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Characterization and identification of *Trichoderma* on shallots isolated from three elevation regions in West Sumatra, Indonesia

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Abstract. *Susila E, Maulina F, Emilda D. 2023. Characterization and identification of* Trichoderma *on shallots isolated from three elevation regions in West Sumatra, Indonesia. Biodiversitas 24: xxxx*. Various microorganisms are found in the rhizosphere of plants. species of *Trichoderma* exhibited many benefits to plants such as biological control of many cr disease and their causing agents. This study aim was to explore, collect, and identify species of *Trichoderma* from shallot rhizosphere based on morphological characters and molecular techniques. The research was carried out from March to October 2021 at Biology Laboratories Agricultural State Polytechnic of Payakumbuh and Plant Protection Laboratory, Indonesian Tropical Fruits Research Institute (ITFRI) for morphological observation, and Molecular Laboratories, ITFRI for molecular identification. Samples were collected from Alahan P ng (1700 m above sea level), Solok (400m asl) and Kambang (< 200 m asl) by using Stratified Random Sampling method. The isolates of *Trichoderma* species were cultured on otato Dextose Agar (PDA). Three *Trichoderma* sp. from shallot rhizosphere were obtained and identified. Four specific primer pairs (T2A F-T2A R, T2 F-T2 R, T1 F-T1 R and Th1 F-Th1 R) were used for molecular identification. The amplification results of three isolates of *Trichoderma* spp. using four pairs of specific primers showed that the three isolates tested amplified only in the *T. asperellum* primer pair. The results showed that *Trichoderma* spp. origin of shallot rhizosphere from three locations (Alahan Panjang, Solok and Kambang) are identified as one species i.e. *Trichoderma asperellum*.

Keywords: Microorganism, molecular, morphology, shallots, *Trichoderma*

INTRODUCTION

Sustainable agriculture takes more attention at latest decades. A massive and widely used of pesticides to overcome plant pests and diseases showed their damage effects to human and environment. Therefore, plant protection practices should be changed toward environmentally friendly agricultural practices. Fungi and their metabolites can be one of the suitable substitute for chemical pesticides. Among different useful fungi, *Trichoderma* spp. are one of best biocontrol agents tha n substitute fungicides. Many species on this genus have been reported as biocontrol agents for many plant diseases (Zin and Badaluddin 2020) and phytoparasitic nematodes (TariqJaveed et al. 2021). This genus also informed as plant growth promoter, natural decomposer and agents for bioremediation (Zin and Badaluddin 2020).

Shallots (*Allium ascalonicum* L.) is an herbaceous plant that has shallow roots. It needs icient water during vegetative stage of its life cycle. Drought is one of the major abiotic constraints, limiting shallots growth and development world-wide Ghodke et al. (2018). Therefore, the cultivation of shallot crops on the dry lands of the lowlands of West Sumatra requires a technology in order to grow it optimally. The use of microorganisms explored from plant rhizosphere itself is one of the alternatives so that plants can grow optimally and increase crop yields.

Apart from being a biological agent, *Trichoderma* spp. acts as a decomposer. It is also reported as endophytic association with number of plant species (Gautam 2014). The species of *Trichoderma* are easy to insulate, has wide adaptability and can grow quickly on various substrates. According to Mukherjee et al. (2022), this fungus also has a wide range of micro parasitism and is not pathogenic in plants. Madbouly (2021) reported competitive potential of *Tricoderma* sp. that means it can suppress the growth and activity of soil infectious pathogens.

Various microorganisms are found in the rhizosphere of plants. Besides Myccorhizal Arbuscular fungi (Susila et al. 2017), one of beneficial soil microorganisms for plants is *Trichoderma* sp. which naturally attacks pathogenic fungi. These fungi found in all soil types l various habitats (Gusnawaty et al. 2014; Tyskiewicz et al. 2022). A wide variety of plants have been reported to be associated with the fungus *Trichoderma* such as in cacao (Mulaw et al. 2013), *Allium sativum* L. (Shentu et al. 2014), and soybean roots (Khaledi and Parisha 2016). The rapid development of this fungus occurs in the root area of the plants, th fungus is generally superior to pathogenic fungi in competition for nutrients and space, therefore it can be used as one of the biological agents to control soil pathogens (Zin and Badaluddin 2020).

As a biological control agent (Consolo et al. 2012; Abo-Elyousr et al. 2014), th species of *Trichoderma* has antagonistic abilities, and able to kill as well as inhibit the growth of pathogenic fungi. *Trichoderm* is parasite also, where with its antagonistic mechanism it can attack and take nutrients from other fungi. Deng et al. (2018) reported that

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the aspartic protease P6281 secreted by the fungus *Trichoderma harzianum* Rifai plays an important role in mycoparasitism on phytopathogenic fungi. The protease significantly inhibited the development of grey mold that causes rotting of cucumber, apple, and orange. Its indicating that rP6281 may be developed as an effective anti-mold agent for fruit storage. Ramona et al. (2022) also reported that *T. harzianum* (Td 22 isolate) was able to suppress *Sclerotinia* minor infection of *Phaseolus vulgarum* plants.

