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Antagonistic of *Trichoderma* spp. Againts the Cause of Fusarium (*Fusarium oxysporum*) wilt on Shallots (*Allium ascalonicum* L.) *IN VITRO.*

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Abstract
Tuber rot is one of the important diseases in onion caused by Fusarium oxysporum. In Indonesia, shallot (Allium ascalonicum L.) is one of the main vegetable commodities and has many benefits. Based on data from The National Nutrient Database, shallots contain carbohydrates, proteins, minerals, sugars, and fatty acids that humans need. In its cultivation is often constrained by pests and diseases, one of them is controlling by using biological agents such as fungi that are antagonistic, for example Trichoderma spp. which has the potential to inhibit the cause of Fusarium wilt in shallot plants. This study aimed to determine the effect of Trichoderma spp isolates and their impact on the growth of F. oxysporum in vitro in onion. Exploration (Trichoderma spp) and observation (antagonist test of Trihoderma spp.) Laboratory and screenhouse experiments were designed respectively a Completely Randomized Design (CRD) with a single factor (3 treatment) repeated six times. There were 3 isolated from Trichoderma with a growth ratio of 4.5 cm/2 days. The best treatment to inhibit the growth of F. oxysporum in vitro is T1, T2, and T3. Observation parameter in this research is Fusarium oxysporum growth inhibition. The 3 types of Trichoderma were able to suppress the pathogenic isolates of Fusarium oxysporum with varying percentages and several mechanisms affecting the inhibition of Fusarium oxysporum, namely competition, antibiosis and mycoparasites. Based on the results of the overall average percentage of inhibitory power, T. harzianum, T. koningii and T. viridae were able to suppress the growth of F. oxysporum with different percentages of inhibitory power. The antagonist interaction showed that the activity of Trichoderma harzianum was very good in inhibiting the growth of Fusarium oxysporum in Vitro with the best inhibition for 3 days of incubation of 89%.
Keywords: *Biological Agents; Inhibition, Pathogenicity, antagonistic, shallot.*

INTRODUCTION

In Indonesia, shallot (*Allium ascalonicum* L.) is one of the main vegetable commodities and has many benefits. Based on data from the nutrient database, shallots contain carbohydrates, proteins, minerals, sugars, and fatty acids that humans need (Waluyo and Sinaga, 2015). Red onion is one of the featured vegetable crops that have long been intensively cultivated by farmers. Shallots belong to a group that serves as a spice seasoning and traditional medicine. National shallot production is sufficient to supply the domestic consumption, but its production fluctuates under abnormal climatic conditions (Zuleika., 2014, Howell et. al., 2004). The disease causes direct damage to the tubers and reduces tuber yield by 50% (Cherkupally, et al., 2017; Cook and Vesth, 1991; Elad, et al.,1982). The cultivation of shallots (*A. ascalonicum* L.) is often constrained by pest and disease attacks. At the begining of growth, one of the important diseases in shallot is a tuber rot caused by *Fusarium oxysporum* (Harman, et al., 2004; Havey.,1999; Jelen, et al.,2014). So far, the control of Fusarium wilt on shallots has been carried out by collecting and destroying diseased plants.

Biological control is an alternative control that can be done without having a negative imfact on the environment and its surroundings. One of them is controlling by using biological agents such as fungi that are antagonistic (Kucuk and Kivanc., 2003; Kullnig, et al.,2000; Kurniawan, et al., 2006).

Pathogen control is generally conducted by utilizing pesticides. Excessive use of pesticides has shown negative impacts such as resurgence, resistance to pests and pathogens, and the death of natural enemies (Kredics, et al.,2003; Nuria, 2009; Krishna, 2016). Currently, pests and pathogens control effort directed at the utilization of natural enemies of pets and pathogens, or better known as biological control. One of the biological control efforts is using *Trichoderma* (Ovilya, 2018 and Ratna, et al., 2014).

