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Fusarium Wilt Disease Control Using Biological Agents *Trichoderma* and *Mycorrhizaeon* Pepper

Diding Rachmawatiand Baswarsiati

Balai Pengkajian Teknologi Pertanian Jawa Timur (BPTP Jatim)
Jl. Raya Karangploso Km. 4 PO Box 188 Malang 65101

*Corresponding author
Email: didingrachmawati@gmail.com
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Abstract

Fusarium oxysporum is the main fungus disease that can wither plants, especially pepper. The fungus spread through diseased soil or already withered plants and then infect plants from its roots. Doing control by using antagonistic fungi such as Trichoderma sp. and Arbuscularmycorrhizae have been widely performed. Trichoderma sp. is a fungus rich with an antifungal activity that produce metabolites, both volatile and non-volatile. These metabolites produced by Trichoderma can diffuse through the dialysis membrane which capable to slow several pathogens growth. Mycorrhizae creates mutual symbiosis between certain types of fungi with roots, also known as biological agents, capable to control F. oxysporum on pepper and to help antibiotics formation. The study was conducted at Laboratorium Hama dan Penyakit BPTP East Java, starting from January to May 2016. This study used completely randomized design (CRD) with treatment consisted of 16 combined doses of Mycorrhizae and Trichoderma, each repeated 4 times that produce 64 test units. Mycorrhizae dose used is 0.0; 1.0; 2.0 and 4.0 grams per polybag, while the Trichoderma dose used is 0.0; 15.0; 30.0; and 45.0 grams per polybag. Data were statistically analyzed by variance analysis and followed by a BNT test of 0.05. The results showed Mycorrhizae 4 g /polybag and Trichoderma 45 g /polybag application could increase the incubation period of F. Oxysporum fungus, shorten xylem discoloration and then reduce wilted plants percentage. Mycorrhizae application can boost Trichoderma fungus in order to reduce wilt disease found in pepper plants.

1. INTRODUCTION

Fusarium oxysporum is the main fungus disease that can wither plants, especially for pepper plants. The fungus spread through diseased soil or already withered plants and then infect other plants from its roots.

Mycelium is located around plant tissues and generally capable to isolate itself from diseased tissue or within the xylem vessels (Frank, 1972 in Isnaini, et al. 2004). The disease can attack all stages of plants from early sprouting or even before to stage when plants start to flowering and fruiting. When young plants are infected, bottom stems become rotten to its wilted and shrink leaves to eventually die (Semangun, 1996).

F. oxysporum spread through already diseased soil which chlamydospore commonly found for then infect roots on pepper plants. This pathogen life cycle undergoes two phases, which are the phase of pathogenesis when the fungus live as a parasite on host plant and saprogenesis phase when fungus live as saprophytes that capable being the source of inoculum. This cycle can also cause disease on other plants and easily transmitted through wind, infected soil and groundwater as well as agricultural tools. (Doolite, et al., 1961 in Winarsih, 1997).

Doing control by using antagonistic fungi such as *Trichoderma* sp. and *Arbuscularmycorrhizae* has been widely performed. *Trichoderma* sp. is a fungus rich with antifungal activity that produce metabolites, both volatile and non-volatile. These metabolites that produced by *Trichoderma* sp. can diffuse through the dialysis membrane which capable to slow several pathogens growth.

Mycorrhizae creates mutual symbiotic between certain types of fungi and roots (Budisma, 2014). This relationship produces very broad spectrum both in terms of host plants, fungi types, mechanisms in associating, effectiveness, microhabitat as well as diffusion. Application of *Mycorrhizae* effectively increasing the nutrient uptake, heightening drought resistance, boosting growth hormone production and its regulator, giving protection from root pathogens and other toxic elements, while mushrooms get the benefit from photosynthetic supplies and evergreens. *Mycorrhizae* application can boost *Trichoderma* sp. in order to reduce wilt disease found in pepper.

