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Effect of *Trichoderma* sp. and Moringa Leaf Extract (*Moringa oleifera* Lam.) for Suppression of Fusarium Wilt Disease in Tomato Plants (*Solanum lycopersicum* L.)

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Abstract: *Tomato (Solanum lycopersicum L.) is a vegetable that has many benefits and various nutritional contents, so tomatoes are in great demand in Indonesia. But in its cultivation, several factors can inhibit the growth of tomato plants, one of which is fusarium wilt disease caused by the fungus Fusarium oxysporum. Controlling wilt disease using synthetic fungicides harms the environment, so other alternatives that are more environmentally friendly are needed, such as the use of botanical pesticides and antagonistic microorganisms as biofungicides. Trichoderma sp. is known to suppress the growth of F. oxysporum because it is mycoparasitic and produces antibiotic compounds. Moringa leaf extract (Moringa oleifera Lam.) is known to contain growth regulators that can increase the growth of tomato plants. This study aims to obtain the best combination of Trichoderma sp. and Moringa leaf extract to suppress the growth of F. oxysporum and increase the growth of tomato plants. The research was conducted in December-March 2024 at the Botany Laboratory, Faculty of Mathematics and Natural Science, University of Lampung using a completely randomized design (CRD) with 7 treatments and 3 replications. The results of ANOVA α = 5% proved that the in vivo results of the combination of 30% moringa leaf extract and Trichoderma sp. had a real effect on the parameters of leaf area, incubation period, disease severity, disease incidence, and chlorophyll content with the best dose found in the P_{T45K} and the P_{T45K} treatment.*

Keywords: *Fusarium oxysporum; moringa leaf extract; tomato plants; Trichoderma sp.*

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I. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the main vegetable crops that are widely cultivated in Indonesia. The high benefits of tomatoes in various fields cause the demand for quality tomatoes to always be high. Demand for tomatoes can be met if factors that can inhibit tomato growth are minimized, especially the problem of disease attack by pathogenic fungi such as fusarium wilt disease caused by *Fusarium oxysporum* f.sp *lycopersici* (Aydi *et al*., 2016). *F. oxysporum* is one of the fungi that cause wilt disease that causes inhibition of tomato plant growth. *F. oxysporum* infects plants through roots, stems, or leaves and causes death (Fadhilla *et al*., 2023). The use of pesticides to control diseases in plants can pollute the environment so the use of biological pesticides has greater prospects and potential to be developed, one of which is the application of biofungicides from antagonistic microorganisms and plant extract (Abhiram and Masih, 2018).

Trichoderma sp. is one of the antagonistic microorganisms that is widely used as a biological control agent to suppress the growth of plant pathogens because it is hyperparasitic against plant pathogens, a decomposer, and can grow quickly so that it can dominate the growth area faster than pathogenic fungi (Narayan *et al*., 2017). Other biological sources that can be utilized as biofungicides are growth regulators and secondary metabolite compounds that play an important role in increasing plant growth and disease resistance. Moringa (*Moringa oleifera* Lam.) is one of the plants that has recently been studied for its potential as a vegetable pesticide material for plant growth because it contains secondary metabolite compounds such as flavonoids, terpenoids, saponins, tannins, glycosides, 48 steroids, and phenols (Kumar *et al*., 2021).

Rahmah *et al*. (2019) prove that the provision of moringa leaf extract can increase the growth of chili plants because it contains cytokinin. Lestari and Panggeso (2022) in their research also proved that moringa leaf extract with the optimal concentration can inhibit the growth of *F. oxysporum* which causes wilt disease in tomato plants.

Considering that the application of *Trichoderma* sp. and moringa leaf extract has a dual function, namely to suppress the growth of *F. oxysporum* and increase the growth of tomato plants, the use of a combination of *Trichoderma* sp. and moringa leaf extract on tomato plants infected with *F. oxysporum* is

expected to suppress the growth of *F. oxysporum* while increasing the growth of tomato plants better than giving them separately.

II. EXPERIMENTAL PROCEDURE

1.1 Preparation of moringa leaf extract

A total of 8 kg of moringa leaves were washed using water and dried in the sun for 2 days then put in an oven at 50-60°C for 48 hours to dry. After completely dry, moringa leaves were mashed until moringa leaf flour was obtained. Extraction of moringa leaf flour was carried out using the maceration method with ethanol. A total of 500 g of moringa flour was macerated with 96% ethanol as much as 5000 ml. Stirring of moringa flour was carried out for 24 hours to separate the residue and filtrate. The filtrate or extraction solution was filtered with filter paper. Moringa leaf extract was then evaporated at 40-50°C and obtained a thick extract (El-Mohamedy and Abdalla, 2014). The thick extract was then diluted to obtain the desired concentration of 30% by dissolving 30 g of moringa leaf thick extract with distilled water until the volume reached 100 ml.

