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UTILIZATION OF ARBUSCULAR MICORRHIZAL FUNGI TO CONTROL FUSARIUM WILT OF TOMATOES

PEMANFAATAN JAMUR MIKORIZA ARBUSKULAR UNTUK MENGENDALIKAN LAYU FUSARIUM PADA TOMAT

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ABSTRACT

Tomato is a vegetable crop which is preferred by the Indonesian people. The problem encountered in tomato production is Fusarium wilt which is known as devastating disease. Studies have been done to solve the problem but effective and inexpensive control technique is still questioned. This study aimed to ascertain the ability of Arbuscular Mycorrhizal (AM) fungi as biological control agent in reducing tomato Fusarium wilt. Research was arranged in a completely randomized design (CRD) consisting of 5 treatments and 10 replications. The treatments were untreated plants, *Fusarium oxysporum* f.sp. *lycopersici* inoculated plants, AM fungi inoculated plants, AM fungi + *F. oxysporum* f.sp. *lycopersici* inoculated plants, *F. oxysporum* f.sp. *lycopersici* + benomyl inoculated plants. The results showed that plants which were inoculated with AM fungi had better growth compared to those which were not inoculated with AM fungi. Moreover, plants which were inoculated with AM fungi showed lower disease intensity compared to untreated plant and inoculated plant with *F. oxysporum* f.sp. *lycopersici* + benomyl application. Orthogonal contrast analysis showed plants treated with AM fungi significantly perform better growth and resistance towards infection compared with other treatments. Thus, it concluded that AM fungi had the potency as biological control agent.

Keywords: AM fungi, disease intensity, Fusarium wilt, tomato

INTISARI

Tomat merupakan tanaman sayuran yang banyak digemari masyarakat Indonesia. Salah satu pengganggu utama pada tomat adalah penyakit layu Fusarium yang disebabkan oleh Fusarium oxysporum *f.sp.* lycopersici *dan menimbulkan masalah yang serius. Kerugian yang ditimbulkan oleh penyakit tersebut sangat besar sehingga perlu dicari cara pengendalian yang murah, efektif, dan aman. Penelitian yang bertujuan untuk mengetahui kemampuan jamur mikoriza arbuskular (JMA) sebagai agens pengendali hayati dalam menekan penyakit layu Fusarium pada tomat ini dilakukan dengan Rancangan Acak Lengkap (RAL) yang terdiri atas 5 perlakuan dan 10 ulangan. Perlakuan meliputi kontrol, inokulasi* F. oxysporum *f.sp.* lycopersici*, inokulasi JMA, inokulasi JMA dan* F. oxysporum *f.sp.* lycopersici*, inokulasi* F. oxysporum *f.sp.* lycopersici *dan aplikasi fungisida benomil. Hasil penelitian menunjukkan bahwa tomat yang diinokulasi JMA memiliki pertumbuhan yang lebih baik dibandingkan yang tidak diinokulasi JMA. Intensitas penyakit pada tomat yang diinokulasi JMA lebih rendah, baik dibandingkan dengan kontrol maupun dengan perlakuan* F. oxysporum *f.sp.* lycopersici *dan aplikasi fungisida. Hasil analisis kontras orthogonal menunjukkan bahwa tomat bermikoriza berbeda nyata bila dibandingkan dengan yang tidak diinokulasi JMA maupun yang diaplikasi benomil. Terjadi peningkatan pertumbuhan tomat dan penekanan intensitas penyakit layu Fusarium, sehingga JMA berpotensi sebagai agens pengendali hayati (APH).*

Kata kunci: intensitas penyakit, JMA, layu Fusarium, tomat

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of important horticultural commodities in Indonesia. Farmers have struggled to increase production from time to time but these efforts have been hampered by disease. An important disease that deters the production is Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lycopersici*. Fusarium causes yellowing on one side of the plant or leaf. Yellowing begins with the older leaves followed by wilting, browning, and defoliation. Growth is typically stunted and little or no fruit develops. Brown, vascular tissue can be found on the base of the infected stem. Infected plants often die before maturing.

