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Research Article

The roles of arbuscular mycorrhizal fungi in the intensity of the foot rot disease on pepper plant from the infected soil

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Abstract : Pepper (*Piper nigrum* L.) is an important spice plant of Indonesia. In Bangka Belitung Province, the main pepper producer, pepper has been the most commonly cultivated commodity. However, the production has declined from time to time. One of the causes of the decline is Pepper Fot Rot, caused by *Phytophthora capsici*. The rapid spread and development of the disease is mainly due to utilization of diseased plant materials for pepper cuttings and infested or diseased plantation soil. The materials used in this research included the infected soil taken from the infected–pepper plantation at Bangka Island with disease intensity of pepper foot rot 60%, inoculum of Arbuscular Mycorrhizal fungi in the zeolite medium, compost, and pepper seedling from Natar variety. This research was done by planting pepper seedling on infected soil and observing plant height, disease intensity, and infection of AM fungi on the roots. The results showed that soil from diseased pepper plants harbored high population of plant pathogens inoculum and caused the death of 9 week-old cuttings and retarded growth of the survivors. Sterilization of the infected soil with hot water vapor for 3 hours still could not control the pathogen. Good growth was observed on one node cutting planted in sterile soil amended with arbuscular mycorrhizal fungi.

***Keywords**: arbuscular mycorrhizal fungi, pepper, pepper foot rot, Phytophthora capsici*

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Introduction

Pepper (*Piper nigrum*) is one of the most important plantation commodities in Indonesia. In the Province of Bangka Belitung, it is the choicest commodity that is being developed nowadays. The Pepper foot rot disease caused by *Phytophthora capsici* is one of the most serious diseases in Indonesia, and sometimes it causes serious problems. The intensity of this disease in Indonesia could reach 63.7% (Bande et al., 2014). The rapid spread and development of the disease is mainly due to the utilization of diseased plant materials for pepper cuttings and infested or diseased plantation soil. Problems challenging pepper farmers in Bangka Belitung are a partial knowledge and limited ability to identify foot rot as well as other predicaments in the field. Evidence indicated that farmers commonly prepare cuttings from pepper veins taken from their own plantation, considering good yield of the parent plants. Obtaining healthy and productive cuttings which is then developed in a controlled Pepper Nursery Farm is a strategic breakthrough to cope with these two major diseases (foot root disease and yellow disease) (Manohara, 2007; Suryanti et al., 2013) and this research is the initial step of the effort. Bande et al. (2014) showed that *P. capsici*, the causal agent of the pepper foot rot disease, besides being a soil-borne pathogen, has the potential of being spread through its propagation materials. Diseased cuttings as propagation materials are very dangerous for the survival of pepper plants.

Swastiningrum (2015) and Putri et al. (2016) proved that Arbuscular Mycorrhizal Fungi could significantly control the disease development, each of which is for the rust disease on sugarcane caused by *Puccinia kuehnii* and leaf spot disease on clove caused by *Colletotrichum gloeosporioides*. The additions of AM Fungi could improve uptake of phosphorus on maize which in turn increase plant yield (Mau and Utami, 2014). This fact needs to be studied for the foot rot disease on pepper.

Materials and Methods

This research was conducted from April to November 2016. The preparation of seedlings and inoculation were done in a screen house in Condongcatur, Sleman DIY (113 m above from sea level). Meanwhile, the laboratory research was conducted at Mycology Laboratory of Agriculture, Department of Plant Pest and Disease, Faculty of Agriculture of Gadjah Mada University, Yogyakarta-Indonesia. The infected soil was taken from the infected—pepper plantation at Bangka Island in the depth of 15-20 cm with disease intensity of pepper foot rot 60%, inoculum of Arbuscular Mycorrhizal AM fungi in the zeolite medium as the collection of Laboratory of Mycology of Agriculture Faculty of UGM, compost, and pepper seeds from Natar variety. The planting of pepper seed was done in the infected soil in the polybags with a capacity of 2 kg, Soil sterilization and inoculation of AM fungi was done in accordance with the type of the treatment. The technique of inoculation was done by spreading 10 g of biological fertilizer AM fungi, fungal spores of 19.57 spores in zeolite to the hole in the polybags containing a mix of sterile soil and compost, before the pepper seeds were planted. Sterilization of infected soil was done using standard sterilization of hot water vapor for a period of three hours at the temperature 100^{o}C. Both the planting of pepper seedlings and inoculation were conducted at 04.00-05.00 pm to accelerate the growth and infection.