1.2 Propagation of *Trichoderma* sp. on rice medium

The rice was weighed as much as 700 g and then steamed for 30 minutes until the rice was half cooked. The steamed rice was then put into a heat-resistant plastic bag and sterilized in an autoclave at 121°C with a pressure of 1 atm for 60 minutes. After the sterilization process is complete, the plastic bag containing the rice is removed from the autoclave and cooled (Novianti, 2018). If the media is too wet, perforate the plastic bag to reduce the amount of water. The *Trichoderma sp.* inoculum used was incubated for 7 days and had a spore density of 10^6 . 10 ml of *Trichoderma* sp. inoculum with a density of 10^6 was put into a plastic bag containing 700 g of rice. The fungus in the rice media was then incubated for 14-21 days until the entire rice media was covered by the greenish color of the fungal hyphae (Prasetyawati and Dania, 2017).

1.3 Inhibition test of *Trichoderma* sp. against *F. oxysporum* growth *in vitro*

The growth inhibition test was carried out by pouring 10 ml of PDA media into a petri dish. After solidifying, *F. oxysporum* was inoculated 3 cm from the right side of the petri dish and *Trichoderma* sp. was inoculated 3 cm from the left side of the petri dish. Petri that had been inoculated with isolates of *F. oxysporum* and *Trichoderma* sp. were incubated at room temperature for one week. The calculation of inhibition is done by measuring the diameter of fungal growth using the formula (Bashir, 2016).

$$\mathrm{L} = \frac{C - T}{C} \times 100\%$$

Where L is the inhibition percentage (%), C is the growth of the pathogen in the control plate (mm), and T is the growth of the pathogen in the dual culture plate (mm).

1.4 *Trichoderma* sp. treatment test *in vivo*

The Trichoderma sp. dosage test was carried out using the method of Sopialena (2015) and Korlina *et al*. (2021) which was modified. *Trichoderma* sp. culture was applied one day before transplanting tomato seedlings by putting the inoculum at the dose according to the treatment (0, 35 g, 40 g, 45 g, and 50 g) into the planting medium in polybags. The *Trichoderma* sp. culture used as inoculum was a culture that was at least 14 days old.

1.5 Inoculation *F. oxysporum* on tomato plants *in vivo*

F. oxysporum culture used to inoculate tomato plants is a culture that has been incubated for 7 days and has a density of 10^6 (Ghufron *et al*., 2017). *F. oxysporum* was inoculated 7 days before transplanting by pouring a 10 mL suspension of *F. oxysporum* on the planting media (Soleha *et al*., 2022).

1.6 Application of moringa leaf extract

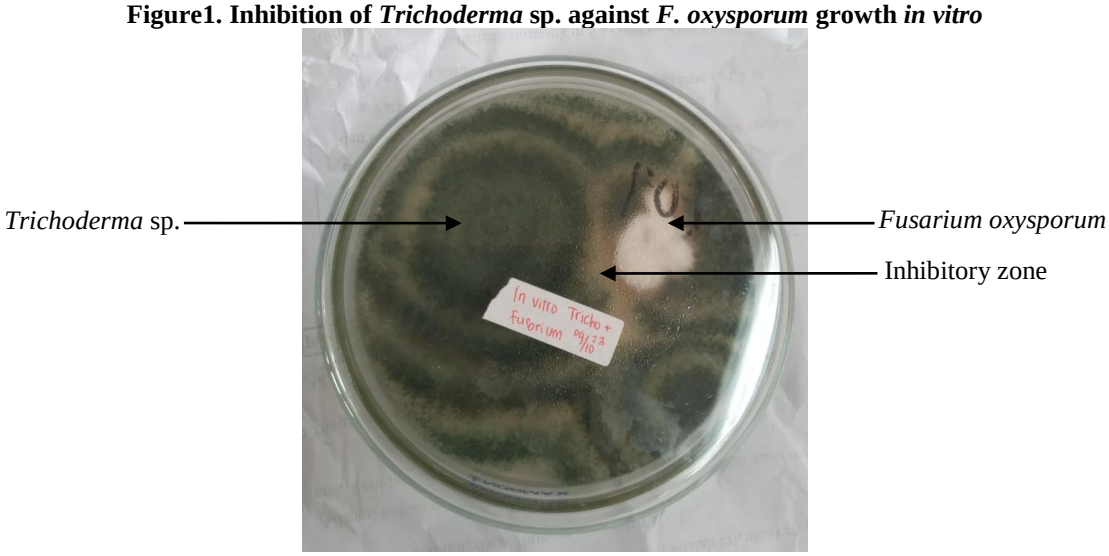
Application of moringa leaf extract was carried out following the method of El-Mohamedy and Abdalla (2014) with modifications. The application of 30% moringa leaf extract was carried out using the spray method on each part of the plant and was carried out 4 times, at 7, 14, 21, and 28 days after planting (Mare *et al*., 2023) with volume 25 ml per plant (Hoque *et al*., 2021).