2. MATERIALS and METHODS

Study was conducted at Laboratorium Hama dan Penyakit BPTP East Java, starting from January to May 2016. This study used completelyrandomized design (CRD) with treatment consisted of 16 combined doses of *Mycorrhizae* and *Trichoderma,* each repeated 4 times that produce 64 test units. *Mycorrhizal* dose used is 0.0; 1.0; 2.0 and 4.0 grams per polybag, while the *Trichoderma* dose used is 0.0; 15.0; 30.0; and 45.0 grams per polybag. Data were statistically analyzed by variance analysis and followed by a LSD test of 0.05.

Increasing *F. oxysporum* inoculum was performed in PDA media as well as increasing *Trichoderma* sp.on a medium-sized corn media grown in plastic bags. The seeds of pepper used are 1 month old or seeds that already passed 15 cm long. *Trichoderma* sp. and *Mycorrhizae* are given simultaneously with pepper seeds planted in poly bags according to respective dose and to each planting hole. Increasing *F. oxysporum* is performed 7 days after planting by immersing the pathogens into the soil with ratio 1 gram for 3 cm deep and then covered with transparent plastic to maintain moisture and to avoid contamination.

3. RESULTS

Mycorrhizae and *Trichoderma* sp. application can greatly affect incubation period of *F. oxysporum* showed in Table 1 that higher dosage of *Mycorrhizae* and *Trichoderma* sp. used in an application, the slower the *F. oxysporum* incubation period too. There is an interaction between *Mycorrhizae* and *Trichoderma* sp. against the incubation period of *F. oxysporum* presumably caused by *Mycorrhizae* dependency of *Trichoderma* sp. to extend the incubation period of *F. oxysporum*. The average incubation period of *F. oxysporum* was 18.50 days at a dose of 4 g/*Mycorrhizae*

polybag and 45 g/Trichoderma sp. polybag, while the incubation period of control treatment without *Mycorrhizae* and *Trichoderma* sp. was 12.17 days.

Table 1. Effect of application of *Mycorrhizae* and *Trichoderma* on the incubation period of *F. oxysporum*

Trichoderma sp. dose (g/polybag)	*Mycorrhizae* Dose (g/poly bag)			
	0,0	1,0	2,0	4,0
0,0	12,17 a	13,67 b	14,42 b	15,75 c
15,0	12,42 a	13,42 a	14,67 b	16,58 d
30,0	12,67 a	13,42 a	15,33 c	17,42 d
45,0	13,08 b	14,08 b	15,08 c	18,50 e

Note : Means followed the same letters not significantly different at 5 % level to LSD

On the other hand, high dose of *Mycorrhizae* applied as single treatment resulted in slower *F. oxysporum* incubation period using4 g/polybag and the incubation period decreased to 17.07 days, while the incubation period of control treatment without *Mycorrhizae* was 12.59 days. Likewise, *Trichoderma* sp. application as single treatment with high dose of *Trichoderma* sp. also slowing the incubation period of *F. oxysporum* fungi using 45 g/polybag decreased the incubation period to 15.19 days while the incubation period of control treatment without *Trichoderma* sp. was 14 days. These events have occurred presumably because *Mycorrhizae* able to create hartique tissue that is difficult to be penetrated by pathogenic fungi, while *Trichoderma* sp. able to slow pathogenic fungi entry and movement. Agrios (1997) stated that environmental conditions that support plant growth and are less supportive for the development of pathogens will slow the incubation period and make pathogens need more time to infect plants (Figure 1 and 2).