Trichoderma spp. has long recognized as a biological agent for controlling plant diseases and helping to promote root growth and development, productivity of plants resistance to a biotic stress as well as absorption and utilization of nutrients (Royse and Ries, 1977; Schirmbock, et al., 1994). *Trichodrma* sp. is and able to control the growth of plant pathogens. Antagonistic mechanism of *Trichoderma* sp. in inhibiting the growth of pathogenic molds, among others, competition, parasitism, antibiosis and lysis (Worasatit, et al., 1994). According to the results of research by (Siti, et al., 2006), *trichoderma* sp. can inhibit the growth of pathogens *C. capsici* with an inhibitory percentage of 68%, *fusarium sp*. with an inhibitory percentage of 53.9% and *S. rolfsii* with an inhibitory of 35.5% in vitro. According to (Wiyatiningsih, et al., 2009) reported that *Trichoderma viride* was the best species to suppress the growth of *Fusarium oxysporum* f.sp. zingiberi with an inhibitory percentage of 88%.

MATERIALS AND METHODS

Materials used in this study were *F. oxysporum* isolates, *Trichoderma* spp, PDA (Potato Dextrose Agar), sterile distilled water, and 70% alcohol. A tool used in this study were petri dish, pipette, needle ose, aluminum foil, cotton, cling wrap, Enkas, autoclave, bunsen burner, microscopes, glass beaker, tweezers, scissors, analytical balance, calipers, and a scalpel blade,

Sterilization Equipment

All the glassware used was washed with running water until clean, then dried. After drying, the apparatus which has a mouth surface was plugged with cotton. Then, all the tools were wrapped in newsprint and sterilized by autoclaving for ten minutes at 121^{o}C.

PDA Media Preperation

About 200 g of potatoes were washed thoroughly, then cut into small pieces and boiled in 1000 ml of distilled water until tender. 20 g dextrose and 20 g agar should be added and stir until blended. After boiling, the PDA then poured into the Erlenmeyer, and the mouth was plugged with cotton and wrapped with aluminum foil. Furthermore, the media was sterilized using autoclave at 2 atm, 121^{0}C for 30 minutes.

Inhibitory Power Test

Antagonism test was carried out with dual culture testing between *Fusarium* sp. with 3 types of *Trichoderma* sp. one colony of the *fusarium* sp. on 3, 5, and 7 days after application by measuring the growth diameter. Antagonist test is conducted to test the ability of antagonistic agents in inhibiting the growth and development of plant pathogens. Pathogenic fungi were also inoculated without antagonist fungi used as control.

RESULTS AND DISCUSSION

Results

Inhibition of the growth of *Fusarium oxysporum* on day 3

The result of analysis of data variance on day 3, day 5 and day 7 showed homogeneous data and giving 3 types of Trichoderma on day 3, day 5 and day 7 had a significant effect in inhibiting the growth of *F. oxysporum* on shallot plants, present in figure 1 below.

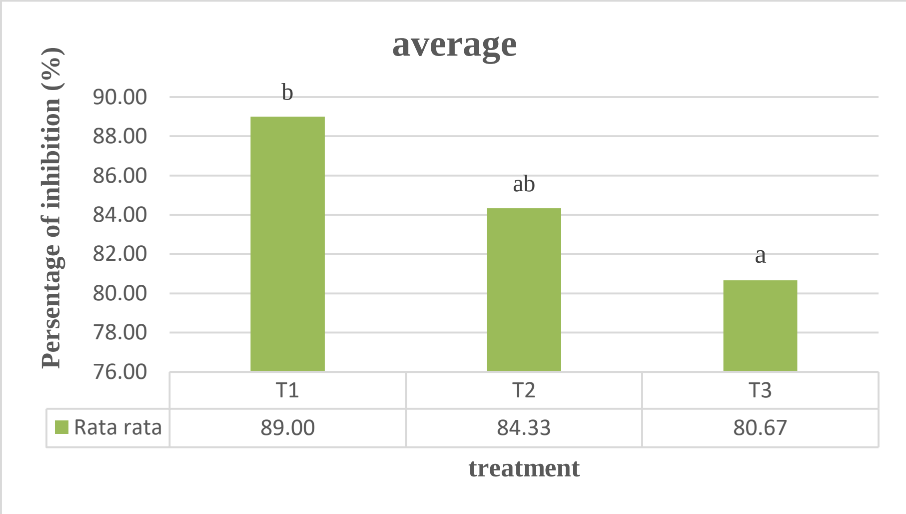


Figure 1. The average inhibition of 3 types of *Trichoderma* spp. Against *F. oxysporum* on the third day. Information: T1; *T. harzianum*, T2; *T. koningii*, T3; *T. viridae*.