Table 1. Code of treatments for growing media

Code	Treatment Description
P0	The pepper seeds without inoculation of AM fungi and fertilizer were planted into the sterile infected soil.
P1	The pepper seeds inoculated with AM fungi and without any fertilizer were planted into the sterile infected soil.
P2	The pepper seeds without inoculation of AM fungi and with fertilizer were planted into the sterile infected soil.
P3	The pepper seeds inoculated with AM fungi and fertilizer were planted into the sterile infected soil
P4	The pepper seeds without inoculation of AM fungi and fertilizer were planted into the infected soil.
P5	The pepper seeds inoculated with AM fungi and without any fertilizer, were planted into the infected soil
P6	The pepper seeds without inoculation of AM)fungi and with fertilizer were planted into the infected soil
P7	The pepper seeds inoculated with AM fungi and fertilizer were planted into the infected soil.

Parameters of observation

The observation included the disease intensity from first weeks of application until the ninth weeks and the infection of AM fungi on the roots of pepper, given with a treatment.

Observation on the emergence and growth of disease

The observation on the growth of the disease was started from the emergence of the symptoms and it was weekly observed for 7 weeks using the scoring. The scoring system used was based upon Manohara (2007) in which: 0: Plants are healthy (not showing any wilting symptoms) with the fresh green leaves and stalk , 1: The symptom occurs on the leaves and stalk of ≤ 10%, 2: The symptoms on the leaves and stalk are the range of 10-50%, 3:50-100% leaves are wilting (plant is dead). The disease intensity was measured using the following formula:

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

Remarks: IP: Disease Intensity: (%), n: Number of plants for each category of attack, N: Number of plants observed, v: Score value of attack

category, and Z: Score value of the highest attack category.

Infection of AM fungi on pepper roots

The observation was conducted by following the method of Kormanik and McGraw (1982):

Percentage of infected roots = [(Number of infected roots/Number of observed roots) x 100%]

The sample roots of the pepper plant at the age of six weeks old were removed and cleaned with the water. Subsequently, the roots were cut into 2 cm approximately. The cuts of the roots were soaked in 10% of KOH in a beaker glass and heated at a temperature of 80-90^{o}C within 10-115 minutes. The roots of pepper were then taken and put into a 50-mL beaker glass and rinsed with water 3-4 times until the water of cleaning was no longer brown. Afterward, they were soaked in 1% of HCl solution within 5 minutes and colored with 0.05 % lactophenoltrypan blue. It would be left for 24 hours before conducting an observation using a binocular microscope to measure the percentage of the infection of mycorrhizae based upon the method of Giovanette and Mosse (1980). The percentage of colonization of the plant roots was measured using the following formula.

Results and Discussion

In the treatment of the infected soil that has been sterilied (P0), it was found that the disease intensity was stable at 40%. The sterilization of infected soil by means of the standard method (3 hours at 100^{o} C) was not effective. This showed that time and type of sterelization for the soil used previously for the program needs to be studied further as something interesting. The role of AM fungi in the disease intensity of pepper foot rot could be seen in the 9th week (Figure 1).

