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The Effectiveness of Liquid *Trichoderma harzianum* Application in Enhancing Tomato Growth (*Solanum lycopersicum* L.) and Suppressing *Fusarium* Wilt Disease

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ABSTRACT
Trichoderma is used as a biological control agent, this fungus has microparasitic and antibiotic properties against soil-borne pathogens. This study aims to investigate the impact of *Trichoderma* on tomato growth and the progression of *Fusarium* wilt disease infection. The method used was the Complete Randomized Design (CRD) method. Data from the observation of the intensity of *Fusarium* wilt disease attack on tomato plants with treatments (P1, P2, P3, P4, P5) showed no significant differences. The treatment of *Trichoderma* on plant height, number of leaves, number of flowers, number of fruits, and intensity of attack did not have a significant effect in suppressing the development of *Fusarium* wilt disease infection in tomato plants. *Trichoderma* application treatment that had the highest effect on tomato plant growth was found in treatment P4 (tomato seedlings soaked for 25 minutes using *Trichoderma*). The treatment with the lowest intensity of *Fusarium* wilt attack was found in treatment P5 (*Trichoderma* incubation in the soil 10 days before planting), showing a better reduction in wilt intensity compared to other treatments.

Keywords: Fungi, Antagonist, In vivo

INTRODUCTION
Fusarium oxysporum is a highly detrimental soil-transmitted fungus that damages root, stem, and leaf tissues, causing yellowing and wilting of tomato plants (Simamora et al., 2021). Wilt diseases caused by *F. oxysporum* can cause reduced yields and economic losses in vegetable crops, especially tomatoes, in Jordan and globally (Hanan Aref, 2020). In India, postharvest losses of tomatoes can reach 17.26% (Gálvez & Palmero, 2022). *Fusarium* fungi have the potential to produce toxins that are harmful to humans and animals if infected plants are consumed (Awuchi et al., 2021). The pathogen *F. oxysporum* has many hosts so it is categorized as a pathogenic fungus (Jangir et al., 2021). *Fusarium* pathogens can survive for long periods of time in soil without a host (Nikitin et al., 2023). According to Lahati & Erwin (2022), the symptoms of wilt disease are characterized by yellowing and falling leaves, and wilting so that the plants are easily uprooted due to root rot and stunted growth. The mode of action of the pathogen can be through conidia or spores from fungi exposed to a favorable environment, such as through infected soil or contaminated agricultural tools (Ekwomadu & Mwanza, 2023). Various efforts are made by farmers to address the problem of wilt disease through the use of synthetic pesticides. However, the use of synthetic pesticides has side effects on

the environment and human health (Tudi et al., 2022). Pesticide poisoning is a global public health issue and causes nearly 300,000 deaths worldwide each year (Sabarwal et al., 2018). In Brazil, Paraná, with a population of 11 million people, contaminated from drinking water due to excessive pesticide use and can increase the risk of cancer (Panis et al., 2022). In Nepal, 27% of the 5,754 cases of pesticide poisoning were recorded in 2019 (Dabholkar et al., 2023). The application of pesticides on wilt disease will lead to the development of resistance to pathogens.

One way to reduce the negative impact of pesticide use is by utilizing antagonistic fungi. Antagonistic fungi are fungi that can inhibit the growth of pathogenic fungi (Mustofa & Hastuti, 2024). One of the antagonistic fungi is *Trichoderma*. This *Trichoderma* fungus is microparasitic and antibiotic against soil-borne pathogens (Dutta et al., 2023). The mechanism of action of *Trichoderma* involves antagonistic mechanisms carried out through competition and antibiosis, which cause lysis in pathogenic fungi (Abbas et al., 2022). In Egypt, the use of *Trichoderma* as a biological control agent can reduce Fusarium wilt disease with an inhibition rate of 61.2% after 10 days of inoculation and reduce colony growth by up to 78.0% (Awad-Allah et al., 2022). According to Sehim (2023), the use of *Trichoderma* can act as a fertilizer by enhancing plant growth. In Greece, the use of *Trichoderma* can inhibit pathogen growth and enhance plant growth, such as plant height and stem weight (Natsiopoulos et al., 2022).