Continuous application of fungicides cause not only plant pathogen resistance but also negative impact towards consumer and environment. Biological control is one of reliable alternative control. Arbuscular

mycorrhiza (AM) fungi has a positive correlation to some aspects of the physiology of host plants, one of which is lessen disease effects (Simanungkalit *et al*., 2006). Mycorrhizae biofertilizer is an environmentally friendly input which supports sustainable agriculture concept. AM fungi are obligate symbionts which required photosyntates of the host plant (Sumiati & Gunawan, 2006). Murniati *et al*. (2008) explained that plant dry weight of onion, chilli, and corn inoculated with AM fungi was higher than uninoculated plants. Setiadi (2002) pointed out that AM fungi infected roots of plants intensively produced interwoven hyphae, which increased plant resistance to drought and pathogen infection and also improve nutrient uptake, especially N, P, K, Zn, and Cu.

The potency of AM fungi as biological control agent needs to be proved to determine its ability to improve growth and yield of tomatoes, as well as effectiveness in controlling Fusarium wilt of tomato.

MATERIALS AND METHODS

This study was conducted in the greenhouse and in the Laboratory of Agricultural Mycology, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Gadjah Mada.

The experimental design used in this research was completely randomized design (CRD) with 5 treatments, i.e.: untreated plants, inoculated plants with *F. oxysporum* f.sp. *lycopersici*, inoculated plants with AM fungi, inoculated plants with AM fungi + *F. oxysporum* f.sp. *lycopersici*, inoculated plants with *F. oxysporum* f.sp. *lycopersici* followed by benomyl spraying at 0.2 g/l. Each treatments was replicated 10 times.

Inoculation of AM fungi to Tomato Seedlings

Tomato seeds were sown in plastic trays. Tomato seedlings of 22 days after planting was transferred into polybags filled with a mixture of sterile soil and compost with a ratio of 1:3, then inoculated with AM fungi. 10 g of AM fungal inocula with spore density of 10−20 spores/g in zeolite was inoculated in a sterile soil-compost mixture. Both planting and inoculation were carried out from 4 to 5 p.m. to accelerate the growth seedlings and AM fungal infection.

Observations were carried out using Kormanik and McGraw (1982) method. Eight weeks after planting, the plant roots were removed and washed with water and cut into pieces of approximately 2 cm in length. Root pieces were soaked in 10% KOH in beaker glass and heated at 80−90^{o}C for 10−15 minutes, placed in a 50 ml beaker glass and rinsed out well at least three times until no brown color appeared in the water. The roots were then soaked in a 1% HCl solution for 5 minutes and stained with 0,05% trypan blue lactophenol and then allowed to stand for 24 hours. Roots were observed under a binocular microscope to calculate of the percentage of mycorrhizal infection, based on the method of Giovanette and Mosse (1980). Colonization percentage of plant roots was calculated using the following formula:

$$\text{Percentage of root infected} = \frac{\text{Number of infected roots}}{\text{Number of roots observed}} \times 100\%$$

Extraction and Spore Density

AM fungi spore extraction was conducted using Daniel and Skipper (1982) method. As much as 100 g of soil taken from tomato roots was put in a beaker glass containing 500 ml of water and stirred for 15 minutes, then the water was filtered with 75 µm sieve. The suspension, were stirred until foamy, then filtered again with a 54 µm sieve and rinsed with water. Spores were deposited on the filter. The spore suspension was poured into centrifuge tubes, and then centrifuged for 5 minutes at 2000 rpm. The supernatant was discarded, and the pellet was suspended with 65% sugar solution and was stirred homogeneously. Tubes were centrifuged for 2 minutes at 2000 rpm. The supernatant was filtered with a 54 µm sieve. The specimen was rinsed with water to remove the sugar solution, then the spores were collected in the petri dish to be observed using a binocular microscope.