Figure 1. shows the everage inhibitory power of Trichoderma sp. Against *F. oxysporum* on the third day, which can be seen in figure 4. T1 treatment had an inhibitory power 89%, T2 treatment 84,33% and T3 treatment 80,67%. T1 and T3 treatment did not show significant differences with T2 treatment, but T1 treatment was siginificantly different from T3 treatment.

The evarage inhibitory power on day 3 can be seen in the picture which show that giving of *T. harzianum* has the best inhibitory power with an evarage of 89%. *T. harzianum* is a biocontrol agent for plant pathogens (Cook and Vesth., 199)1. The antagonistic method of this fungus is by producing antibiotics and fungal cell wall degrading enzymes such as chitinase, glucanase and protease (Elad, et al., 1982).

Growth inhibition of F. oxysporum on day 5 and day 7

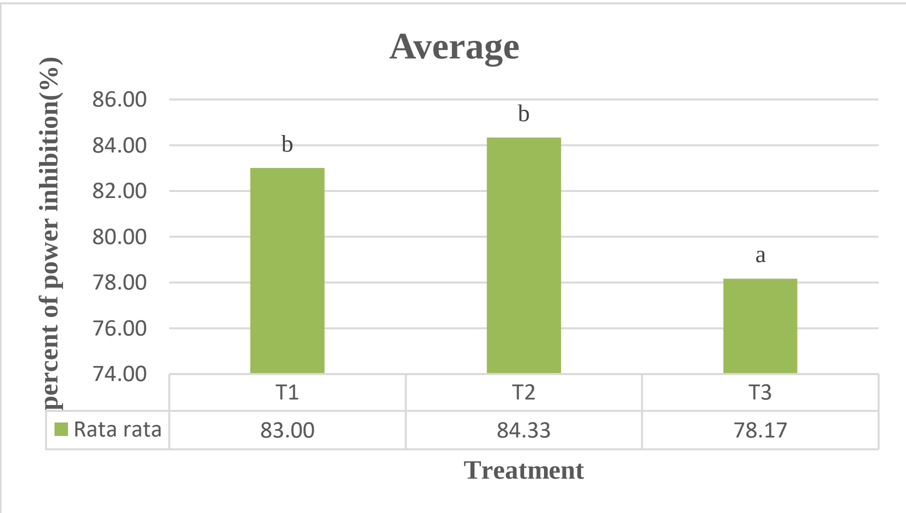


Figure 2. Average inhibibitory power of 3 types of Trichoderma sp. Against *F. oxysporum* on day 5.

The everage inhibitory power of 3 types of Trichoderma sp. Against *F. oxysporum* on day 3 can be seen in figure 4. The T1 treatment had an inhibitory power of 83%. T2 treatment was 84,33% and T3 treatment was 78,17% Treatments T1 and T2 showed significantly from treatment T3, but treatment T1 was different from T2. The everage inhibitory power on day 5 can be seen in the picture showing that giving of *T. koningii* has the best inhibitory power with an average of 84,33%.