Based on research conducted by Arsyadmunir (2024) at Trunojo University, Madura, the use of *Trichoderma* in powder form to control downy mildew in corn has an inhibition rate of 42.3% and disease severity of 44.99%. However, research on the effectiveness of liquid *Trichoderma* is still limited, especially in direct application on tomato plants infected with *F. oxysporum*. Therefore, this study aimed to determine the effect of liquid *Trichoderma* application on tomato plant growth and the development of Fusarium wilt disease infection.

MATERIAL AND METHOD

The research was conducted from June to October 2023 in the research experimental field and the Phytopathology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Sriwijaya University.

The method used in the research employs the Completely Randomized Design (CRD) method which consists of 5 treatments with 5 replications, resulting in 30 experimental plant units with a plant spacing of 50x50 cm. The treatments tested are:

P1 = Control
P2 = Application of *Trichoderma* at the time of planting
P3= *Trichoderma* incubation without fertilizer 10 days before planting
P4 = Tomato seedlings soaked for 25 minutes using *Trichoderma*
P5 = Incubation of *Trichoderma* in the soil 10 days before planting

Preparation

The preparation of the planting medium was done using non-sterilized soil that had been supplemented with manure as initial nutrition. The seeds used are the Karuna tomato variety, which is sown in trays measuring 35x 27x6 cm with 90% soil. After 2-3 weeks and having 4-5 leaves, the seedlings are transferred to polybags containing planting media. Watering is done in the morning and evening if it doesn't rain, while weeding is done once a week or when weeds grow around the polybags. For research purposes, PDA (Potato

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Dextrose Agar) media was prepared by dissolving 20 grams of instant PDA in 500 mL of aquadest, heated, sterilized in an autoclave at 121ºC, then antibiotics were added and poured into petri dishes spread evenly.

The propagation of *F. oxysporum* isolates begins with the exploration of fungi from plants showing disease symptoms. The symptomatic parts of the plant were cut and sterilized using sodium hypochlorite and alcohol and then rinsed with distilled water. The samples were planted on PDA media in a Laminar Air Flow and incubated for 7 days before being identified using a microscope. The isolate was then subcultured on PDA media for 7-10 days to ensure there were no contaminants. Dilution was performed by adding aquadest to the culture, homogenized using a shaker for 3 minutes, and then further diluted to the desired volume. The calculation of spore density was performed using a hemocytometer with a 40x lens magnification. 4×10^6 Implementation Observations were conducted by measuring the height of the plants from the beginning of planting until the generative phase, as well as recording the number of leaves, flowers, and fruits each week. The intensity of Fusarium wilt disease was also observed based on a severity scale (0-4), which indicates the level of plant damage due to the infection. This measurement aims to determine the effect of *Trichoderma* application on the growth and resistance of tomato plants to *F. oxysporum*. The severity of the disease is calculated using the formula.

$$I \frac{\sum(nxv)}{Z\ x\ N}\ x\ 100\%$$

The scoring system used is based on research (Jamil, 2021).

Table 1. Scoring Table

Scale	Crop Damage
0	Healthy plants show no symptoms.
1	1-20% of the leaves are temporarily wilted
2	21-40% of the leaves are temporarily wilted
3	41-60% of the leaves are temporarily wilted
4	61-100% of the leaves show permanent wilting

RESULT AND DISCUSSION

The *Fusarium* isolates used on the petri dish have the macroscopic morphological characteristic of colonies that are yellowish-white, and microscopically, it has crescent-shaped conidia with 3-4 septa. The *Trichoderma* isolate used has the macroscopic morphological characteristic of green colonies with white edges, and microscopically, it has conidia with a specific shape.

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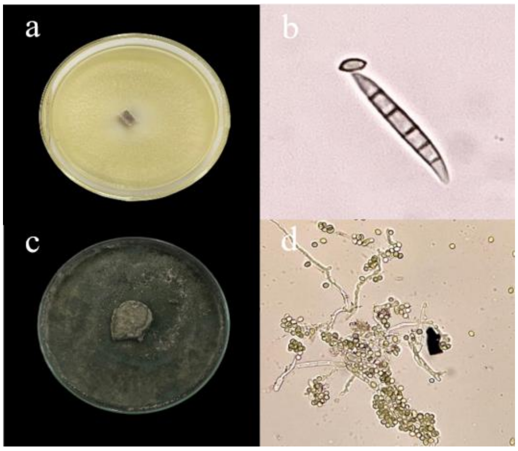

Figure 1. Isolat *Fusarium oxysporum* (a), konidia *Fusarium oxysporum* (b), Isolat *Trichoderma*. (c), konidia *Trichoderma* (d).