Inoculation of **F. oxysporum *f.sp*. lycopersici** *to Tomato*

This study was conducted in the greenhouse using tomato seedlings which were planted in sterile soil. After 22 days, the seedlings were carefully removed and transferred into polybags containing 5 kg of sterile soil. The tips of tomato roots were cut. After 2 weeks, the plants were inoculated with 5−10 ml of *F. oxysporum* f.sp. *lycopersici* spore suspension at 10^6 conidia/ml. Inoculation was carried out at 4–5 pm.

Observation of Disease Development

Observation of the disease were started after the first symptoms appeared, and observed every week for 7 weeks by using a scoring system. The disease development was scored using the rating system of Ambar (2010): 0: healthy plants (no symptoms of wilting), 1: 1−25% leaves wilted, 2: 26− 50% leaves wilted, 3: 51−75% leaves wilted, 4: 76−100% leaves wilted (dead plants).

The intensity of the disease was calculated by the following formula:

$$IP = \frac{\sum (n \times v)}{N \times Z} \times 100\%$$

IP: disease intensity (%); n: number of plants per infected score; N: number of plants observed; v: infected value based on scoring categories; Z: the infected score highest value.

Statistical Analysis

All data ware analysed using Analysis of Variance (ANOVA) and, if there was a significant difference, it was continued with orthogonal contrasts at P = 95%.

RESULTS AND DISCUSSION

AM Fungal Infection and Spore Density

AM fungal inspection and spore density on treated tomato roots were 80% and 9.4 spore per g soil, respectively. Plants treated with AM fungi and *F. oxysporum* f.sp. *lycopersici* had 44% AM fungi infection, with spore density of 6 spore per g soil, whereas in the untreated plants, inoculated plants only with *F. oxysporum* f.sp. *lycopersici,* and treated plants with benomyl showed no AM fungal infection (Figure 1 and 2).

Orthogonal test showed higher root infection and spore density of AM fungi in AM fungi treated plants regardless single or mix inoculation. However benomyl-mixed treatment showed less spore density than AM fungi treatment. AM fungal infection on tomato roots increases nutrient uptake by external mycelia due to expansion area and chemical compounds released to soil. AM fungi infect the host plant

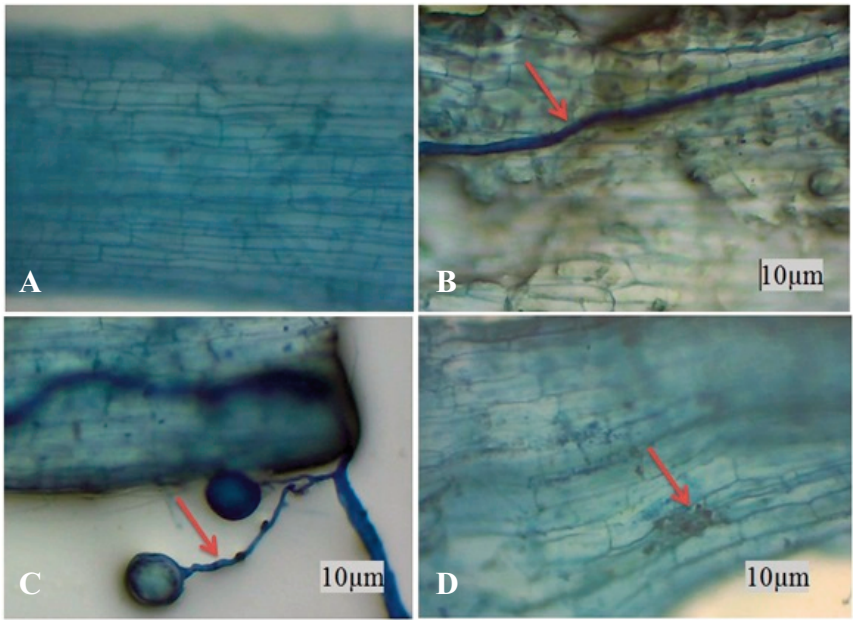

Figure 1. Arbuscular mychorrhizal (AM) fungi infection in tomato root: root without AM fungi infection (A); internal hypha (B); vesicular (C); arbuscular (D)