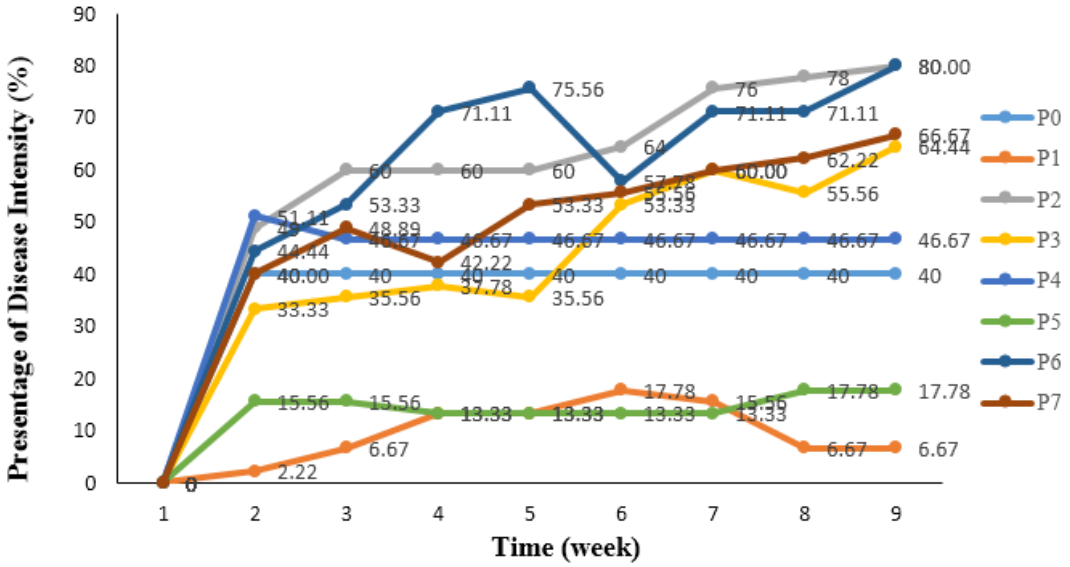

Figure 1. The development of disease intensity of pepper foot rot for 9 weeks.

Figure 1 showed that application of AM fungi on sterile soil (P1) and non-sterile soil (P5) gave a good effect on the percentage of disease intensity than other treatments. It also revealed that application of AM fungi with compost on sterile soil (P3) and non-sterile soil (P7) had a higher score of disease intensity but lower than application on the seedlings without AM fungi on both the sterile soil (P2) and non-sterile soil (P6). The results of this study expressed that the effect of mycorrhizae inhibited the rate of disease progress compared to others treatments. It indicated that on sterile soil and amendment of compost were not able to inhibit the rate of disease infection after 9th observation. Figures 1 and 2 show that the provision of AM fungi in inhibit the disease intensity of pepper foot rot, particularly in the treatment of P1 (6.67%) and P5 (17.78%). Djunaedy (2008) stated that mycorrhizal fungi could increase the ability in the nutrient absorption and the nutrient provision for the plant thus enabling the mycorrhizal fungi to be used as the biological fertilizer and to increase the soil fertility. Mycorrhizal fungi can be also used as one of the soil-borne pathogen controlling agents. Mycorrhizal fungi are able to make the plants more resistant to or tolerant of disease due to the improvement of nutrient on the host plant,

competition between pathogen and mycorrhizae in obtaining the photosynthate and infection area, and the change of anatomy and morphology on the roots for experiencing the lignification causing the roots thicker. Mycorrhizae makes the mechanism of the resistance to the attack of soil-borne pathogen active due to the increase of amino acid and phenol compound that is toxic towards the pathogen (Rozy et al., 2004). Combination of mycorrhizae and compost did not show lower score of disease intensity than single treatment of AM fungi because compost had a high content of organic materials providing nutrients for the pathogens. The infected soil from pepper plant contains the inoculum of *Phytophthora capsici* (Figure 3), the causal agent of foot rot disease.

The disease intensity in this research developed rapidly because, as stated by Manohara (2007), the leaves infected by *P. capsici* would show the symptom after 24 hours, the diseased leaves would fall three days after the infection and it could be dead shortly.