Fusarium wilt disease on tomato plants can cause the plants to wilt. The secondary symptoms of wilt disease attack start from the oldest or lowest leaves. Leaves affected by *Fusarium* wilt can exhibit symptoms characterized by yellow to brown leaves, causing temporary wilting to permanent wilting (Figure 2a). The primary symptoms of the wilt disease attack are found on the inner part of the stem, where cross-sectioning reveals black lesions resembling rings (Figure 2b).

Figure 2. Secondary symptoms of *Fusarium* wilt attack (a), primary symptoms of *Fusarium* wilt attack (b).

The research results show that the treatment with *Trichoderma* does not have a significant effect on the intensity of wilt disease. Wilting symptoms appeared in the fourth week of observation in the P4 treatment (tomato seedlings soaked with *Trichoderma*). The treatment with the highest wilt disease intensity was found in treatment P1 (Control) with an average of 65.67. Meanwhile, the treatment with the lowest wilt disease intensity was found in treatment P5 (Incubation of *Trichoderma* in the soil 10 days before planting) with an average of 25.70.

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Table 2 . The intensity of wilt disease in tomato plants observed from week 1 to week 7

Treatment	The intensity of the wilt disease in tomato plants observation to…(%						
	1	2	3	4	5	6	7
P1	0	0	0	0	10,86±0,43	24,91±6,56	65,67±13,08
P2	0	0	0	5,50 ±4,92	20,20±9,46	32,23±15,41	49,62±13,39
P3	0	0	0	0	9,49±0,76	12,04±0,33	26,20±6,79
P4	0	0	0	0	7,47±1,69	18,26±3,06	33,91±4,63
P5	0	0	0	0	9,18±0,08	20,26±3,57	25,70±6,88
F hitung	-	-	-	1^{ns}	$1{,}11^{ns}$	$0{,}75^{ns}$	$2{,}49^{ns}$
F tabel	-	-	-	2,87	2,87	2,87	2,87
BNJ 5%							

Notes: ns = not significantly different, * = significantly different; P < 0.05

The research results show that treatment with *Trichoderma* does not have a significant effect on plant height. In the treatment with *Trichoderma* from observations 1-7, the treatment with the highest plant height was obtained in treatment P4 (tomato seedlings soaked for 25 minutes using *Trichoderma*) with the highest average of 77.00, while the treatment with the lowest plant height was obtained in treatment P1 (Control) with an average of 69.20. In the observation from days 5-7, the height of the plants increased slightly and even experienced a decrease. This is because the tomato plants have entered the generative phase, marked by the presence of flowers and fruits.

Table 3. Height of tomato plants observed from weeks 1 to 4

Perlakuan	Height of tomato plants observed from week 1 to..4±SE			
	1	2	3	4
P1	18,80±1,04	28,40±1,73	46,60±3,00	57,60±4,22
P2	20,80±0,87	27,20±0,95	42,80±2,27	62,50±2,25
P3	19,60±1,00	27,60±2,13	48,40±3,76	63,60±6,49
P4	17,40±0,61	26,80±1,99	48,40±3,65	64,40±5,72
P5	18,20±0,44	27,20±1,63	50,00±3,79	63,00±3,76
F hitung	$1{,}97^{ns}$	$0{,}10^{ns}$	$0{,}49^{ns}$	$0{,}79^{ns}$
F tabel	2,87	2,87	2,87	2,87
BNJ 5%	-	-	-	-

Notes: ns = not significantly different, * = significantly different; P < 0.0

Table 4. Height of tomato plants observed from weeks 5-7

Treatment	Height of tomato plants observed from week 5 to 7 .. ±SE		
	5	6	7
P1	67,20±3,69	68,60±4,92	69,20±5,05
P2	74,00±4,68	74,50±4,30	74,00±4,21
P3	70,00±7,04	70,20±6,38	72,40±5,47
P4	76,40±4,66	77,00±3,76	77,00±3,44
P5	67,80±5,35	71,00±4,06	70,20±4,06
F hitung	$0{,}69^{ns}$	$0{,}74^{ns}$	$0{,}80^{ns}$
F tabel	2,87	2,87	2,87
BNJ 5%	-	-	-