III. RESULTS AND DISCUSSIONS

3.1. Inhibition test of *Trichoderma* sp. against *F. oxysporum* growth *in vitro*

The observation results of the *Trichoderma* sp. inhibition test against the growth of *F. oxysporum in vitro* can be seen in Figure 1. below.

Figure1. Inhibition of *Trichoderma* sp. against *F. oxysporum* growth *in vitro*

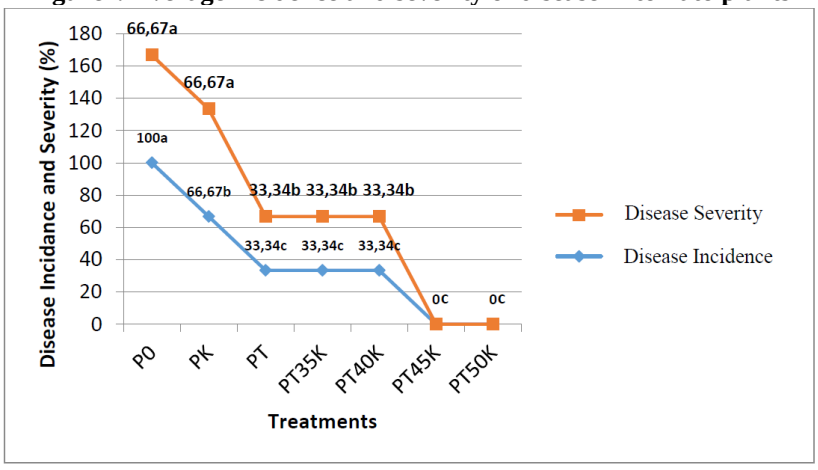


The observation in Figure 1. shows that *Trichoderma* sp. can inhibit the growth of *F. oxysporum* on PDA media. The inhibition of *F. oxysporum* by *Trichoderma* sp. is evidenced by the inhibition zone and the average growth diameter of *Trichoderma* sp. which is wider than *F. oxysporum*, which is 9 cm while the growth diameter of *F. oxysporum* is 2 cm with a percentage of inhibition 77.78%. These results are in accordance with the results of research by Abhiram and Masih (2018) which prove that the inhibition zone of *Trichoderma* sp. occurs because *Trichoderma* sp. is mycoparasitic, which can inhibit the growth of pathogens through the process of hyphal entanglement so that the hyphae of *Trichoderma* sp. can penetrate the hyphae of *F. oxysporum*. The entanglement and penetration of pathogenic fungal hyphae causes changes in cell wall particles so that it can affect the permeability of the pathogen cell wall which will then cause lysis of the pathogen wall. *Trichoderma* sp. can produce antibiotic compounds such as gliotoxin, viridin, and chitinase enzymes that can cause lysis of the pathogen cell wall so that the growth of *F. oxysporum* is inhibited.

3.2. Disease incidence and disease severity

The data in Figure 2. shows that the higher the dose of *Trichoderma* sp. and moringa leaf extract is given, the more optimal it is in minimizing disease incidence and severity due to the inhibited growth of *F. oxysporum*.

Figure2. Average incidence and severity of disease in tomato plants



Based on the results in Figure 2, it can be seen the highest disease incidence was found in the P_0 at 100%, while the lowest average disease incidence was found in the P_{T45K} and P_{T50K} treatments at 0%. Based on the average value of disease severity, it is known that the highest disease severity was found in the P_0 and P_K at 66.67%, while the lowest disease severity was obtained in the P_{T45K} and P_{T50K} treatments at 0%. It can be seen that the P_{T45K} and P_{T50K} treatment has the best effect in minimizing the incidence and severity of disease.