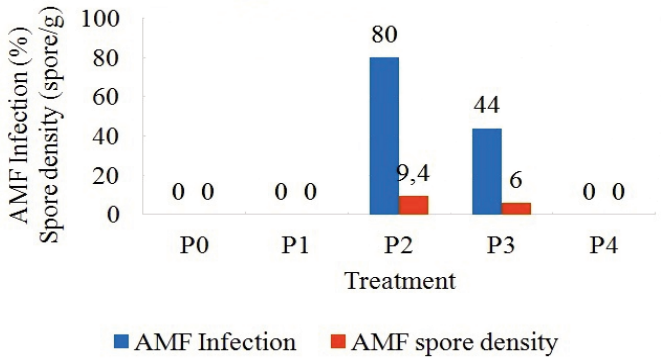

Figure 2. Arbuscular mychorrhizal fungi (AMF) infection (%) in tomato roots and spore density (spore/g); tomato without AM fungi and *Fusarium oxysporum* f. sp. *lycopersici* infection (P0); tomato inoculated with *F. oxysporum* f.sp. *lycopersici* (P1); tomato inoculated with AM fungi (P2); tomato inoculated with AM fungi and *F. oxysporum* f.sp. *lycopersici* (P3); tomato inoculated with *F. oxysporum* f.sp. *lycopersici* and treated with benomil 0,2 g/l (P4)

through several stages, initially with recognition, which is a stage when spores come across root surface and recognize it. When a host is compatible with AM fungi spore, the spore will germinate and form an appressorium on the epidermal root cells and penetrate the cortex and form a coil or a tube to pass between cells and form arbuscular and vesicular inside host cells (Brundrett, 2002; Peterson *et al*., 2004).

AM fungi provide an advantage for plant by helping the roots to improve the absorption of nutrients by breaking the nutrients into forms that are easier to absorb, for example polyphosphates are decomposed into P element that can be immediately absorbed by plant roots (Ohtomo & Saito, 2005). Furthermore, Hodge *et al*. (2010) illustrated that AM fungi were also were able to obtain N from the decomposition of organic matter and distributed N to the plant. AM fungal infection and spore density on inoculated plants without AM fungi and without inoculated pathogens did not significantly different between those that were given with fungicide and those that were not.

Disease Intensity

The disease intensity of Fusarium wilt in the plants inoculated with AM fungi showed the lowest (IP 9%) in comparison with those of untreated plants (IP 27%), plants inoculated with *F. oxysporum* f.sp. *lycopersici* (IP 35%), plants treated with benomyl (IP 18%), and plants inoculated with AM fungi and *F. oxysporum* f.sp. *lycopersici* (IP 22.5%) (Figure 3 and 4). Reduced disease intensity was due to interaction occurred between tomato roots and AM fungi, followed by growth of mutualistic symbionts which potentially suppressed Fusarium wilt intensity (Mosse, 1981).

Orthogonal contrast showed that the plants inoculated with AM fungi had lower disease intensity compared to those which were not inoculated, and those which were not applied with benomyl fungicide. Tomato plants inoculated with AM fungi had lower disease intensity compared to those inoculated with *F. oxysporum* f.sp. *lycopersicum* and applied with benomyl fungicide. According to Talanca and Adnan (2005), plants infected with mycorrhizae had greater resistance than those without mycorrhizae. Plants