The effectiveness of each *Trichoderma* species in the control of pathogenic fungi varies. due to the morphology and physiology of each species. Madbouly (2021) stated that different species are very important biocontrol agents against several phytopathogenic fungi. Thus it can function as a bio-fungicide. Various studies have been reported regarding the *Trichoderma* sp., as reported by Es-Soufi et al. (2020), *T. harzianum* TR is effective for the biological control of anthracnose, gray mold and powdery mildew on strawberries grown in field conditions, and Kandula et al. (2015) reported that *Trichoderma atroviride* P.Karst. isolates were selected for field assessment as biocontrol agents of soil borne pathogens of pasture species. Pimentel et al. (2022) also reported that *Trichoderma* spp. could reduce soybean root rot caused by *Fusarium vigulforme* associated microparasites and induce genes related to plant resistance.

The ability of *Trichoderma* sp. in suppressing diseases in various plants is also thought to be able to suppress soil infectious diseases in shallots. The limitations of the indigenous *Trichoderma* isolate cause the lack of application this fungus in reducing the risk of yield loss in shallots. Therefore, it is necessary to explore and identify *Trichoderma* sp. from various shallot growing locations in West Sumatra. Identification of the fungus Trichoderma sp. conventionally can be done morphologically. Identification is conventionally based on macroscopic and microscopic characteristics, namely by looking at the development of colony growth in the growing medium and looking at the part of the fungus under a microscope. *Trichoderma* is one of the most common fungi in soil. Despite the high significance of this species, it has no known taxonomic boundaries due to its complexity and is highly capable of living in diverse habitats (Chaveri and Samuel 2013). Conventionally identification relies heavily on morphology and characterization of isolates. However, biological restrictions at the species level make it difficult to identify only by relying on morphological identification. The development of science and technology in the field of genetics is very supportive of molecular observations. Advances in modern methods can identify *Trichoderma* using Polymer Chain Reactions (PCR) and sequence analysis using common primers for fungi, namely Internal Transcribed Spacer (ITS) or can use specific primers. This study aim was to explore, collect, and identify species of Trichoderma from shallot rhizosphere based on morphological characters and molecular techniques.

MATERIALS AND METHODS

Determination of sample locations

Research was conducted from May 2021 to October 2021 at Biology Laboratories, Agricultural State Polytechnic of Payakumbuh and Laboratory of Plant Protection, the Indonesian Tropical Fruits Research Institute (ITFRI) for morphological observation, and the Laboratory of Molecular, ITFRI for molecular identification. *Trichoderma* spp. isolated from soil rhizosphere of shallots at three locations in West Sumatra. A Stratified Random Sampling was used for sample collection based on three elevations i.e. Alahan Panjang (high elevation region, 1,700m above sea level), Solok (medium elevation region, 400m asl) and Kambang (low elevation region, <200m asl) (Figure 1).

Isolation of Trichoderma spp. from shallots rhizosphere

Before isolating Trichoderma isolates, the tools to be used were sterilized. The heat-resistant tools such as beakers, Erlenmeyer tubes, glass spatulas, object glasses, drip pipettes and others were sterilized into an autoclave at 121°C with a pressure of 1 atm for 15 minutes. Tools that are not heat resistant were sterilized using 70% alcohol. Samples were isolated on Potato Dextrose Agar (PDA) and purified on the same PDA medium.

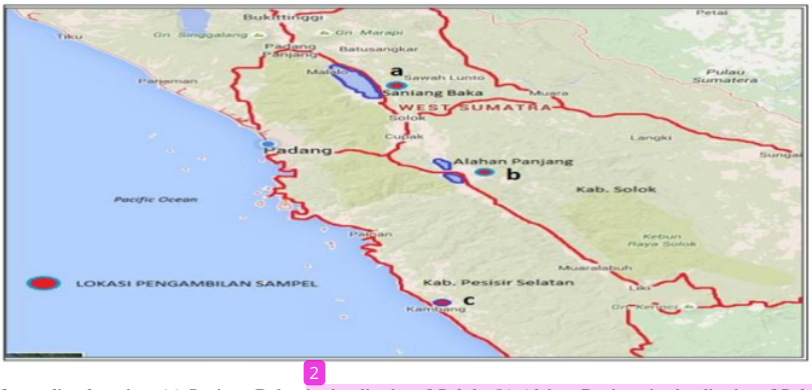

Figure 1. Map of sampling location: (a) Saniang Baka, in the district of Solok, (b) Alahan Panjang in the district of Solok, (c) Kambang, in the district of Pesisir Selatan, Indonesia (Susila et al. 2018)

Isolates of *Trichoderma* spp. obtained from shallot production centers in West Sumatra at different elevation (Alahan Panjang-high elevation region, Solok-medium elevation region and Kambang-low elevation region). Soil samples were obtained from a 15 cm depth, placed in sterile bags and transferred to the laboratory for isolation process. Isolation of *Trichoderma* spp. from shallot rhizosphere soil samples was carried out by serial dilution technique. Furthermore, the microbes that were found and suspected to be Trichoderma isolates were continued with re-isolation until pure isolates were obtained.

