Isolation And Characterization Of *Trichoderma* Sp. And Its Antagonism Activity Against Fusarium Wilt In Shallots

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Abstract.

Moler disease in shallots, particularly during the rainy season, caused a significant decline in production. As a solution, biological control using indigenous Trichoderma sp. was needed, as it functioned both as a biocontrol agent and a plant growth promoter. This study aimed to isolate and characterize Trichoderma sp. from the BTP Study Program Pilot Garden and to evaluate its antagonistic potential against moler disease in vitro. The research was conducted at the Agronomy Laboratory of Samarinda State Agricultural Polytechnic from July to August 2024. Observed variables included macroscopic and microscopic characteristics, as well as inhibition tests. Characterization data were analyzed descriptively, while inhibition test results were analyzed using analysis of variance (ANOVA), followed by a P-value of 0.05. Based on the isolation results, four isolates of Trichoderma sp. were isolated from the vegetation of palm oil plants, rubber plants, and pepper plants, exhibiting morphologically and microscopically distinct characteristics. Based on the morphological and microscopic characters, the TP2 isolate was suspected to be Trichoderma sp., the TH2 isolate was identified as T. hamatum, the TE1 isolate was assumed to be T. harzianum, and the TE2 isolate was thought to be T. koningi. The results of the antagonist test showed that the Trichoderma sp. isolates could act as biological controllers, as shown by the inhibition test results ranging from 35.16% to 69.46%. These findings indicated that the indigenous Trichoderma isolates had promising potential as biological control agents and could be further developed for field application to manage moler disease in shallots effectively.

Keywords: *Antagonism; biocontrol; Fusarium oxysporum; moler and Trichoderma sp.*

I. INTRODUCTION

Current agricultural practices face many problems, especially stress, including biotic stress caused by attacks by various plant diseases caused by pathogenic microorganisms that result in crop loss. So far, to overcome this, farmers have used many synthetic pesticides. The use of synthetic pesticides, although very effective, but their use in the long term or continuously can have negative impacts on the environment, human health, and damage the soil [1]. In addition, it can also cause resistance to pathogens and result in the loss of beneficial soil microbes. One type of disease that often attacks plants is the pathogen Fusarium oxysporum, which causes fusarium wilt disease. This pathogen can cause damage to various cultivated plants. One of the plants that is often damaged by *F. oxysporum* attacks is the shallot plant, which can result in a significant decrease in harvest yields [2]. Further research reported that yield losses due to this pathogen attack reached 30-50% [3]. The increasing global food needs and economic impacts of plant diseases require effective and environmentally friendly control strategies to support the implementation of sustainable agriculture. A safer alternative approach method in sustainable agricultural crop cultivation, one of which is through the use of beneficial biocontrol agents in the form of Trichoderma sp fungi [4–6]

Trichoderma sp. is a soil fungus with a wide spectrum of adaptability and is found in many types of soil and associated with plants. [7, 8]. This fungus is also known to have the ability to act as an antagonistic agent against various plant pathogens, through various mechanisms such as competition for growing space and nutrient acquisition, mycoparasitism, and production of antimicrobial compounds [9, 10]. The use of

Trichoderma sp. has been widely reported to act as a plant growth stimulant [11, 12], and as a biological control [13–15].Therefore, various beneficial microbes in the form of Trichoderma sp. are needed, which can play a specific role in the location. Its ability in the field is very much determined by the suitability of its original habitat. Trichoderma isolates from local agroecosystems have better ability or adaptability as biocontrol agents [13, 14]. This study aims to isolate and characterize *Trichoderma* sp. found in the Samarinda State Agricultural Polytechnic Pilot Garden and test its antagonistic activity in controlling fusarium wilt disease caused by the pathogen *Fusarium oxysporum* in shallot plants.

