colour from above and below) and colony shape on PDA medium. This description is important as a preliminary step in the classification and selection of isolates with potential as biological agents.

Table 3. Macroscopic Morphological Characteristics of Fungal Colonies

Isolate Code	macroscopically visible		colony shape	Macroscopic Image
	Top View	Bottom view		
Larvae 1	Light green with a white circle	Light green with a white circle	The surface of the colony is irregularly shaped small circles with a smooth texture.	
Larvae 2	Black with white circular borders	Cloudy white	The surface of the colony is irregularly circular and has a fine, powdery texture.	
Larvae 3	Cloudy white	Cloudy white	The surface of the colony is cotton-like with fine threads	
Larvae 4	Dark Green	Cloudy white	The surface of the colony forms small irregular circles with a smooth texture.	
Larvae 5	Dark Green	Dark Green	The surface of the colony forms a perfect circle with a dark green micellium circle.	
Larvae 6	Dark green	Green to brass	The surface of the colony forms an irregular circle.	

Spore Density Of Isolates

Larvae Isolate 4 (presumed Metarhizium sp.) and Larvae 2 (A. niger) showed the highest density. The high spore density in the suspected Metarhizium sp. isolate indicates good sporulation capacity, which is an excellent trait for development as a bioinsecticide. However, high spore density alone does not guarantee efficacy, so further pathogenicity testing is absolutely necessary.

Spore density calculations were performed at three dilution levels (10^4, 10^6, and 10^8). The results showed that all isolates had spore concentrations of more than 10^7 conidia/mL at the initial dilution (10^4), indicating high potential for use in subsequent bioassay effectiveness tests. At a dilution of 10^4, the best isolate codes were isolate larva 4 (1.40×10^7), suspected to be Metarhizium sp, and larva 2 (A. niger), which showed the highest density. The high spore density in the suspected Metarhizium sp. isolate indicates good sporulation capacity, which is an excellent trait for development as a bioinsecticide. However, high spore density alone does not guarantee efficacy, so further pathogenicity testing is absolutely necessary.

Table 4. Results of Spore Density Calculations (conidia/mL)

Isolate Code	Dilution		
	10^4	10^6	10^8
Larvae 1	1.04×10^7	0.30×10^7	0.11×10^7
Larvae 2	1.39×10^7	0.91×10^7	0.58×10^7
Larvae 3	1.22×10^7	0.77×10^7	0.75×10^7
Larvae 4	1.40×10^7	0.55×10^7	0.37×10^7
Larvae 5	1.05×10^7	0.26×10^7	0.21×10^7
Larvae 6	1.30×10^7	0.57×10^7	0.54×10^7

Of the total isolates obtained, three isolates have been morphologically identified as Aspergillus flavus, Aspergillus niger, and Trichoderma sp., and one isolate shows strong evidence of being Metarhizium sp. Two isolates have not yet been identified (PCR). Based on the results of spore suspension calculations, all isolates had sufficiently high densities, making them potential candidates for testing their effectiveness in biological control of leaf miner flies (Liriomyza spp.) in the next phase.

Discussion

Microbial exploration of the local shallot farming ecosystem in Guntarano Village yielded a diversity of entomopathogenic and antagonistic fungal isolates. Of the total isolates obtained during four stages of exploration and screening, six isolates exhibited distinctive morphological characteristics and stable colony growth. Some of them have been morphologically identified as Aspergillus flavus, Aspergillus niger, and Trichoderma sp., while one isolate showed morphology resembling the genus Metarhizium and two isolates remain unidentified. The presence of Trichoderma and Metarhizium fungi in shallot farming soil indicates that location-specific entomopathogenic microbes naturally exist in the environment and have the potential to be used as biological agents. This is in line with previous reports by Zimmermann (1986) and Goettel et al. (2005), which stated that Metarhizium anisopliae and Trichoderma harzianum are commonly found in tropical agricultural soils and are capable of effectively infecting various insect pests. In contrast, isolates from the genus Aspergillus, such as A. flavus and A. niger, tend to be saprophytic and are generally not major entomopathogens. However, their presence indicates that the microbiome in agricultural land is quite complex, and further selection is needed to distinguish isolates that are antagonistic to pests from those that are merely contaminants or saprophytes.

Macroscopic and microscopic analysis of the colonies showed that the Trichoderma sp. isolate and suspected Metarhizium sp. had morphology consistent with descriptions in the literature. Trichoderma sp. colonies have a symmetrical circular surface with a characteristic dark green colour, while Metarhizium shows dark green colony growth with irregular edges and a smooth texture, characteristic of M. anisopliae (Samson et al., 1988). Other isolates that have not yet been identified and are still awaiting PCR confirmation need to be further investigated, as they may belong to a group of lesser-known entomopathogenic fungi with hidden potential (hidden diversity).

Spore density is one of the initial indicators of the viability and infection potential of fungal isolates. The results of spore density calculations show that all isolates have a concentration of more than 10^7 conidia/mL at a dilution of 10^4. This value indicates that the isolates obtained have high sporulation ability, which is important for successful infection of the target host (Liriomyza spp.) (Arfan et al., 2020). Isolates with high sporulation ability tend to have better efficacy in field applications because they can spread and infect a wider pest population. In this context, isolates from the second and fourth instars showed the highest density and have the potential to be the main candidates for bioassay testing in the next phase of research.

The discovery of location-specific entomopathogenic microbes in Guntarano Village supports an adaptive and sustainable biological control approach. This approach not only reduces dependence on synthetic pesticides, but is also in line with the principles of Integrated Pest Management (IPM) and conservation of natural enemies. The results of this study contribute to the vision of the National Research Master Plan (RIRN) in creating safe and environmentally friendly food security technologies.

Furthermore, the development of formulations based on local microbes such as Metarhizium anisopliae and Trichoderma harzianum has been proven successful in several previous studies and can be transformed into local bioinsecticides that are suitable for the agroecosystem conditions in Guntarano.

One limitation at this stage is that isolate identification is still limited to morphological characteristics, so molecular analysis (PCR and sequencing) is required to accurately confirm the taxonomy of the isolates. In addition, no efficacy tests have been conducted on Liriomyza spp. larvae, either in vitro or in vivo, which are important for determining the actual efficacy of the isolates obtained. Nevertheless, the results of this exploration have paved the way for the development of local microbial-based biological control agents that are not only effective but also ecologically and economically relevant to shallot farmers in Central Sulawesi.

Conclusion

Microbial exploration of the shallot farming ecosystem in Guntarano Village yielded six fungal isolates, namely Aspergillus flavus, Aspergillus niger, Trichoderma sp., and one isolate suspected to be Metarhizium sp. All isolates had high spore densities (>10^7 conidia/mL), thus meeting the initial criteria as candidates for biological agents. Based on the identification results, Trichoderma sp. and the suspected Metarhizium sp. showed the highest potential for

development as biological control agents against Liriomyza spp. pests on onion plants.

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Author Contributions

Arfan was responsible for conceptualisation, research design, supervision, funding acquisition, conducting experiments, data collection, and data analysis. Ratnawati developed the methodology, conducted experiments, collected data, and contributed to the initial draft. Hasmari performed data analysis, validated the results, and edited the manuscript. All authors were actively involved in discussing the results, reviewing, and approving the final manuscript.

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Conflicts of Interest

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