The serial dilution technique carried out on shallot rhizosphere soil samples from three locations in West Sumatra i.e. Alahan Panjang, Solok and Kambang found seven isolates suspected of being *Trichoderma*. Furthermore, re-isolation of each isolate was carried out to obtain pure *Trichoderma* culture. The characterization carried out shows that the isolate consists of three different types.

Morphological characterization

Three 19) *Trichoderma* spp. isolates as a result of screening were grown back on PDA media and incubated for 7 days. Furthermore, pure isolates on 7th day after incubation (DAI) were diluted 10^{-5} and then spread on new PDA media to obtain a single colony of *Trichoderma* spp. Making a single spore culture aims to get spores from the same type.

Propagation was carried out by taking 1 cork borer for each isolate to be cultured on PDA media and incubated for seven days. Each isolate was repeated three times and in each replication, there were three units so that a total of 27 research units. Daily observations for seven days on the culture of the fungus *Trichoderma* spp. were carried out macroscopically on the appearance and growth of the colonies. Observation of colony color, colony shape and growth diameter was carried out as per Watanabe (2010).

Microscopic observations were conducted on conidiophore, phialides, and conidia shape of *Trichoderma* spp. isolates by using slide culture method. Fungal colony was placed on microscope slide and added with distilled water or lactophenol blue. Observation was performed under microscope with 100x magnificent. Identification of isolates was done by using Trichoderma identification book of Watanabe (2010).

Molecular identification

Extraction of *Trichoderma* spp. genomic DNA from screening results (*Trichoderma* sp. I, *Trichoderma* sp. II and *Trichoderma* sp. III) was performed by using Zymo Quick DNATM Fungal/Bacterial Miniprep Kit (Cat. No. D6005). Tissue Lyser II Qiagen was used for fungal tissue lysis at frequency 25/s for 5 minutes. DNA quantity and quality were checked by using Bio spectrometer. MyTaqTM Red Mix (Bioline) was used for DNA amplification. Primer pairs used in this study as described at Table 1. PCR process as 30 ow: initial denaturation 95°C (1 minute), followed by 30x cycles 33 denaturation at 95°C (15 seconds), annealing at 54°C (15 seconds), extension at 72°C (10 seconds) and final cycle at 72°C (10 minutes).

Electrophoresis process used SB buffer on 1.2% agarose gel at voltage 50V for 60 minutes. Coloring using a solution of ethylene bromide. Gel Doc 2000 Video Gel Documentation Sys m was used for visualization of these bands.

Four sets of primers (T2A F-T2A R, T2 F-T2 R, T1 F-T1 and Th1 F-Th1 R) used in this multiplex PCR technique were combined in one tube for simultaneous identification of four different *Trichoderma* species. Under optimal conditions, multiplex PCR produced specific amplicon with the expected size with each DNA plate (Figure 2). The advantage is that the multiplex reaction does not generate extra amplicon with non-target DNA. This technique can show that four different species of *Trichoderma* can be identified simultaneously in one PCR (Prabhakaran et al. 2014). However, in this study, we used only each single primer pairs and mix of two primer pairs which were: 1. Primer pairs for *Trichoderma asperellum,* 2. Primer pairs for *T. harzianum,* 3. Primer pairs for *Trichoderma longibrachiatum,* 4. Primer pairs for *Trichoderma virens,* 5. Mix of Primer pairs for *T. asperellum* and *T. harzianum* (mix of primer pairs 1 and 2) and 6. Primer pairs for *T. longibraciatum* and *T. virens* (mix of primer pairs 3 and 4).

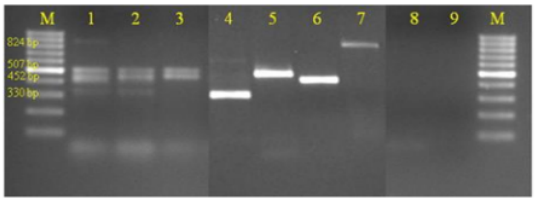

Figure 2. Multiplex PCR produced specific amplicon with expected size with respective DNA plates (tef1 and rpb2 genes amplified by *Trichoderma* species with amplicon (4) *T. virens* (330bp), (5) *T. asperellum* (507bp); (6) *T. longibrachiatum* (452bp) (7) *T. harzianum* (824bp) (Source data: Prabhakaran et al. (2014)).

Table 1. Primer pairs used in this study (Prabhakaran et al. 2014)

Species	Gene	Primer	Sequen (5'-3')	Product (bp)
Trichoderma asperellum Samuels, Lieckf. & Nirenberg	*tef1*	T2A F	5′- CTCTGCCGTTGACTGTGAACG -3'	507
		T2A R	5′-CGATAGTGGGGTTGCCGTCAA -3′	
Trichoderma harzianum Rifai	*rpb2*	Th1 F	5′-TTGCATGGGTTCGCTAAAGG-3′	824
		Th1 R	5′-TCTTGTCAGCATCATGGCCGT-3′	
Trichoderma longibrachiatum Rifai	*tef1*	T1 F	5′- CCGTGAGTACACACCGAGCTT -3'	452
		T1 R	5′- CGGCTTCCTGTTGAGGGGA -3′	
Trichoderma virens (J.H.Mill., Giddens & A.A.Foster) Arx	*tef1*	T2	5′- CCGTTTGATGCGGGGAGTCTA-3′	330
		T2 R	5′- GGCAAAGAGCAGCGAGGTA-3′	

Note: *tef1*: *translation elongation factor* 1a, rpb2: *rna polymerase II*

RESULTS AND DISCUSSION

Isolation of *Trichoderma* spp.