II. METHODS

The research was conducted at the Pilot Plantation Garden and Agronomy Laboratory of the Plantation Crops Cultivation Study Program, Samarinda State Agricultural Polytechnic, from July to September 2024. The research was conducted using a quantitative research approach (experimental method). The research was conducted in stages, starting with sampling, isolation, and characterization, followed by antagonistic testing of the identified isolates against pathogens in vitro.

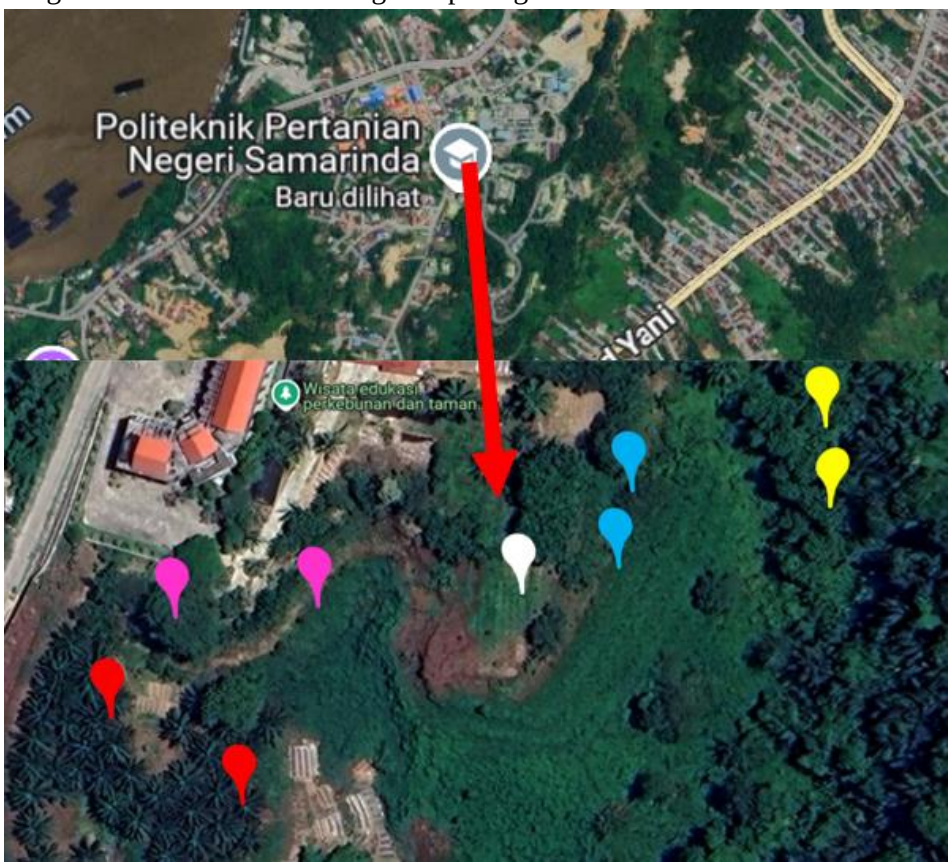

Fig 1. Red (palm oil plant), purple (coffee plant), white (pepper plant), blue (cocoa plant), and yellow (rubber plant) markers are the locations for taking samples of *Trichoderma* sp. in the Field Laboratory of the Demonstration Garden of the Department of Plantation Crop Cultivation

Isolation and Characteristics of *Trichoderma* sp.

The study began with isolation and laboratory. Before the isolation and characterization of *Tricoderma* sp., the media for propagation of *Tricoderma* sp. was first made using PDA (Potato Dextrose Agar) media. Weighing 40 g PDB and 20 g agar and adding 1000 ml of distilled water. The mixture of ingredients was then cooked until boiling using a hot plate. After boiling, the PDA media were put into an Erlenmeyer flask and then covered with plastic wrap. The media were then sterilized in an autoclave for approximately 15 minutes at a temperature of 121 ^{o}C at a pressure of 1.25 atm. After being sterilized, the PDA media can be directly poured into a cup aseptically in the LAFC until solid.The next step is the exploration of *Tricoderma* sp. carried out by the Plantation Crops Cultivation Experimental Garden (oil palm, rubber, coffee, cocoa, and pepper vegetation). The process of trapping Tricoderma sp. using the baiting

method, namely coconut media that is immersed in the soil in a closed manner until the fruit flesh is submerged and stored for 4-7 days [15].