Notes: ns = not significantly different, * = significantly different; P < 0.05

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The research results show that the treatment with *Trichoderma* did not have a significant effect on the number of leaves. The treatment with the highest number of leaves was found in treatment P5 (Incubation of *Trichoderma* in the soil 10 days before planting) with an average of 114.80, while the treatment with the lowest number of leaves was found in treatment P1 (Control) with an average of 84.00. In observations 1-5, the number of leaves continued to increase, but in observations 6-7, the number of leaves decreased because the tomato plants had entered the generative phase and the older leaves were showing symptoms of *Fusarium* wilt disease.

Table 5. Number of tomato leaves observed from weeks 1 to 4

Treatment	Several tomatoes leaves observation.. ±SE			
	1	2	3	4
P1	23,20±1,18	39,00±2,45	58,40±2,22	93,60±6,59
P2	24,60±1,04	34,60±2,66	60,20±6,97	99,75±7,44
P3	27,00±1,23	40,40±1,59	69,60±7,35	95,00±11,21
P4	25,20±1,97	40,00±2,12	75,80±4,28	109,20±7,25
P5	25,60±0,83	43,00±1,02	70,80±6,70	116,20±7,53
F hitung	0,87ns	1,77ns	1,13ns	1,15ns
F tabel	2,87	2,87	2,87	2,87
BNJ 5%	-	-	-	-

Notes: ns = not significantly different, * = significantly different; P < 0.0

Table 6. Number of tomato leaves observed from weeks 5 to 7

Treatment	Several tomatoes leaves observation.. ±SE		
	5	6	7
P1	106,20±7,19	105,00±5,24	84,00±6,57
P2	120,00±12,74	122,50±12,62	106,75±15,07
P3	104,00±15,18	105,00±13,13	94,00±12,47
P4	146,20±8,35	131,60±10,17	113,40±12,58
P5	134,60±9,20	135,80±9,82	114,80±10,02
F hitung	1,37ns	1,03ns	0,87ns
F tabel	2,87	2,87	2,87
BNJ 5%	-	-	-

Notes: ns = not significantly different, * = significantly different; P < 0.0

The research results show that the treatment with *Trichoderma* did not have a significant effect on the number of fruits. The treatment with the highest number of fruits was obtained in treatment P3 (*Trichoderma* incubation without fertilizer 10 days before planting) with an average of 6.00. Meanwhile, the treatment with the lowest number of fruits was obtained in treatment P2 (Application of *Trichoderma* at the time of planting) with an average of 2.75.

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Table 7. The number of tomato plant fruits observed from weeks 1 to 7

Treatment	Several tomato fruits observation... ±SE						
	1	2	3	4	5	6	7
P1	0	0	0	1,00±0,57	3,00±0,40	3,40±0,36	4,20±0,18
P2	0	0	0	0,00±0,00	2,50±0,22	3,50±0,92	2,75±0,37
P3	0	0	0	0,80±0,52	5,80±1,80	5,80±1,73	6,00±1,81
P4	0	0	0	0,40±0,22	3,40±0,88	4,80±0,59	5,40±0,46
P5	0	0	0	1,40±0,36	5,20±1,04	5,00±0,49	5,60±0,67
F hitung	-	-	-	$2{,}21^{ns}$	$1{,}42^{ns}$	$1{,}24^{ns}$	$2{,}21^{ns}$
F tabel	-	-	-	2,87	2,87	2,87	2,87
BNJ 5%	-	-	-	-	-	-	-

Notes: ns = not significantly different, * = significantly different; P < 0.05

The research results show that the treatment with *Trichoderma*did does not have a significant effect on the number of flowers. The treatment with the highest number of flowers was obtained in treatment P4 (Tomato seedlings soaked). For 25 minutes using *Trichoderma*). with an average of 21.00. Meanwhile, the treatment with the lowest number of flowers was found in the P1 (Control) treatment with an average of 14.80. Table 8.