Serial delusion technique performed on shallot rhizosphere soil samples from three locations in West Sumatra, namely AP, SLK, KMB found seven isolates suspected of being *Trichoderma* spp. Re-isolation was carried out until pure *Trichoderma* isolates were obtained which showed that of the seven isolates, there were three types of *Trichoderma* spp. that had macroscopic differences (table 3), namely *Trichoderma* sp. I, *Trichoderma* sp. II, and *Trichoderma* sp. III.

Macroscopic characterization

Various Tricoderma species have been reported such as *T. harzianum*, *Trichoderma viride* Pers., *Trichoderma koningii* Oudem., *Trichoderma hamatum* Bonord.) Bainier, *Trichoderma polysporum* (Link) Rifai and, *Trichoderma aureoviride* Rifai which are widespread in various cultivated plants (Gusnawaty et al. 2014). However, the use of biological agents in the control of plant diseases is specific. Erwanti (2003) stated that biological control is locally specific, namely antagonistic microorganisms found in an area will only provide good results in their native regions. As reported by Prayudi et al. (2000) that there are differences in the ability of *Tricoderma sp* from rice fields in South Kalimantan with Tricoderma from Jogjakarta in suppressing leaf blight on rice leaf sheaths in tidal fields of South Kalimantan. Therefore, it is necessary to increase the exploration and identification of Trichoderma from various plant rhizosphere so that its utilization as a biological agent is even wider. Morphological identification both macroscopically and microscopically can be carried out to identify and verify *Trichoderma* species. However, there are many obstacles in making accurate identification such as the lack of laboratory tools that support morphological observations. The influence of the environment on spore growth at the location of origin is also thought to affect the morphological characters of *Trichoderma* so that the identification results are far from accurate. Advances in technology have made this identification activity easier and more accurate in a short period of time. However, morphological characterization cannot be left as a first step in identification activities. Macroscopic characters of *Trichoderma* spp. were observed including propagule shape and color as stated at Table 3.

Table 3 shows that of the three isolates of *Trichoderma* spp. from shallot rhizosphere which is characterized based on its morphology. There was a development of different colony colors from first day until 7th day. Colony color development begins with white, whitish green, whitish dark green and dark green after 7 days after inoculation. The results of macroscopic observations for three isolates of *Trichoderma* spp. have a specific morphological structure (color and shape of the colony) that is generally similar. Even though on the *Trichoderma* sp. II isolate the color of the colony on the 2nd until the 5th day is whitish yellow green, but on the 7th day the color and shape of the third colony of the isolate becomes the same.

Growth differences are also seen in colony growth (colony diameter). The growth rate of colony diameter in *Trichoderma* sp. I isolate is the highest rate compared to two other isolates, namely *Trichoderma* sp. II and *Trichoderma* sp. III. Average full-growing colonies reach the periphery of the petri dish within 3 days after cultured, while *Trichoderma* sp. II and *Trichoderma* sp. III isolates within 4 days after cultured (Table 3). The results of the observation of the growth form of *Trichoderma* colonies on *Trichoderma* sp. I and *Trichoderma* sp. III isolates have a similarity, namely forming a circle, dark green in each circle. It is different from the form of colony growth in *Trichoderma* sp. II isolates that grow evenly on the surface of the medium (Figure 3). The morphological characteristics of *Trichoderma spp*. are complete in Table 3. Macroscopic and microscopic characteristics of *Trichoderma* isolates from shallots (*Trichoderma* sp. I, *Trichoderma* sp. II, *Trichoderma* sp. III) 7 days after culture can be seen in Figure 3.

Microscopic characterization

Microscopic characterization of *Trichoderma* spp. isolates had been done for conidiophore, phialides, conidium and hypha of these fungi by using *Trichoderma* identification book by Watanabe (2010). The observation results as stated at Table 4.

Based on the result of microscopic observations (Table 4 and Figure 4), the three *Trichoderma* isolates of shallot rhizospheres have similarities (conidiophore, phialides, conidium and hypha). The conidiophore for three *Trichoderma* isolates is erect, branched, phialides are thick and short, the shape of the conidium is oval, the walls of the conidium are thick, the color of the conidia is green, and the hypha has something in common that is hyaline and insulated. Taribuka et al. (2016), obtained the same morphological characteristics as the morphological characters of two *Trichoderma* isolates on bananas, namely Ksn and Psr 1. The difference is only between phialids size and conidia size that is not far apart. After molecular observations obtained the results that the Ksn isolate was *T. asperellum* and Psr 1 was *T. harzianum*.