Furthermore, Tricoderma sp. that is trapped is marked by the presence of greenish-yellow hyphae. *Trichoderma* sp. that is trapped is placed below in the Laboratory for isolation. Isolation of Trichoderma sp. media using solid PDA media. *Trichoderma* sp. is taken using a 5 mm diameter cork borer and grown on PDA media aseptically in LAFC, incubated at room temperature until the mycelium fills the cup. Furthermore, *Tricoderma* sp. can be characterized. *Trichoderma* sp. characteristics are carried out by characterizing the macroscopic *Trichoderma* sp., which is observed visually which including the color, texture, and diameter of colony growth. Observations are made every 24 hours by measuring the diameter of the colony until the mycelia cover the entire surface of the cup. Furthermore, microscopic characterization is carried out by taking hyphae and placing them on a glass slide, then dripping water and covering it with a cover glass, and then observing it under a microscope at up to 40x magnification. Microscopic characterization was carried out on conidiophores, phialids, conidia, hyphae, and chlamydiospores. After being characterized, identification was carried out on the type of *Trichoderma* obtained based on the mushroom identification book [16].

Antagonistic activity of *Trichoderma* sp.

Trichoderma sp. antagonistic activity test using the dual culture method. In PDA medium in a petri dish, inoculation was carried out in two different places, both with the antagonistic fungi F. oxysporum. Then incubated for 7 days at room temperature [17]. The following is the layout of the antagonistic test of *F. oxysporum* disease using *Trichoderma* sp. (**Fig. 2**).

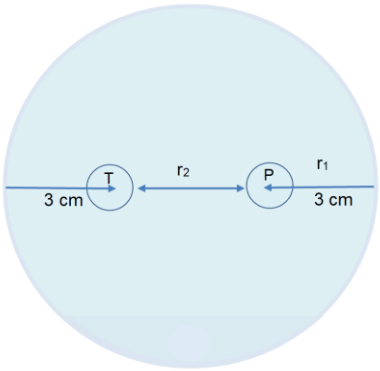

Fig 2. The layout of the antagonistic test of *Trichoderma* sp. (T) on *F. oxysporum* disease (P)

`The characterization data were analyzed descriptively, while the observations of the antagonist tests obtained were analyzed using analysis of variance (ANOVA) and followed by the Tukey test with a P-value of 0.05, using statistical software.

III. RESULT AND DISCUSSION

The results of the study on the isolation and characteristics of *Trichoderma* sp. and its antagonistic activity against fusarium wilt disease in shallots are as follows:

Table 1. *Trichoderma* sp. isolates in various vegetation in the Plantation Crop Cultivation Pilot Garden

No.	Kode Isolat	Asal Vegetasi
1.	TP2	Pepper Plants
2.	TH2	Rubber Plants
3.	TE1	Oil Palm Plants
4.	TE2	Oil Palm Plants
5.	-	Coffee Plants
6.	-	Cocoa Plants

Keterangan : (-) not found

The results of the study in **Table 1** show that there are 4 types of *Trichoderma* sp. at the Plantation Crop Cultivation Pilot Garden, including one in pepper plant vegetation, one in rubber plant vegetation, and two in palm oil plant vegetation, but in coffee plant vegetation and cocoa plant vegetation locations were not found. Kahadiran mikroba tanah sangat ditentukan kelimpahan nutrisi dan kerapatan vegetasi menentukan

kepadatan populasi dari mikroba tanah [5, 18–20]. This is what happens to coffee vegetation, where on the land there are very few coffee plants, and on the land the plants are very rare, and land clearing is often carried out, which has an impact on the presence of microbes.