Table 8. The number of tomato plant flowers observed from weeks 1 to 7

Treatment	Number of tomato flowers observation... ±SE						
	1	2	3	4	5	6	7
P1	0	0	0	4,4±0,67	8,00±1,17	12,20±1,18	14,80±1,07
P2	0	0	0	4,25±0,37	8,75±0,73	13,50±0,97	16,75±0,58
P3	0	0	0	7,40±1,66	8,40±2,38	13,80±3,78	14,60±3,75
P4	0	0	0	6,20±1,31	13,20±1,37	14,80±2,37	21,00±3,85
P5	0	0	0	6,80±1,11	12,40±2,05	15,80±3,39	19,20±3,48
F hitung	-	-	-	$1{,}66^{ns}$	$1{,}60^{ns}$	$0{,}40^{ns}$	$0{,}68^{ns}$
P value	-	-	-	0,20	0,21	0,81	0,61
BNJ 5%	-	-	-	-	-	-	-

Notes: ns = not significantly different, * = significantly different; P < 0.05.

Trichoderma is an antagonistic fungus widely used as a biological control agent and plays a role in enhancing plant growth (Poveda, 2021). The research results show that the application of *Trichoderma* treatment does not have a statistically significant effect on plant growth. Nevertheless, some treatments showed improvements in several other parameters. The variable plant height with treatment P4 (seedling immersion for 25 minutes in *Trichoderma* solution) resulted in the highest average plant height compared to other treatments (Table 3&4). This is because soaking the seeds after planting can help the plants and accelerate the vegetative phase. In the variable of the number of leaves, the P5 treatment (*Trichoderma* incubation in the soil 10 days before planting) showed a better increase in the number of leaves compared to other treatments (Tables 5&6). The variable of the number of flowers and fruits (Table 8) in treatment P4 resulted in the highest number of flowers, and the highest number of fruits was found in treatment P3. This

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application of *Trichoderma* with seed soaking and soil incubation treatments can help increase plant productivity.

The research results show that the application of *Trichoderma* in suppressing *Fusarium* wilt disease did not yield significant results. However, on the P5 variable, it can inhibit *Fusarium* attacks and has the lowest intensity compared to other treatments. This is consistent with the research by Nurnawati and Hidaya (2022) that the longer the incubation period, the more effective *Trichoderma* is in inhibiting wilt disease. This is due to the competition for nutrients and food sources in the soil between *Trichoderma* and *Fusarium* (Kim & Knudsen, 2016). According to Baldoni *et al.*, (2020), the ability of *Trichoderma* to inhibit pathogen growth is due to *Trichoderma's* ability to produce chitinase enzymes. This enzyme can cause cell damage up to cell death. The growth of *Trichoderma* affects plant growth. If the environmental conditions are not.

Supportive, the *Trichoderma* applied to the plants cannot function optimally (Di Lelio et al., 2021). According to Sudantha & Suwardji (2021), *Trichoderma* can affect plant growth if the environmental conditions support the survival of pathogens. According to Wulandari dan Widyawati (2023), plants require large amounts of nutrients during every growth stage, especially in the vegetative phase such as stems and leaves. The mechanism of action of *Trichoderma* in controlling *Fusarium* disease is through competition, parasitism, antibiosis, and lysis. *Trichoderma* produces hydrolytic enzymes such as chitinase, glucanase, and cellulase. According to Liasaputri (2023), the antagonistic mechanism of *Trichoderma* is carried out by releasing toxins in the form of 1,3 such as glucanase, chitinase, and cellulase, which can inhibit the growth and development and can kill pathogens. Therefore, *Trichoderma* has been widely used in the biological control of plant diseases, including controlling *Fusarium* wilt disease caused by the pathogen *F. oxysporum* (Jamil et al., 2021).

CONCLUSION

Based on the research results, the application of *Trichoderma* on the growth of tomato plants and the intensity of wilt disease attacks did not have a significant effect. However, the treatment of *Trichoderma* on tomato growth was highest in treatment P4 (Tomato seedlings soaked for 25 minutes using *Trichoderma*.), while the lowest *Fusarium* wilt attack was found in treatment P5 (*Trichoderma* incubation in the soil 10 days before planting).

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