Table 2. Screening for *Trichoderma* species from 3 shallot rhizosphere locations in West Sumatra, Indonesia

Location sample	Elevation	Origin	Isolate	Characterization	Species
AP	High (1700m asl)	Alahan Panjang	AP1	AP1I	*Trichoderma* sp. I
			AP2	AP2I	
SLK	Medium (400m asl)	Solok	SLK1	SKL1II	*Trichoderma* sp. II
			SLK2	SLK2II	
KMB	Low (<200m asl)	Kambang	KMB1	KMB1II	*Trichoderma* sp. III
			KMB2	KMB2III	
			KMB3	KMB3III	

Based on the morphological characters of these three isolates in general, it is almost similar but have not yet been determined at the species level. To be sure up to the species level requires more complete observation parameters such as spore size, phialid thickness and various other microscopic observations. The three *Trichoderma* isolates (*Trichoderma* sp. I, *Trichoderma* sp. II, and *Trichoderma* sp. III) in Figure 4 show green conidiophores with thick walls, the shape of the conidia was oval. Phialides were short and thick, conidiophores were upright and branching. Therefore, molecular identification was continued to confirm the species and answer whether the three isolates were the same or different species.

Molecular identification of *Trichoderma* species

The taxonomic history of *Trichoderma* shows that it is very difficult to define biological species at the genus and species level if only relying on morphological identification. According to Zin and Badaluddin (2020), differentiation among *Trichoderma* species based on morphological characters had difficulties because several characters among them were non-differentiable. Molecular identification was developed to overcome this difficulty. Molecular identification can be carried out using universal primers or species-specific primers. The first method uses universal primers using the ITS region of ribosomal DNA sequences. Universal primers that are usually used are primers ITS 1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (Abdel-Lateif and Bakr 2018).

Carrying out molecular identification generally uses these following methods namely: 1) DNA extraction using a kit (protocol according to the manufacturer's provisions) and tissue destruction using Tissue Lyser II with a frequency of 25/s for 5 minutes. 2) Check the quantity and quality of DNA. 3) Perform PCR using selected primers. 4) Electrophoresis. 5) Taking pictures to find out if the intended gene is amplified. 6) Sequencing of the resulting PCR products (can be ordered from other parties. 7) Check the quality of the sequencing results. 8) Alignment (BLAST) of sequenced results with previously reported sequences (NCBI). 9) Perform phylogeny analysis using certain software (such as Mega 6 or other software). 10) The results of the phylogenetic analysis will show that the tested isolates are grouped with species that have been reported. All the steps above need to be done for molecular identification with universal primers. As the study reported by Taribuka et al. (2016), identified endophytic *Trichoderma* on banana plant roots molecularly using universal primers ITS 1 and ITS 2. Furthermore, the DNA sequences obtained were analyzed and compared with existing data in NCBI with the BLAST-N. However, in this study we used specific primers for *Trichoderma* species identification which were published by Prabhakaran et al. (2014), therefore the steps that needed to perform only number 1 to 5.

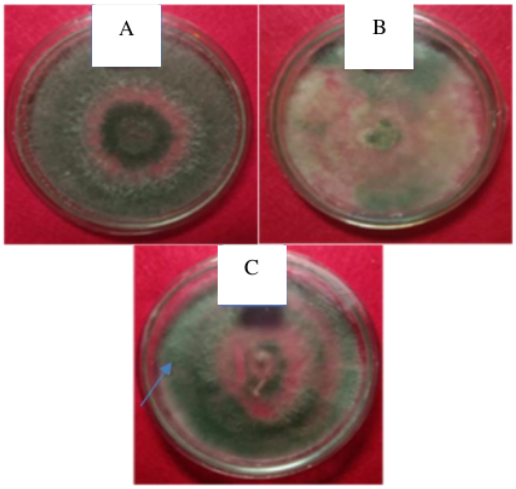

Figure 3. Macroscopic appearances of *Trichoderma* spp. isolated from the root of shallot. These isolates collected from Alahan Panjang at elevation 1.700 m above sea level- *Trichoderma* sp. I (A), Solok at elevation 400m asl-*Trichoderma* sp. II (B) and Kambang at elevation <200m asl-*Trichoderma* sp. III (C) (Source; primary data)

Table 3. Development of *Trichoderma* spp. propagule color and diameter at 1-7 days after isolation cultured on 9 cm petri dish containing PDA medium and incubated at room temperature

Isolat	Observation period (days after isolation)							Propagule shape
	1	2	3	4	5	6	7	
Trichoderma sp. I	White	Whitish green	Whitish green	Whitish green	Whitish dark green	Dark green	Dark green	Round Forming a circle
	5.25cm	7.25cm (d)	9cm (d)	9cm (d)	9cm (d)	9cm (d)	9cm (d)	
Trichoderm sp. II	White	Whitish yellow	Whitish yellow green	Whitish yellow green	Whitish yellow green	Green	Dark green	Grow evenly on the surface
	2.5cm	5.5cm	6.8cm	9cm	9cm	9cm	9cm	
Trichoderma sp. III	White	Whitish green	Whitish green	Whitish green	Dark green	Dark green	Dark green	Round Forming a circle
	3.35cm	6.05cm	7.9cm	9cm	9cm	9cm	9cm	

The second way of molecular identification is by using species-specific primers. If using species-specific primers, then steps 6 to 10 are not necessary. When the gene is amplified, it means that the isolate is the isolate shown by the specific primer Prabhakaran et al. (2014) reported multiplex PCR for detection and identification of four *Trichoderma* species including *T. asperellum*, *T. harzianum*, T. *longibrachiatum* and *T. virens*. This specific pair of primers was used in this study to improve the accuracy and efficiency of *Trichoderma* identification. Multiplex PCR technique provides a fast, simple, and reliable alternative as a new method for the identification of different *Trichoderma* species (*T. asperellum, T. harzianum, T. longibrachiatum*, dan *T. virens* based on the amplicon band pattern) in a single reaction.