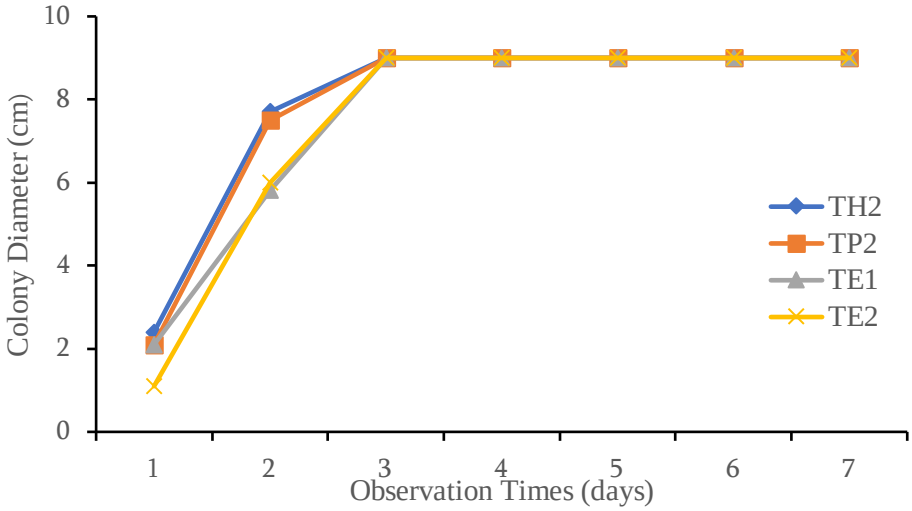

Fig 3. Colony growth of *Trichoderma* sp.

The research results in **Fig. 3** show that the diameter of the four isolates had filled the petri dish on the third day, where Isolate TP2 with colony growth on the first day had a diameter of 2.1, on the second day had a diameter of 7.5, and on the third day it had filled the dish with a diameter of 9.0. TH2 isolate with colony growth on the first day had a diameter of 2.4, on the second day a diameter of 7.7, and on the third day it had filled the petri dish with a diameter of 9.0. TE1 isolate with colony growth on the first day had a diameter of 2.1, on the second day a diameter of 5.8, and on the third day it had filled the petri dish with a diameter of 9.0. TE2 isolate with colony growth on the first day had a diameter of 1.1, on the second day a diameter of 6.0, and on the third day it had filled the diameter of the petri dish. The growth rate of *Trichoderma* sp. in filling the growing space is one of the mechanisms of *Trichoderma* in competition. The higher the growth rate of *Trichoderma*, the higher the ability to dominate the growing space, which will then have an impact on the ability to suppress the growth of pathogens [13, 21].

Table 2. Macroscopic identification of *Trichoderma* sp. from the Plantation Crop Cultivation Pilot Garden

Isolates Code	**Morphological Characters**								
	Colour							**Teksture**	**Colony Shape**
	1	2	3	4	5	6	7		
TP2	White		Greenish White		Dark Green	Dark Green	Dark Green	Thick, A bit rough	Round
TH2					Dark Green	Dark Green	Dark Green	Thin, Smooth	Round
TE1					Greenish White	Green	Green	Thick, Rough	Round
TE2					Greenish White	Greenish Yellow	Greenish Yellow	Thick, Rough	Round

The results of the study in Table 2 show the macroscopic characteristics of the four isolates, where Isolate TP2 with changes in colony color from white, greenish white, and dark green, thick texture, slightly rough with round colony shape. Isolate TH2 with changes in colony color from white, greenish white, and dark green, a thin, smooth texture, with a round colony shape. Isolate TP2 with changes in colony color from white, greenish white, and dark green, thick, a bit rough texture, slightly rough, and with a round colony shape. Isolate TE1 with changes in colony color from white, greenish white, and green, thick, rough texture, with a round colony shape. Isolate TE2 with changes in colony color from white, greenish white, and yellowish white, slightly greenish, thick, rough texture, with a round colony shape. Morphologically, each species of *Trichoderma* fungus has several similarities and specific differences that can be used as a basis for determining the differences between species [22, 23].