Figure 2. Pepper seed in some treatments. P0, P1, P2, P3, P4, P5, P6 and P7 are as presented in Table 1

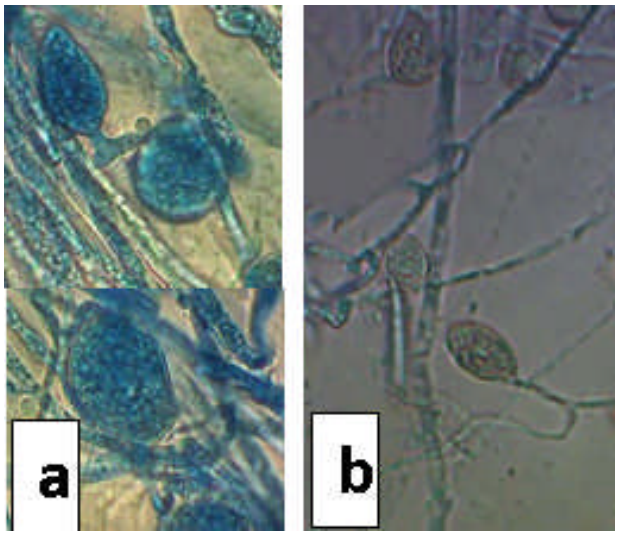

Figure 3. Sporangium of *Phytophthora capsici;* a) Sporangium of *Phytophthora capsici* from samples of infected soil, b) Sporangium of *Phytophthora capsici* from symptomatic roots and leaves after treatment.

The results of contrast analysis

The plants inoculated with AM fungi showed a better height compared to the ones without inoculation of AM fungi. The disease intensity on the plants inoculated with AM fungi was lower than the one without the inoculation of AM fungi.

In contrast, on the infection of AM fungi to the lower roots, its high disease intensity was affected by the lower infection of AM fungi in the pepper plants (Table 2). AM fungi are able to enhance the growth of plants including the plant height, number of leaves, and the root length on the plants (Prasasti et al., 2013; Wicaksono et al., 2014). Application of 20 g mycorrhizae increased plant height, leaf area, numbers of leaf, index of chlorophyll, and proline content of tomato plant (Damaiyanti et al., 2015). The inoculation of AM fungi on onion plants is able to control the intensity and the growth rate of soil borne pathogen and can be used as the trigger of resistance on the pepper plants to the soil borne disease (Halim et al., 2016; Sari et al., 2016).

Table 2. The effect of provision of AM Fungi to the infected soil on the plant height, infection of AM fungi on the plant roots, and disease intensity at 9 weeks after inoculation

Variable	**Treatment**	
	Without Inoculation of AM Fungi (P0, P2, P4, P6)	**Inoculated with AM fungi (P1, P3, P5, P7)**
Plant height, cm	4.64[b]	8.03[a]
Infection of AM Fungi on the roots,	14.93[b]	49.44[a]
Disease intensity, %	61.67[a]	38.89[b]

Remark: the number followed by a different letter shows a significant difference in the orthogonal contrast test

The provision of compost to the infected soil did not give any effects on the height of pepper plants as the disease intensity tended to be high; thus, the growth of the plants was disturbed. The provision of compost increased the disease intensity on the pepper plants in the infected soil (Table 3). However, the provision of compost was not effective in controlling the pepper foot rot disease on the infected soil.

Table 3. The effect of the compost provision to the infected soil on the plant height, infection of AM fungi on the plant roots, and disease intensity at 9 weeks after inoculation

Variable	**Without inoculation of AM fungi**	
	Without Compost (P0, P4)	**Compost (P2, P6)**
Plant height, cm	5.36[b]	3.93[b]
Infection of AM Fungi on the roots , %	9.85[a]	20.00[b]
Disease intensity, %	43.33[b]	80.00[a]

Remark: the number followed by a different letter shows a significant difference in the orthogonal contrast test

The treatment of AM fungi at the same time as the compost could not restrain or control the intensity of the pepper foot rot disease. The percentage of disease intensity with the treatment of AM fungi and the compost was higher than the treatment of AM fungi without the compost Table 4. As reported by Stone et al. (2001), organic materials such as compost are inconsistent in controlling the soil borne pathogen such as *Pythium ultimum* dependent upon the quality and quantity of the compost as the organic materials.