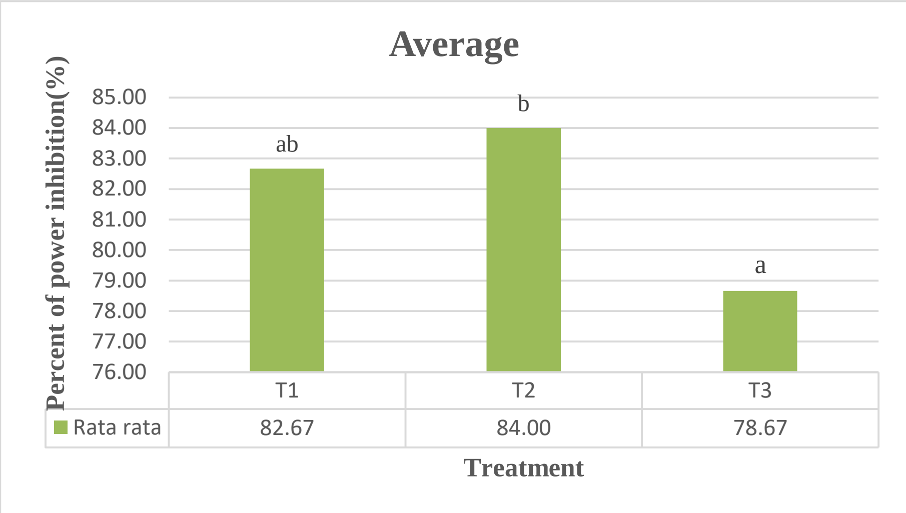
‘ 

Figure 3. Average inhibibitory power of 3 types of Trichoderma sp. Against *F. oxysporum* on day 5.

Figure 3 shows the average inhibitory power of 3 types of Trichoderma sp. Against *F.oxysporum* on the third day which can be seen in Figure 4. Treatment T1 had an inhibitory power of 82.67%, treatment T2: 84.00% and treatment T3: 78 .67%. Treatment T2 and treatment T3 were not significantly different from treatment T1, but treatment T2 was significantly different from treatment T3. The average inhibitory power on day 7 can be seen in the picture which shows that giving of *T. koningii* has the best inhibitory power with average of 84.00%. The best percentage in inhibiting the growth of *F. Oxysporum* on days 5 and 7 was owned by *T. koningii* isolates, compared to *T. harzianum* and *T. viride*. The *T. koningii* isolate had the best ability to inhibit *F. oxysporum* where on day 3 and day 5 the *T. koningii* isolate had an inhibitory percentage of 84.33% while on day 7 it had an inhibitory percentage of 84.00%. The antagonistic mechanism of *T. koningii* as a biological agent is related to its ability to produce 6-pentyl alpha pyrone which is an inhibitory compound and trichokonins compounds which have antimicrobial activity (Kurniawan, et al., 2006).

Discussion

F. oxysporum is a soil-borne fungus that causes rot at the base of plant stems. *F. oxysporum* has a wide host range, one of which is onion plants. Root rot disease caused by *F. oxysporum* f. specialist. cepae is a disease that is often found in onion plants that grow throughout the world (Cramer., 2000). Control of *F. oxysporum* in the field is carried out by collecting and destroying infected plants. Apart from that, control is carried out by spraying chemical fungicides, continuous use can cause residue in the environment. So it is necessary to carry out controls that do not leave residue in the environment. One of them is the use of biological agents in the form of antagonistic fungi such as Tricohderma spp. which is known as an antagonistic agent capable of suppressing Fusarium sp (Harman, et al., 2004).

The antagonist test is the first step to determine the inhibitory power of antagonist agents in suppressing and inhibiting the growth of pathogens. In this research, an antagonist test was carried out using the Dual culture method or double test to determine the inhibitory power of 3 types of Trichoderma isolates against *F. oxysporum* on shallot plants. Fusarium control is used as a comparison where fusarium growth occurs in vitro without the presence of Trichoderma. The 3 types of Trichoderma sp. used are *T. harzianum*, *T. koningii* and *T. viridae*. According to (Cherkupally, et al., 2017), Trichoderma sp. is one type that is often found on all types of soil and in various habitats. Trichoderma sp. It is used as a biological agent to control soil-borne pathogens and has become an important concern in the last few decades due to its ability to act as a biological control against several plant pathogens.

F. oxysporum antagonist test with 3 types of Trichoderma and growth of control *F. oxysporum* was incubated for 7 days at room temperature 33-34^{o}C. Observations of the antagonist test for 3 types of Trichoderma against *F. oxysporum* were carried out on day 3, day 5 and day 7. Sivan, and Chet, 1986 State that temperature is one of the factors that can change the antagonistic potential of biocontrol agents against pathogens. Previous studies showed the effect of temperature on the growth and antagonistic activity of

Trichoderma spp. against various pathogens. Stated that (Soesanto, et al., 2013) the optimum temperature for the growth of Trichoderma sp. ranges from 15-35°C. The antagonistic effect in the dual culture test or double test can be seen visually in Figure 4. The results show that isolates of *T. harzianum*, *T. koningii* and *T. viridae* are able to suppress the growth of *F. oxysporum* mold in the dual culture method or test double. In accordance with research by, it shows that the Trichoderma sp. is proven to be superior because its growth is faster than *F. oxysporum* f.sp melongenae. This is related to differences in levels of hydrolytic enzymes produced by each type of Trichoderma spp.