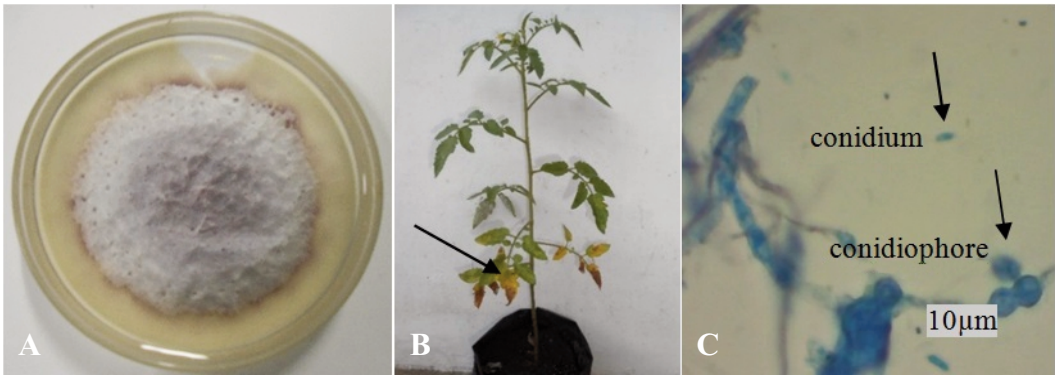

Figure 3. Fusarium wilt disease on tomatoes: *F. oxysporum* f. sp. *lycopersici* colony on PDA (A); Fusarium wilt symptom after inoculation (B); Fusarium conidiophore and conidium (C)

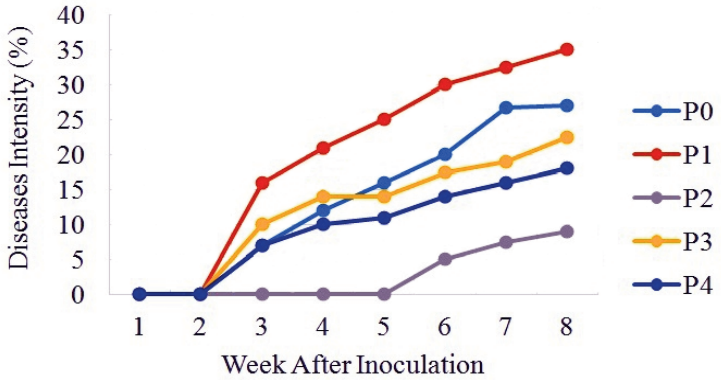

Figure 4. Percentage of disease intensity on tomato wilt after inoculation: untreated plants (P0); plants inoculated with *F. oxysporum* f.sp. *lycopersici* (P1); plants inoculated with AMF (P2); plants inoculated with AMF and *F. oxysporum* f.sp. *lycopersici* (P3); plants inoculated with *F. oxysporum* f.sp. *lycopersici* followed with benomil 0,2 g/l (sprayed) (P4)

with mycorrhizae produce substances such as phenol, phytoalexin, and quinone, and develop morphological changes by cell wall thickening as a result of lignification (Soenartiningsih, 2011).

F. oxysporum f.sp. *lycopersici* inoculated plants (Figure 3) showed higher disease intensity, including in mix inoculation of benomyl + *F. oxysporum* f.sp. *lycopersici*. Fungicide did not show effectiveness in reducing the intensity of Fusarium wilt in tomato, while plants inoculated with AM fungi showed lower disease intensity (Figure 4). This indicates that AM fungi acts as potential biological agent to suppress Fusarium wilt of tomato. The suppression of disease intensity is possible to be made because of the ability of AM fungi to live in soil and colonize the roots, and thereby protecting the roots from *F. oxysporum* f.sp. *lycopersici* infection.

CONCLUSION

Tomato plants which were inoculated with arbuscular mycorrhizal (AM) fungi had better performance compared to untreated plants, plants which were inoculated with *F. oxysporum* f.sp. *lycopersici* and even plants treated with benomyl fungicide. Disease intensity of Fusarium wilt on inoculated plants with AM fungi was lower than that of those which were not inoculated with AM fungi. These results indicate that AM fungi had a potential as a biological control agent towards *F. oxysporum* f.sp. *lycopersici*.

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