The results revealed the identification of *T. asperellum* as one species of three *Trichoderma* isolates on shallots (Figure 5). Dewi et al. (2021) evaluated *T. asperellum* for the control of various plant diseases include: the following recent examples: Fusarium wilt in *Stevia rebaudiana*, *Pratylenchus brachyurus* in soybeans, and pearl millet downy mildew caused by *Sclerospora graminicola*. Taribuka et al. (2016) reported *T. asperellum* as one of fungi on banana.

Trichoderma asperellum reported as biocontrol agent for rot root disease on cocoyam caused by *Pythium myriotylum* and wilt disease on tomato caused by *Fusarium oxysporum*. sp. *lycopersici* (Zin and Badaluddin 2020). *T. asperellum* has also been reported to increase local defense responses in cucumber plants (Alfiky and Weisskopf 2021). No information obtained about the role of *T. asperellum* on shallots growth and diseases. Therefore, these aspects need to be further explored.

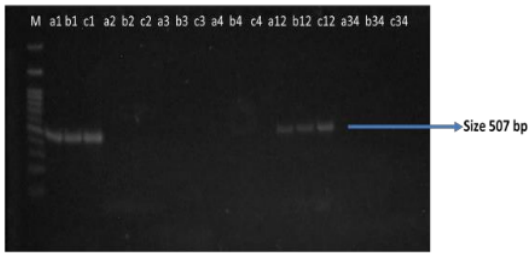

Figure 5. PCR products derived from amplification of *Trichoderma* sp genes by using 4 specific primer pairs for *T. asperellum, T. harzianum, T. longibrachiatum and T. virens*. M = marker, a = Isolate *Trichoderma sp.* I, b = Isolate *Trichoderma* sp. II, c = Isolate *Trichoderma* sp. III, 1 = specific primer pairs for *T. asperellum*, 2 = specific primer pairs for *T. harzianum*, 3 = specific primer pairs for *T. longibrachiatum*, 4. specific primer pairs for *T. virens* (Source; primary data)

Table 4. Microscopic observation of *Trichoderma* spp. from rhizosphere of shallots isolated from three locations at different elevations in West Sumatra, Indonesia

Isolates	Microscopic observation					
	Conidiophore	Phialides	Conidium shape	Conidium wall	Conidium color	Hypha
Trichoderma sp. I	Upright, branching	Short, thick	Oval	Thick	Green	Hyalin, septate
Trichoderma sp. II	Upright, branching	Short, thick	Oval	Thick	Green	Hyalin, septate
Trichoderma sp. III	Upright, branching	Short, thick	Oval	Thick	Green	Hyalin, septate

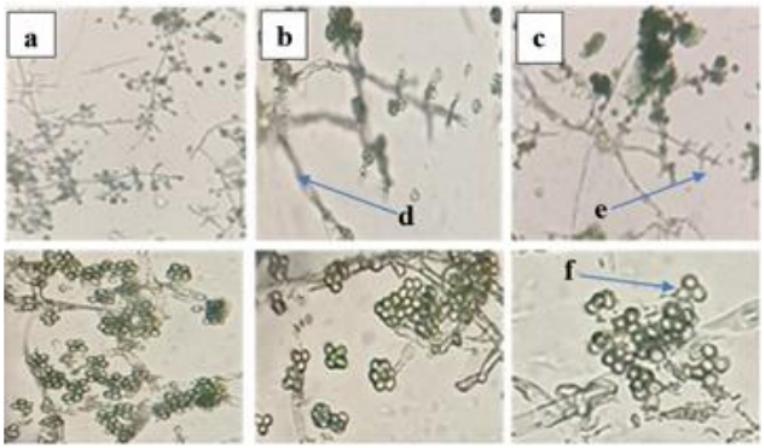

Figure 4. Microscopic appearances of *Trichoderma sp* isolated from the root of shallots. These isolates collected from Alahan Panjang at elevation 1.700 m above sea level- *Trichoderma* sp. I (a), Solok at elevation 400m asl- *Trichoderma* sp. II, (b) and Kambang at elevation <200m asl- *Trichoderma* sp. III, (c) koniodofor (d), phialides (e), conodium shape (f) (Source; primary data)

The results of the study by El Kommy et al. (2015) reported that *T. asperellum* as a biocontrol agent was effective for controlling tomato wilt caused by *Fusarium oxysporum f. sp. lycopersici* (FOL). Random amplified polymorphic DNA (RAPD) method was used to observe genetic variability among 30 *asperellum* isolates. From the results, it was observed that all *T. asperellum* isolates could reduce the growth of the mycelium of FOL isolates, however the decrease in FOL growth varied significantly. There are six isolates of *T. asperellum* which are highly antagonistic to FOL and have the potential to be developed commercially for wilt control in tomatoes. Understanding that genetic variation in *Trichoderma* isolates and their different chemical abilities, it is needed in the selection of effective strains to be used as biocontrol agents. Therefore, the development of studies in the identification of *Trichoderma* both morphologically and molecularly needs to be developed. As a recent study by Abadi et al. (2022) carried out identification of this fungi based on morphology and molecular level (PCR) to obtain soil fungi isolated from natural forests for bioremediation of the Mancozeb fungicide on potato fields. From the several soil fungi evaluated, *Trichoderma* was found as bioremediation in potato fields.