The difference in growth speed between Trichoderma types was visible at the beginning of the observation, namely on day 3 at a room temperature of 33°C. On day 5 at a room temperature of 33°C Trichoderma grew spread on PDA media and began to inhibit the growth of *F. oxysporum*. On day 7 at a room temperature of 34°C Trichoderma filled the PDA medium and inhibited the growth of *F. oxysporum*. *F. oxysporum* experienced growth stagnation on day 5 and day 7, thought to be the result of growth and antagonistic mechanisms by Trichoderma. Castle, et al., (1998) stated that optimum temperature regulation for fungal growth is related to the formation of special structures for survival. For example, conidium formation in Trichoderma sp. which is a specialized structure for reproduction. In addition, temperature influences the metabolite compounds produced, including enzymes, which determine the distribution of fungal species in nature. The mechanism thatstate by Sriwati, (2017), can be seen macroscopically in this research is the competition mechanism which is shown by the growth speed of Trichoderma resulting in less availability of nutrients and growing space for pathogens. Trichoderma itself has a competition mechanism in getting nutrients and living space.

The difference in growth speed between the 3 types of Trichoderma was seen on the 3rd day after incubation at room temperature 33°C. It was known that the growth of *T. harzianum* was faster than *T. koningii* and *T. viridae*. On the 5th and 7th day after incubation at room temperature 33°C-34°C the growth of *T. harzianum* and *T. viridae* slowed down compared to the growth of *T. koningii* (Sriwati., 2017), differences in mycelium growth speed of Trichoderma spp. due to changes in the results of metabolites that should be used to produce fungus organs, switching to producing other secondary metabolites. Apart from that, due to the different subcultures between Trichoderma sp., the more frequently subculturing is carried out, the more likely it is to change the growth rate and the lower the ability of Trichoderma to inhibit the growth of pathogens.

In the antagonist test between Trichoderma and *F. oxysporum*, an inhibition zone or clear zone was formed. The inhibition zone or clear zone is the length of the area between Trichoderma and *F. oxysporum* in a petri dish that is not grown by both isolates. The formation of an inhibitory zone or clear zone between *T. harzianum*, *T. koningii* and *T. viridae* and *F. oxysporum* can be thought to be the result of an antibiosis mechanism. The working of the antibiosis mechanism is strengthened by suppressing the growth of pathogenic fungi on PDA media. Trichoderma has the ability to produce secondary compounds, antibiotics and enzymes that break down fungal cell walls. According to Nuria., 2009, the antibiosis mechanism of Trichoderma spp. This is the ability of Trichoderma to produce secondary compounds such as viridin, trichomidine, and gliotoxin which are usually combined with cell wall degrading enzymes so that they are able to penetrate pathogenic hyphae with antibiosis properties. Previous research showed that before fungal mycelia interact, Trichoderma sp produces low amounts of extracellular exocytination (Kullnig, et al., 2000).

Observations on day 7 of the Trichoderma antagonist test with *F. oxysporum* showed that *T. koningii* hyphae were attached or contacted to *F. oxysporum* hyphae after the formation of an inhibitory zone between the two microorganisms. According to (Kucuk and Kivanc., 2003), the occurrence of hyphae attachment is the third stage of the mycoparasitism interaction pattern possessed by Trichoderma spp. The first stage of the mycoparasitism interaction pattern between Trichoderma spp. with pathogenic fungi is chemotropic growth, where at this stage there is a process of chemical stimulation of the host against antagonistic fungi. The second stage is recognition, which occurs in some cases and is specific so that the antagonistic nature of Trichoderma spp. only effective against certain pathogenic fungi. The fourth stage is the breakdown of the pathogen cell wall, related to the enzymes produced. The ability of Trichoderma spp. in producing cell wall degrading enzymes (chitinolytic, cellulolytic and glucanolytic) its capabilities have been researched and tested.