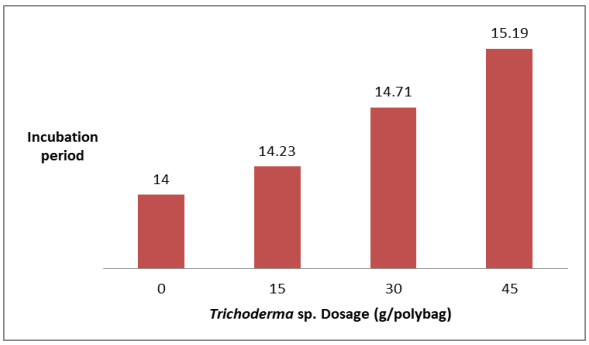

Figure 1. The incubation period of F. oxysporum with Trichoderma sp. application

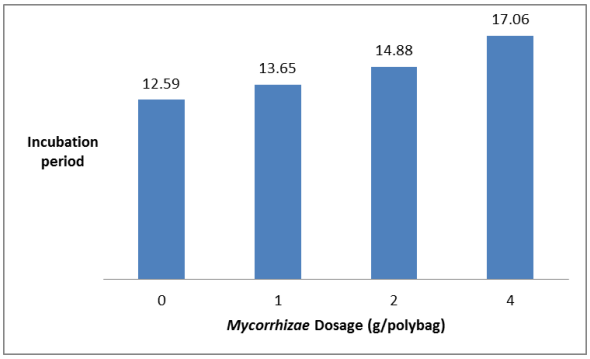

Figure 2. The Incubation period of F. oxysporum with Mycorrhizae application.

Application of *Trichoderma* sp. and *Mycorrhizae* also affect xylem discoloration length. Using both as single treatment resulted in slowing down the fungal infection at plant root done by *F. oxysporum* while *Mycorrhizae* work to suppress xylem discoloration length. Higher dosage of *Trichoderma* sp. and *Mycorrhizae* usage can shorten the length of discolored xylem and in some cases, there are even plants that grow without infected by *F.*

oxysporum. *Trichoderma* sp. application with 45 g/polybag dosage resulted in shortest discolored xylem which was 3.69 cm, while control treatment without *Trichoderma* sp. resulted 12.46 cm. Another application of *Mycorrhizae* with 4 g/polybag dosage also resulted in shortest discolored xylem that was 3.92 cm, while control treatment without *Mycorrhizae* resulted 7.05 cm (Figures 3 and 4).

These events occurred presumably by *Trichoderma* sp. activities that are able to slow the development of *F. oxysporum* and *Mycorrhizae* that able to create an environment suitable for *Trichoderma* sp.to develop. Increased population yet antagonistic activity has a positive effect on plants because reducing pathogens means better growth and development plants on vegetative and generative phases (Chongkapakorn & Sivasithamparam 1986 in Yuflida and Rustam 2003).