Table 3. Microscopic identification of *Trichoderma* sp. from the Plantation Crop Cultivation Pilot Garden

Isolates Code	Microscopic Characters			Species Similarity
TP2	Konidiofor	:	Erect branched	*Trichoderma* sp.
	Fialid	:	Short, thick	
	Konidia	:	Oval	
	Hifa	:	Septate	
	Klamidiospora	:	-	
TH2	Konidiofor	:	Erect branched	*T. hamatum*
	Fialid	:	Short, curved	
	Konidia	:	Round	
	Hifa	:	Septate	
	Klamidiospora	:	Slightly round at the center of the hyphae	
TE1	Konidiofor	:	Erect branched	*T. harzianum*
	Fialid	:	Short, thick, curved	
	Konidia	:	Round/Oval	
	Hifa	:	Septate	
	Klamidiospora	:	Slightly round at the center of the hyphae	
TE2	Konidiofor	:	Erect branched	*T. koningi*
	Fialid	:	Short, thick	
	Konidia	:	Elliptical	
	Hifa	:	Septate	
	Klamidiospora	:	Slightly round at the center and tip of the hyphae	

Based on the identification results, the *Trichoderma* isolates found have similar characteristics to several *Trichoderma* species. Isolate TP2 has similar microspecies with *Trichoderma* sp. (**Fig. 3**), namely, erect, branched conidiophores, short and thick phialids, oval conidia, septate hyphae, but chlamydiospores were not found. Isolate TH2 has similar microspecies characteristics to *T. hamatum* (**Fig. 4**), namely, erect, branched conidiophores, short, curved phialids, round conidia, septate hyphae, and slightly rounded chlamydiospores in the middle of the hyphae. Isolate TE1 has similar microspecies with *T. harzianum* (**Fig. 5**), namely, erect, branched conidiophores, short, thick, and curved phialids, round/oval conidia, septate hyphae, and slightly round at the center and tip of the hyphae. Isolate TE2 has similar microspecies with *T. koningii* (**Fig. 6**), namely, erect, branched conidiophores, short and thick phialids, elliptical conidia, septate hyphae, and slightly round at the center and tip of the hyphae [16]. Previous research results show that *Trichoderma* has many species that can be distinguished morphologically and microscopically [17, 24–26]

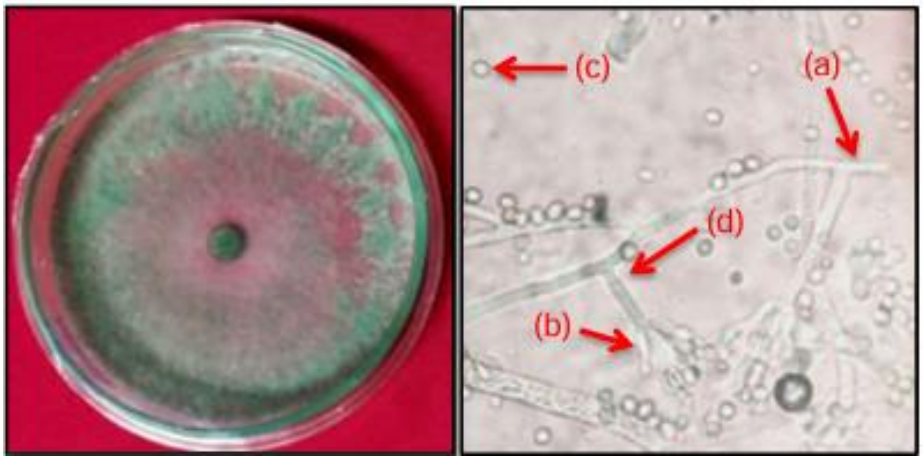

Fig 3. Characteristics of *Trichoderma* sp. TP2. Note: (a) Conidiophores, (b) phialids, (c) conidia, (d) hyphae