Based on the results of the data analysis carried out, it was found that there were differences in the percentage of inhibitory power of *T. harzianum*, *T. koningii* and *T. viridae* on the growth of *F. oxysporum* on day 3, day 5 and day 7. On day 3, Trichoderma antagonist testing with *F. oxysporum* It is known that the average percentage of inhibition of *T. harzianum* on the growth of *F. oxysporum* is 89.00%. The average percentage of inhibition of *T. koningii* on the growth of *F. oxysporum* was 84.33%. The average percentage of inhibition of *T. viridae* on the growth of *F. oxysporum* was 80.67%.

Observations on day 5 showed that the average percentage of *T. harzianum* inhibition on *F. oxysporum* growth was 83%. The average percentage of inhibition of *T. koningii* on the growth of *F. oxysporum* was 84.33%. The average percentage of inhibition of *T. viridae* on the growth of *F. oxysporum* was 78.17%. On the 7th day of observation, it was discovered that the average percentage of inhibition of *T. harzianum* on the growth of *F. oxysporum* was 82.67%. The average percentage of inhibition of *T. koningii* on the growth of *F. oxysporum* was 84.00%. The average percentage of inhibition of *T. viridae* on the growth of *F. oxysporum* was 78.67%.

The best percentage in inhibiting the growth of *F. oxysporum* on day 3 belonged to *T. harzianum* with an inhibition percentage of 89%, followed by *T. koningii* with an inhibition percentage of 84.33%. According to (Cook and Vesth., 1991), *T. harzianum* is a biocontrol agent for plant pathogens. The antagonistic method that this mold has is by producing antibiotics and enzymes that break down fungal cell walls such as chitinase, glucanase and protease (Elad, et al., 1982). From several studies, *T. harzianum* showed chitinase and gluconase activity when tested with *Sclerotium rolfsii* (Elad, et al., 1982), *F. oxysporum*, *Rhizoctonia solani* (Kumar, 2013) and *Botrytis cinerea*. The production of hydrolytic enzymes is influenced by growing conditions and the pathogen host (Sulistiyono, 2015).

The best percentage in inhibiting the growth of *F. oxysporum* on days 5 and 7 belonged to the *T. koningii* isolate, where this isolate had more ability to inhibit the growth of *F. oxysporum* compared to *T. harzianum* and *T. viridae*. The *T. koningii* isolate had a constant inhibitory value against *F. oxysporum*, which on day 3 and day 5 had an inhibitory percentage of 84.33%. however, on day 7, the percentage of inhibition decreased by 84.00%. The antagonistic mechanism of *T. koningii* as a biological agent is related to its ability to produce 6-pentyl alpha pyrone which is an inhibitory compound and trichokonins compounds which have antimicrobial activity. According to (Schirmbock, et al.,1994), the compound 6-pentyl alpha pyrone denatures proteins, disrupts the lipid layer and causes cell wall damage. Apart from that, according to (Jelen, et al., 2014), 6-pentyl alpha pyrone is a compound that plays a role in inhibiting the *F. oxysporum* pathogen. 6-PAP has a role in fighting pathogenic molds by reducing deoxynivalenol produced by *F. oxysporum*.

In the antagonist test at a room temperature of 33^{o}C-34^{o}C, *T. harzianum* and *T. viridae* isolates experienced a decrease in inhibitory power on day 5 and day 7. However, in *T. koningii* isolates there was a decrease in the percentage of inhibitory power on day 7. Results of research by (, stated that temperature is an important parameter for manipulating growth, sporulation and also the ability of saprophytes to produce non-volatile metabolites, involved with their ability to take up nutrients and space, mycoparatitisism and the formation of extracellular enzymes that destroy the cell walls of fungi. At a temperature of 35^{o}C the growth of Trichoderma sp. decrease. The optimal temperature for growth differs between Trichoderma isolates, although most Trichoderma strains are mesophilic (Kredics, et al., 2003).