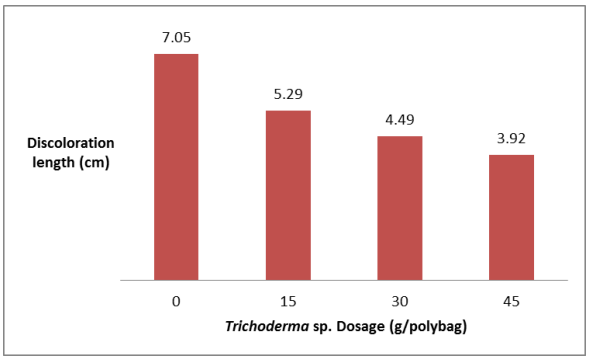

Figure 3. Length of Xylem discolored with Trichoderma sp. Dosage

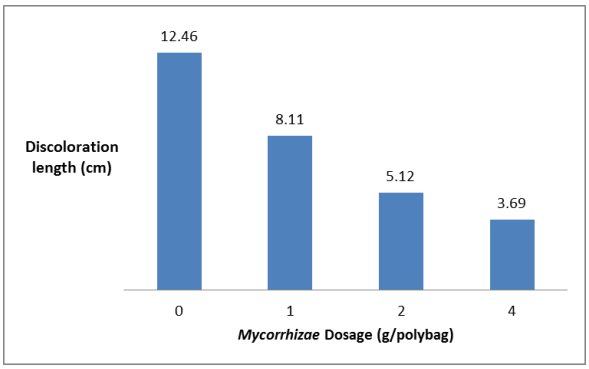

Figure 4. Length of Xylem discolored with Mycorrhizae dosage

The lowest wilted percentage was shown by single treatment using *Mycorrhizae* at 4 g/polybag dosage with 44.68% while control treatment without *Mycorrhizae* resulted 59.41% (Figures 5 and 6). Likewise, single treatment using *Trichoderma* sp. with 45 g/polybag dosage resulted in 35.81% while control treatment without *Trichoderma* sp. is 77.59%. These events occurred because *Mycorrhizae* are able to increase the number of activity of antagonistic fungi inside soil yet able to reduce *Fusarium* activity in infecting plants. Cantoso, et al. (1997) stated that such conditions increase the competition between antagonists and pathogens inside soil causing pathogenic to lose. On the other hand, high dosage of *Trichoderma* sp. able to reduce disease progression more quickly and its chance to develop wider. Semangun (1996) stated that *Fusarium* fungi can infect through the tip of uninfected roots, where fungi develop in the parenchymal tissue and then settle to develop in the vascular bundles.

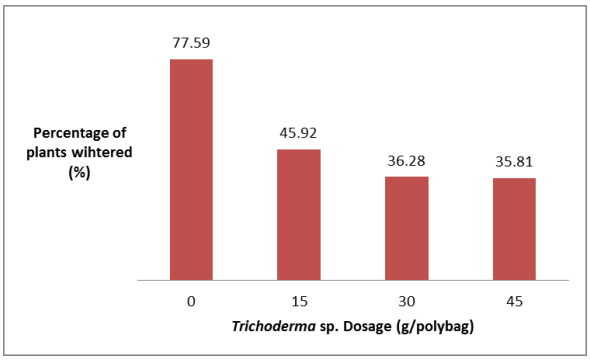

Figure 5. Percentage of plants withered with *Trichoderma* sp. dosage

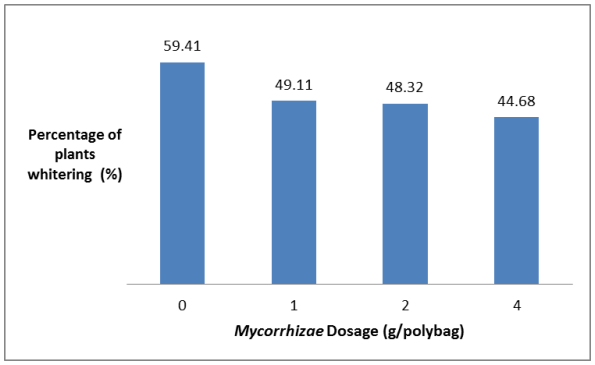

Figure 6. Percentage of plants withering with Mycorrhizae dosage

4. DISCUSSION

Papavizas (1985) stated that *Trichoderm*a sp. capable to suppress the development of pathogens in several ways which are antagonist, parasitism and competition. Besides that, the environment can also affect the development of *Mycorrhizae* and *Trichoderma* sp. to suppress the rate of development of pathogenic fungi. The faster incubation period triggers faster *F. oxysporum* to infect the xylem vessels and resulted in longer discolored xylem. According to Semangun (1996) that *Fusarium* fungal infection starts from the roots, goes up to the base of the stem with white hyphae started to appear covering the stem skin, and continues to run to the top of the stem. Hyphae color will gradually changeto blackishor brownish when the infected area became greater and longer. By giving *Trichoderma* sp. as an antagonistic fungus for soil, it is expected to suppress the development of disease from inside soil. According to Sivan (et al 1987), *Trichoderma* sp. will develop primarily on the surface or the tip of the root so that it slows down contact and infection of the disease.

5. CONCLUSION

Application of *Mycorrhizae* 4 g/polybag and *Trichoderma* sp. 45 g/polybag are able to increase the incubation rate of fungus *F. Oxysporum*. Meanwhile, application of *Mycorrhizae* 4 g/polybag are able to reduce discoloration on Xylem by 44,68% rather than on control by 59,41%. On the other hand, application *Trichoderma* sp. 45 g/polybag are also able to reduce discoloration on Xylem by 35,81% rather than on control by 77,59%.

6. ACKNOWLEDGEMENTS

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