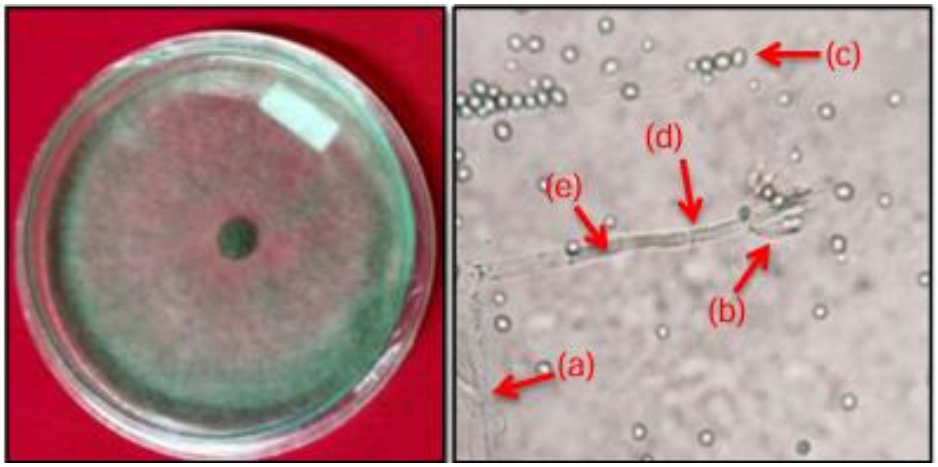

Fig 4. Characteristics of *T. hamatum* TH2. Note: (a) Conidiophores, (b) phialids, (c) conidia, (d) hyphae, (e) chlamydiospores

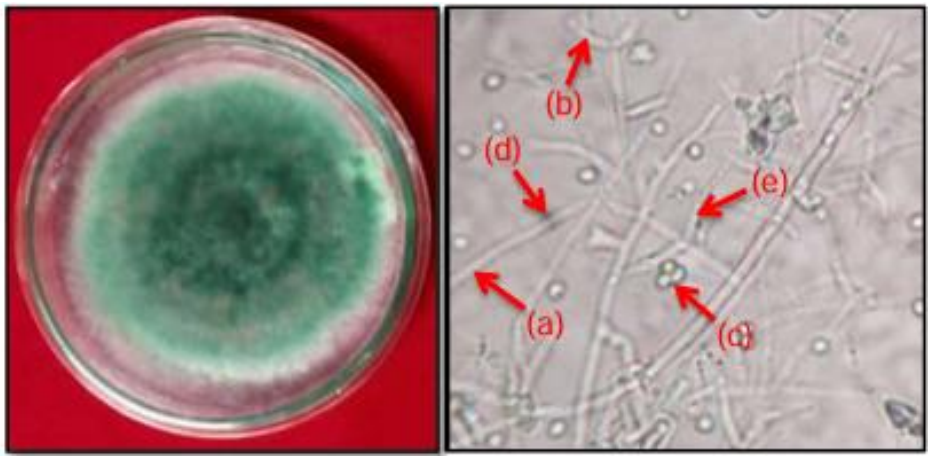

Fig. 5. Characteristics of *T. harzianum* TE1. Note: (a) Conidiophores, (b) phialids, (c) conidia, (d) hyphae, (e) chlamydiospores