Table 4. The effect of provision of AM fungi and compost to the infected soil on the plant height, infection of AM fungi on the plant roots, and disease intensity at 9 weeks after inoculation

Variable	**Inoculated with AM fungi**	
	Without compost (P1, P5)	**Compost (P3, P7)**
Plant height, cm	9.71[a]	6.35[b]
Infection of AM Fungi on the roots, %	76.67[a]	22.22[b]
Disease Intensity, %	12.22[b]	65.56[a]

Remark: the number followed by a different letter shows a significant difference in the orthogonal contrast test

Table 5 shows that the disease intensity in the infected soil with the sterilization treatment (P0) is lower than that of the infected soil without any sterilization (P4). The soil sterilization could control the population of pathogen as it can terminate the pathogen in the infected soil (Rebecca et al., 2005). However, data presented in Table 5 show that soil sterilization did not effective to terminate the pathogen because it was not able to suppress the overall populations of pathogens. Longer period of soil sterilization might be able to effectively suppress the pathogens. The treatment of sterilization with the provision of compost to the infected soil cannot control but increase the population of soil borne pathogen as seen in Table 6. *P. capsici* as the causal agent of pepper foot rot disease can live as saprophyte in the remains of plant (Manohara, 2007); thus, with the provision of compost, the pathogen can increase in the infected soil for obtaining the nutrients from the compost.

Table 5. The effect of sterilization to the infected soil on the plant height, infection of AM fungi on the plant roots, and disease intensity at 9 weeks after inoculation

Variable	**Without inoculation of AM fungi**	
	Sterilization (P0)	**Without Sterilization (P4)**
Plant height, cm	5.95[a]	4.78[b]
Infection of AM Fungi on the roots , %	16.67[a]	3.03[b]
Disease Intensity , %	40.00[b]	46.67[a]

Remark: the number followed by a different letter shows a significant difference in the orthogonal contrast test

Table 6. The effect of sterilization and the provision of compost to the infected soil on the plant height, infection of AM fungi on the plant roots, and disease intensity at 9 weeks after inoculation

Variable	**Without Inoculation of AM fungi**	
	Sterilization + Compost (P2)	**Without Sterilization + Compost (P6)**
Plant height, cm	3.83[b]	4.00[b]
Infection of AM Fungi on the roots, %	10.00[b]	30.00[a]
Disease intensity, %	80.00[b]	80.00[b]

Remark: the number followed by a different letter shows a significant difference in the orthogonal contrast test

The provision of AM fungi to the infected soil sterilized and infected without sterilization showed the low level of disease intensity as shown in Table 7. This was because sterilization was able to control the soil-borne pathogen and the roots of the pepper plant were protected by AM fungi, which can be seen from the data on the high infection of AM fungi on the roots. There was no any difference in the result of the research to the provision of AM fungi, sterilization of infected soil and the provision of compost with the provision of AM fungi, infected soil without sterilization and the provision of compost as shown in Table 8. The provision of compost has caused the quite high disease intensity in spirit of having been inoculated with AM fungi and the treatment of sterilization

Table 7. The effect of provision of AM fungi and sterilization to the infected soil on the plant height, infection of AM fungi on the plant roots, and disease intensity at 9 weeks after inoculation

Variable	**Inoculation of AM fungi**	
	Sterilization (P1)	**Without Sterilization (P5)**
Plant height, cm	9.93[b]	9.49[b]
Infection of AM Fungi on the roots,%	83.33[a]	70.00[b]
Disease intensity , %	6.67[b]	17.78[b]

Remark: the number followed by a different letter shows a significant difference in the orthogonal contrast test

Table 8.`The effect of the provision of AM fungi, provision of compost and sterilization to the infected soil on the plant height, infection of AM fungi on the plant roots, and disease intensity at 9 weeks after inoculation.

Variable	AM fungi Inoculation	
	Sterilization + Compost (P3)	**Without Sterilization+ Compost (P7)**
Plant height, cm	7.16[b]	5.54[b]
Infection of AM Fungi on the roots, %	21.10[b]	23.33[b]
Disease intensity, %	64.44[b]	66.67[b]

Remark: the number followed by a different letter shows a significant difference in the orthogonal contrast test

Conclusion

This research concluded that the use of Arbuscular Mycorrhizal fungi could control the disease intensity of the pepper foot rot on the pepper seedlings planted in the infected soil. The single treatment of mycorrhizae was more effective in suppressing the rate of disease progress. Sterilization of the infected soil with hot water vapor for 3 hours still could not control the pathogens. There was a negative correlation between the infection of AM fungi on the roots and the intensity of the pepper foot rot disease.