This study concluded that three isolates of *Trichoderma* from rhizosphere of shallots was isolated. Those isolates identified as *T. asperellum* based on molecular identification, using four pairs of specific primers. These *Trichoderma* had different growth rates even though they were including in one species. The fastest growth rate isolate was isolate *Trichoderma* sp. I isolated from rhizosphere of shallots at Alahan Panjang region.

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REFERENCES

Abadi AL, Choliq FA, Oktavianita M, Arinata N, Hadi MS, Setiawan Y. 2022. Screening of soil fungi as bioremediation fungicide and its effect on growth of potato plants. Biodiversitas 23 (3): 1605-1610. DOI: 10.13057/biodiv/d230351.

Abdel-lateif KS, Bakr RA. 2018. Internal Transcribed Spacers (ITS) based identification of *Trichoderma* isolates and biocontrol activity against *Macrophomina phaseolina*, *Aspergillus niger* and *Meloidogyne incognita*. Afr J Microbiol Res 12 (30): 715-722. DOI: 10.5897/AJMR2018.8915.

Abo-Elyousr KAM, Abdel-Hafez SII, Abdel-Rahim IR. 2014. Isolation of *Trichoderma* and evaluation of their antagonistic potential against *Alternaria porri*. J Phytpathol 162 (9): 567-574. DOI: 10.1111/jph.12228.

Alfiky A, Weisskopf L. 2021. Deciphering *Trichoderma*-plant-pathogen interactions for better development of biocontrol applications. J Fungi 7 (1): 61. DOI: 10.3390/jof7010061.

Chaverri P, Samuels GJ. 2013. Evolution of habitat preference and nutrition mode in a cosmopolitan fungal genus with evidence of interkingdom host jumps and major shifts in ecology. Evolution 67 (10) : 2823-2837. DOI: 10.1111/evo.12169.

Consolo VF, Mónaco CI, Cordo CA, Salerno GL. 2012. Characterization of novel *Trichoderma* spp. isolates as a search for effective biocontrollers of fungal diseases of economically important crops in Argentina. World J Microbiol Biotechnol 28 (4): 1389-1398. DOI: 10.1007/s11274-011-0938-5.

Degani O, Khatib S, Becher P, Gordani A, Harris R. 2021. *Trichoderma asperellum* secreted-6-pentyl-α-pyrone to control *magnaporthiopsis maydis*, the maize late wilt disease agent. Biology 10 (9): 897. DOI: 10.3390/biology100908997.

Deng JJ, Huang WQ, Li ZW, Lu DL, Zhang Y, Luo XC. 2018. Biocontrol activity of recombinant aspartic protease from *Trichoderma harzianum* against pathogenic fungi. Enzyme Microb Technol 112: 35-42. DOI: 10.1016/j.enzmictec.2018.02.002.

El-Komy MH, Saleh AA, Eranthodi A, Molan YY. 2015. Characterization of novel *Trichoderma asperellum* isolates to select effective biocontrol agents against tomato Fusarium Wilt. Plant Pathol J 31 (1): 50-60. DOI: 10.5423/PPJ.OA.09.2014.0087.

Erwanti, Mardius Y, Habazar T, Bachtiar A. 2003. Studi kemampuan isolat-isolat jamur *Trichoderma spp*. yang beredar di Sumatra Barat untuk mengendalikan jamur patogen *Sclerotium roflsii* pada bibit cabai. Prosiding Kongres Nasional XVI dan Seminar Ilmiah PFI, Bogor, Indonesia. [Indonesian].

ES-Soufi R, Tahiri H, Azaroual L, Oualkadi AE, Martin P, Badoc A, Lamarti A. 2020. Biocontrol potential of *Bacillus amyloliquefaciens* Bc2 and *Trichoderma harzianum* TR against strawberry anthracnose under laboratory and field conditions. Agric Sci 11 (3): 260-277. DOI: 10.4236/as.2020.113017.

Gautam AK. 2014 Diversity of fungal endophytes in some medicinal plants of Himachal Pradesh, India. Arch Phytopathol Plant Prot 47 (5): 537-544. DOI: 10.1080/03235408.2013.813678.

Ghodke PH, Andhale PS, Gijare UM, Thangasamy A, Khade YP, Mahajan V, Singh M. 2018. Physiological and biochemical responses in onion crop to drought stress. Intl J Curr Microbiol App Sci 7 (1): 2054-2062. DOI: 10.20546/ijcmas.2018.701.247.