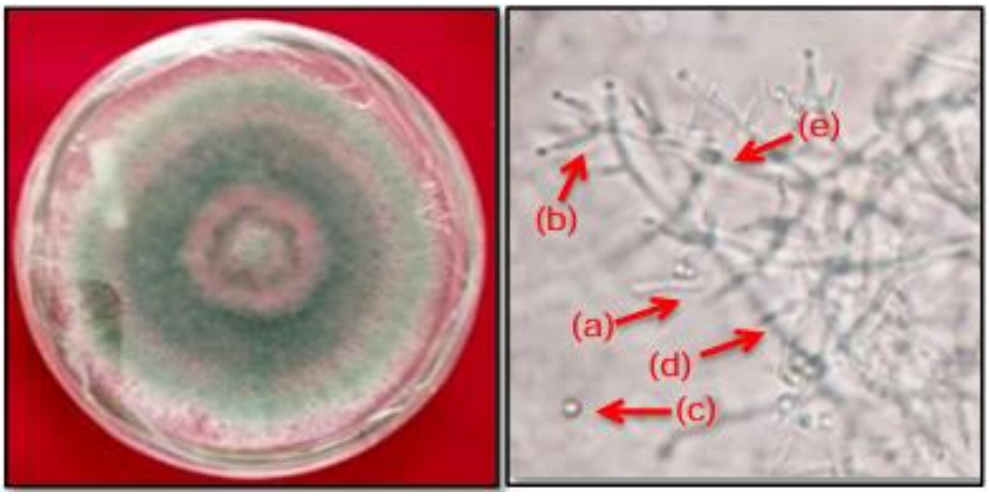

Fig 6. Characteristics of *T. koningii* TE2. Note: (a) Conidiophores, (b) phialids, (c) conidia, (d) hyphae, (e) chlamydiospores

Table 4. In vitro antagonistic activity of *Trichoderma* sp. against the *F. oxysporum* pathogen

Isolates	Inhibition (%)						
	1	2	3	4	5	6	7
T. harzianum TE1	2.78	13.06	39.97[a]	42.62[a]	54.60[a]	56.82[a]	57.78[b]

T. hamatum TH2	5.56^{tn}	14.93^{tn}	25.54^{b}	34.77^{ab}	41.85^{b}	47.28^{b}	69.46^{a}
Trichoderma sp. TP1	2.78	8.98	24.13^{b}	29.78^{bc}	42.00^{b}	49.29^{ab}	64.15^{ab}
T. koningii TE2	3.80	10.31	21.45^{b}	24.07^{c}	31.54^{b}	33.22^{c}	35.16^{c}

Note: Numbers followed by the same letter in the same column are not significantly different according to the Tukey test with a P-value of 0.05.

The results of the inhibition test showed that there was a difference in the inhibition of each isolate. This difference is thought to be caused by the ability of each isolate. In addition, based on the results of the inhibition test, it can be seen that there was an increase in inhibition from the first day to the seventh day. This is because the longer *Trichoderma* sp. experiences growth, and also because of the presence of certain compounds produced by *Trichoderma* sp. [27–29]. *Trichoderma* sp. is reported to be able to produce enzymes that can degrade the growth of pathogens [30–33]. Other studies have reported that *T. harzianum* is reported to be able to play a role as a plant disease controller [34, 35]. *T. hamatum* can play a role in controlling the pathogen *F. oxysporum*, which causes root rot disease in shallot plants. [3]. In addition, *Trichoderma* sp. is reported to act as a plant growth stimulant [11, 12, 36–39].

IV. CONCLUSION

The isolation results obtained four isolates of *Trichoderma* sp. were isolated from the vegetation of palm oil plants, rubber plants, and pepper plants, exhibiting morphologically and microscopically distinct characteristics. Based on the morphological and microscopic characters, the TP2 isolate was suspected to be *Trichoderma* sp., the TH2 isolate was identified as *T. hamatum*, the TE1 isolate was assumed to be *T. harzianum*, and the TE2 isolate was thought to be *T. koningii*. The results of the antagonist test showed that the *Trichoderma* sp. isolates could act as biological controllers, as shown by the inhibition antagonism activity ranging from 35.16% to 69.46%. These findings indicated that the indigenous *Trichoderma* isolates had promising potential as biological control agents and could be further developed for field application to manage moler disease in shallots effectively.

V. ACKNOWLEDGMENTS

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