Based on the results of the overall average percentage of inhibitory power, *T. harzianum*, *T. koningii* and *T. viridae* were able to suppress the growth of *F. oxysporum* with different percentages of inhibitory power between Tricoderma types. According to Krishna, 2016, states that every Trichoderma sp. has maximum and minimum resistance values as a biocontrol agent, beside that, the biocontrol ability of Trichoderma sp. varies depending on the pathogen and culture conditions. The antagonistic interaction showed that the activity of *T. koningii* was very good in inhibiting the growth of *F. oxysporum* in vitro for 7 days of incubation with an inhibitory percentage of 84.00%. This is in accordance with the opinion of, antagonistic fungi have a minimal inhibitory effect on attacking the growth of pathogenic fungi, if the percentage of inhibition is less than 60% of the surface of the petri dish. If the inhibition percentage is more than 60% of the surface of the petri dish, then the antagonist fungus is said to be able to attack and inhibit the growth of the pathogenic fungus optimally (Krishna, 2016).

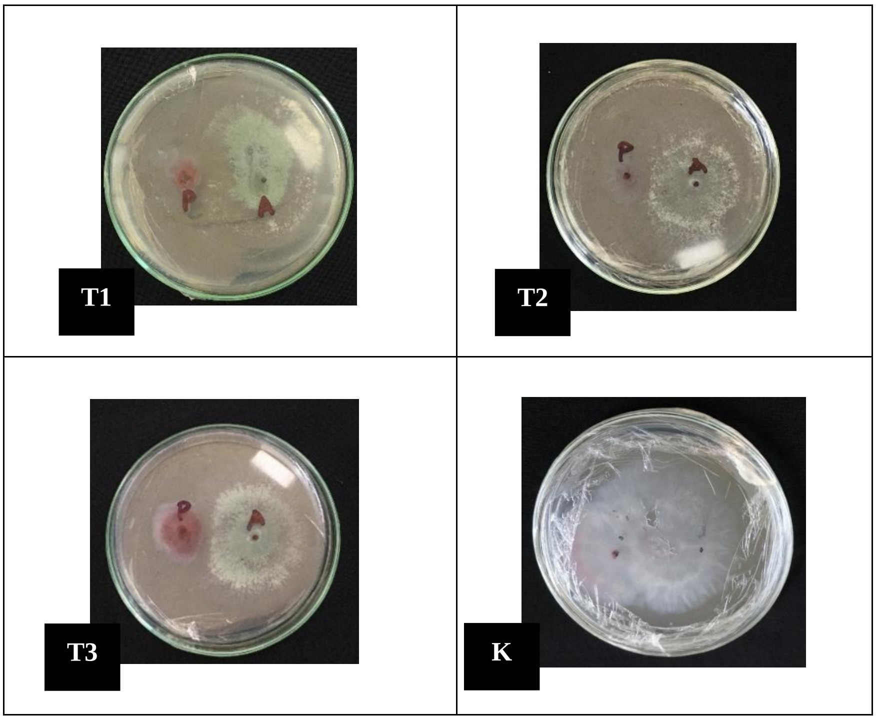


Figure 4. Antagonist test of 3 types of *Trichoderma* spp. against *F. oxysporum*. Information: T1; *T. harzianum*, T2; *T. koningii*, T3; *T. viridae*, K; *F. oxysporum* (control).

CONCLUSIONS

The 3 types of Trichoderma sp. are able to suppress *F. oxysporum* disease pathogen isolates with varying percentages and several inhibitory mechanisms for the 3 types of Trichoderma which influence the growth of the cause of fusarium wilt (*F. oxysporum*) in vitro, namely competition, antibiosis and mycoparasites. The *T. harzianum* and *T. koningii* isolate are trichoderms isolate that had the best potential in inhibiting the cause of fusarium wilt in shallot plants in vitro, with the highest inhibitory power during 3 days. The best percentage in inhibiting the growth of *F. oxysporum* on day 3 belonged to *T. harzianum* with an inhibition percentage of 89%, followed by *T. koningii* with an inhibition percentage of 84.33%.

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