Gusnawaty HS, Taufik M, Triana L, Asniah. 2014. Karakterisasi morfologis *Trichoderma* spp. Indigenus Sulawesi Tenggara. Jurnal Agroteknos 4 (2): 88-94. DOI: 10.56189/ja.v4i2.211. [Indonesian].

Kandula DRW, Jones EE, A. Stewart A, McLean KL, Hampton JG. 2015. *Trichoderma* species for biocontrol of soil-borne plant pathogens of pasture species. Biocontrol Sci Technol 25 (9): 1052-1069. DOI: 10.1080/09583157.2015.1028892.

Khaledi N, Taheri P. 2016. Biocontrol mechanisms of *Trichoderma harzianum* against soybean charcoal rot caused by *Macrophomina phaseolina*. J Plant Prot Res 56 (1): 21-31. DOI: 10.1515/jppr-2016-0004.

Madbouly AK. 2021. Biodiversity of Genus *Trichoderma* and Their Potential Applications. in: Industrially Important Fungi for Sustainable Development. Springer Nature Switzerland Press, Switzerland. DOI: 10.1007/978-3-030-67561-5_13.

Mukherjee PK, Mendoza-Mendoza A, Zeilinger S, Horwitz BA. 2022. Mycoparasitism as a mechanism of *Trichoderma* mediated suppression of plant diseases. Fungal Biol Rev 39: 15-33. DOI: 10.1016/j.fbr.2021.11.004.

Mulaw TB, Druzhinina IS, Kubicek CP, Atanasova L. 2013. Novel endophytic *Trichoderma spp*. isolated from healthy *Coffea arabica* roots are capable of controlling coffee tracheomycosis. Diversity 5 (4): 750-766. DOI: 10.3390/d5040750.

Pimentel MF, Arnão E, Warner AJ, Subedi A, Rocha LF, Srour A, Bond JP, Fakhoury AM. 2020. *Trichoderma* isolates inhibit *Fusarium virguliforme* growth, reduce root rot, and induce defense-related genes on soybean seedlings. Plant Dis 104 (7): 1949-1959. DOI: 10.1094/PDIS-08-19-1676-RE.

Prabhakaran N, Prameeladevi T, Sathiyabama M, Kamil D. 2014. Multiplex PCR for detection and differentiation of diverse *Trichoderma* species. Ann Microbiol 65 (3): 1591-1595. DOI: 10.1007/s13213-014-0998-5.

Prayudi B, Budiman A, Rystham MA, Rina Y. 2000. *Trichoderma harzianum* isolat Kalimantan Selatan agensia pengendali hawar pelepah daun padi dan layu semai kedelai di lahan pasang surut.

Prosiding Simposium Penelitian Tanaman Pangan IV. Banjar Baru, Indonesia. [Indonesian].

Ramona Y, Darmayasa IBG, Line MA. 2022. Biological control of *Sclerotinia minor* attack on pyrethrum plants by *Trichoderma harzianum* in glasshouse experiment. Biodiversitas 23 (6): 3264-3269. DOI: 10.13057/biodiv/d230655.

Shentu X, Zhan X, Ma Z, Yu X, Zhang C. 2014. Antifungal activity of metabolites of endophytic fungus *Trichoderma brevicompactum* from garlic. Braz J Microbiol 45 (1): 248-254. DOI: 10.1590/S1517-83822014005000036.

Susila E, Anwar A, Syarif A, Agustian. 2017. Population and diversity of indigenous arbuscular mycorrhizal fungi from the rhizosphere of shallots at different altitudes in West Sumatra. Intl J Adv Sci Eng Inf Technol 7 (5): 1886-1893. DOI: 10.18517/ijaseit.7.5.1592.

Susila E, Anwar A, Syarif A, Agustian. 2018. Selection of six types of isolates of indigenous arbuscular mycorrhizal fungi for growth, yields and essential oil content of shallots (*Allium ascalonicum* L.). Intl J Adv Res 6 (7): 856-864. DOI: 10.21474/IJAR01/7434.

Taribuka J, Sumardiyono C, Widyastuti SM, Wibowo A. 2016. Eksplorasi dan identifikasi *Trichoderma* endofitik pada pisang. J HPT Tropika 16 (2): 115-123. DOI: 10.23960/j.hptt.216115-123. [Indonesian].

TariqJaveed M, Farooq T, Al Hazmi AS, Hussain MD, Rehman AU. 2021. Role of *Trichoderma* as a Biocontrol Agent (BCA) of phytoparasitic nematodes and plant growth inducer. J Invertebr Pathol 183: 107626. DOI: 10.1016/j.jip.2021.107626.

Tyśkiewicz R, Nowak A, Ozimek E, Jaroszuk-Ściseł J. 2022. *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. Intl J Mol Sci 23 (4): 2329. DOI: 10.3390/ijms23042329.

Watanabe T. 2010. Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species. Third Edition (Mycology) 3rd Edition. CRC Press, Boca Raton, Florida.

Zin NA, Badaluddin NA. 2020. Biological functions of *Trichoderma spp.* for agriculture applications. Ann Agric Sci 65 (2): 168-178. DOI: 10.1016/J.AOAS.2020.09.003.

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