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References

Bande, L.O.S., Hadisutrisno, B., Somowiyarjo, S. and Sunarminto, B.H. 2014. Detection and quantification of *Phytophthora capsici* in soil using black pepper leaf baiting. *Journal of Agroteknos* 4:160-166.

Damaiyanti, D.R.R., Aini, N. and R. Soelistyono, R. 2015. Effects of arbuscular mycorrhizae inoculation on growth and yield of tomato (*Lycopersicum esculentum* Mill.) under salinity stress. *Journal of Degraded and Mining Lands Management* 3: 447-452.

Djunaedy, A. 2008. Applications systemic fungicide and utilization of mycorrhizae for control of soil borne pathogens on soybean crop (*Glycine max* L.). *Embryo*. 5: 149-157.

Giovannette, M. and Mosse, B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist* 84:489-500.

Halim, M., Karimuna, L. and Hasid, R. 2016. The role of mycorhizal arbuscular in the incidence of foot rot disease on pepper plant. *Jurnal Fitopatologi Indonesia* 12: 178-184.

Kormanik, P.P. and McGraw, A.C. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: Schenck, N.C. (ed). Methods and Principles of Mycorrhizal Research. APS Press.

Manohara, D. 2007. Phytophthora leave spots as the source of inoculums of foot rot disease on black pepper (*Piper nigrum* L.**).** *Bulletin of Research on Spice and Medicinal Plants* 18: 177-187.

Mau, A.E. and Utami, S.R. 2014. Effects of biochar amendment and arbuscular mycorrhizal fungi inoculation on availability of soil phosphorus and growth of maize. *Journal of Degraded and Mining Lands Management*. 1: 69-74.

Prasasti, O.H., Purwani, K.I. and Nurhatika, S. 2013. Effect of glomus *Mycorrhizae fasciculatum* against the vegetative growth of peanut plants were infected by the pathogen *Sclerotium rolfsii*. *Jurnal Sains dan Seni Pomits* 2: 2337-3520.

Putri, A.O.T., Hadisutrisno, B. and Wibowo, A. 2016. The effects of arbuscular mycorrhizal inoculation on the growth of clove seedling and leaf spot intensity. *Jurnal Pemuliaan Tanaman Hutan* 10: 145-154.

Rebecca, E.D., Duncan, R.A. and Scow, K.M. 2005. Soil sterilization and organic carbon, but not microbial inoculants, change microbial communities in replanted peach orchards. *California Agriculture*. 59: 176-181.

Rozy, F., Liestiany, A. and Maftuhah. 2004. Ability controlling of mycorrhizal on *Rhizoctonia solani* Kuhn at soybean. *Agroscientiae* 11: 96-97.

Sari, M.P., Hadisutrisno, B. and Suryanti. 2016. Suppressing of purple blotch disease development on shallot by arbuscular mycorrhizal fungi. *Jurnal Fitopatologi Indonesia* 12: 159-167.

Stone, A.G., Traina, S.J. and Hoitink, H.A.J. 2001. Particulate organic matter composition and pythium damping-off of cucumber. *Soil Science Society of America Journal* 65:761–770.

Suryanti, Hadisutrisno, B., Mulyadi and Widada, J. 2013. Survey of distribution pepper yellowing disease and pathogens associated. *Jurnal Budidaya Pertanian* 9: 60-63.

Swastiningrum, A. 2015. The mechanism of arbuscular mycorrhizal fungi in suppressing the development of disease in sugarcane seedlings. Thesis. Universitas Gadjah Mada. Yogyakarta.

Wicaksono, M.I., Rahayu, M. and Samanhudi. 2014. Effect of mycorrhizal and organic fertilizer on the growth of garlic. *Jurnal Ilmu Ilmu Pertanian*. 29: 34-43.

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