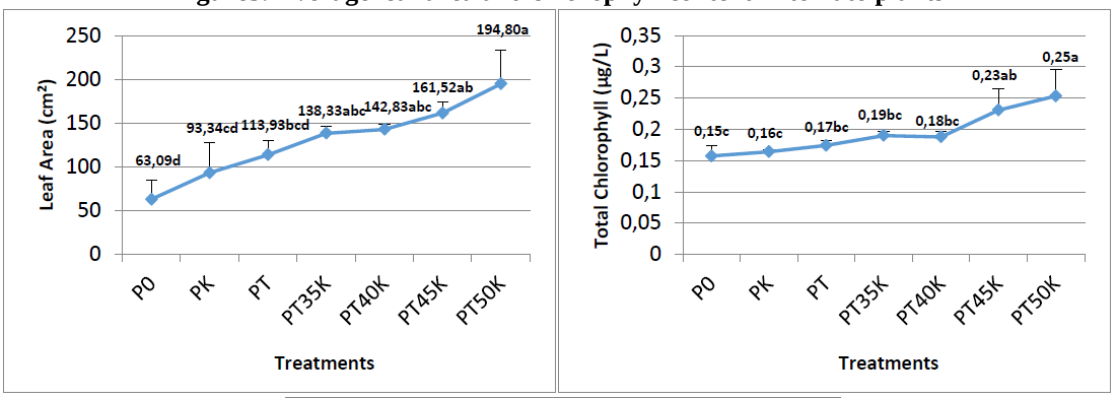
These results are in accordance with the results of research by Nahswa *et al*. (2019) which prove that the application of *Trichoderma* sp. can reduce the disease severity in tomato plants with high control effectiveness. *Trichoderma* sp. can grow faster than pathogenic fungi so that it can dominate space and nutrients that can inhibit the growth of pathogenic fungi. *Trichoderma* sp. also can use a variety of substrates to grow and produce antibiotic compounds in the form of viridin, gliotoxin, and chitinase enzymes that can inhibit the growth of pathogens by lysing the cell walls of pathogenic fungi. The toxin produced by *Trichoderma* sp. will inhibit the growth of *F. oxysporum* in the soil.

Secondary metabolite compounds contained in moringa leaf extract can also play a role in minimizing the incidence and severity of disease in tomato plants infected with *F. oxysporum*. This result is supported by the research of Ibiam *et al*. (2022) which proves that moringa leaf extract contains secondary metabolite compounds in the form of saponins, glycosides, terpenoids, alkaloids, flavonoids, steroids, phenols, and tannins which are antifungal and play a role in inhibiting the growth of *F. oxysporum*. Moringa leaf extract also contains growth regulators, namely cytokinins and macro and micronutrients that can increase plant growth. Cytokinin can increase plant growth because it plays a role in cell division and enlargement so that it can spur shoot growth. In addition, macro and micronutrients contained in moringa leaves also play a role in the process of plant growth and development. If the growth and development of plants increase, it can affect the readiness of plants to grow well and not be easily infected with disease.

3.3. Leaf area and chlorophyll content

The data in Figure 3 shows that the higher the dose of *Trichoderma* sp. and moringa leaf extract is given, the more optimal in increasing the parameters of leaf area, chlorophyll content, and dry weight of tomato plants.

Figure3. Average leaf area and chlorophyll content in tomato plants



Based on the results in Figure 3, the average leaf area, dry weight, and chlorophyll levels in tomato plants with a combination treatment of *Trichoderma* sp. 50 g and 30% moringa leaf extract gave the best effect in increasing the parameters of leaf area, dry weight, and chlorophyll levels of tomato plants. These results are in accordance with the research of Madina and Koesriharti (2023) which proves that the provision of moringa leaf extract with a maximum concentration can increase leaf area in plants. This is related to the content of nutrients and cytokinin growth regulators contained in the moringa leaf extract. The cytokinin content in moringa leaf extract plays a role in stimulating bud formation, preventing aging, and assisting in cell division and enlargement. According to Ayuningsari *et al*. (2017), the increased leaf area is the result of cell elongation and enlargement. Niknejhad and Pirdashti (2012) mentioned that cytokinins can increase leaf area by increasing the number of cells so that leaves with a larger area are produced.

The increase in plant leaf area is also related to the increase in chlorophyll content. The larger the plant leaf area, the more light will be captured and the higher the chlorophyll content produced. The high content of chlorophyll produced will increase the photosynthesis process. Batool *et al*. (2020) in their research stated that giving moringa leaf extract to plants can increase plant chlorophyll levels. This can occur because moringa leaves contain nutrients, especially N and P, which play a role in the process of chlorophyll formation needed in the photosynthesis process.

The application of *Trichoderma* sp. on plants is also thought to increase leaf area and chlorophyll levels in plants. These results are in accordance with the results of research by Azamri *et al*. (2011) which proved that the application of *Trichoderma* sp. on plants can increase leaf area and chlorophyll levels significantly. The growth of *Trichoderma* sp. in the planting media will be able to decompose organic matter in the soil so that the absorption of nutrients for plants becomes more optimal. The decomposition will release N and P nutrients that play a role in the formation of chlorophyll needed in the photosynthesis process (Cahyani *et al*., 2021).

IV. CONCLUSION

Based on the results of the study, it can be concluded that the application of *Trichoderma* sp. and moringa leaf extract has a significant effect on suppressing the growth of *F. oxysporum* that infects tomato plants *in vitro* through inhibition test parameters and *in vivo*, namely disease incidence, disease severity, leaf area, and chlorophyll content with the best dose found in the P_{T50K} treatments.

Conflict of interest

There is no conflict to disclose.

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