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Research Article

Beneficial Effects of Arbuscular Mycorrhizal Fungi and *Trichoderma* on Diseased Shallot

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ABSTRACT

Purple blotch and fusarium basal rot are important shallot diseases which have caused significant yield loss. An alternative control method for these diseases is the use of biocontrol agents, such as arbuscular mycorrhizal fungi (AMF) and *Trichoderma.* The objectives of this study were to determine the effects of AMF and *Trichoderma* sp. on shallot growth and disease suppression. The experiment was set as a Complete Randomized Block Design with three treatments and three replications. The treatments were AMF, *Trichoderma* sp., and control. Each treatment was applied to a row and 15 shallot plants were taken as samples for observation. Disease severity of purple blotch and fusarium basal rot, plant height, number of leaves and shallot resistances to *Fusarium solani* were observed during this study. Results demonstrated that at seven weeks after planting, the application of AMF and *Trichoderma* sp. tended to suppress fusarium basal rot to 0.89% and 1.78% respectively, but only the application of AMF that suppressed purple blotch disease to 0.44%. The application of AMF and *Trichoderma* sp. also tended to increase plant height, number of leaves, leaf fresh weight and dry weight, root length, and bulb weight. In addition, AMF and *Trichoderma* sp. application increased shallot resistances against *Fusarium solani*.

Keywords: biocontrol agent; Fusarium basal rot; plant disease management; purple blotch

INTRODUCTION

Production of shallot in 2015–2018 was fluctuative. Production of shallot in 2015 decreased by 0.39 percent compared to production in 2014. Production growth of shallot in 2018 compared to 2017 increased by 2.26 percent (Badan Pusat Statistik, 2018). Fungal diseases, such as purple blotch *(Alternaria porri)*and fusarium basal rot, are one of the problems in shallot production. Continuous disease control using chemical fungicides may have detrimental effects to the environment by contaminating soil and water (Sudirman *et al.,* 2011). Therefore, it is necessary to use alternative control methods, such as biological control using Arbuscular mycorrhizal fungi (AMF) and *Trichoderma*.

Arbuscular mycorrhizal fungi can increase water and nutrient absorption; thus, increasing plant growth (Salisbury & Ross, 1995). The fungi can also inhibit *Fusarium* infection by colonizing roots and promoting plant growth and root volume (Al-Hmoud & Al-Momany, 2015). In addition to their effects on soil-borne pathogen, AMF (*Glomus intraradices*) has ability to inhibit air borne disease such as early blight disease (*Alternaria solani)* on tomatoes (Jung *et al.,* 2012). Sari (2016) also reported that shallots treated with AMF showed lower purple blotch severity compared to the untreated control.

Another fungus that also has beneficial effect on plant growth and suppresses fungal disease is *Trichoderma*. Interaction of *Trichoderma* with the host plants can increase plant growth, yield, nutrient availability, and resistances to plant pathogens (Naguleswaran *et al.,* 2014). *Trichoderma* is recognized as an antagonistic fungus against several soil-borne pathogens, such as *Fusarium, Pythium, Sclerotinia, Rhizoctonia, Gaeumannomyces* (Howell, 2007). *Trichoderma* has been reported to inhibit *Alternaria* sp. in vitro and the development of purple blotch disease in onion (Ghanbarzadeh *et al.,* 2016). Combination between *Trichoderma harzianum* and *Glomus mosseae* also decreased severity fusarium basal rot and purple blotch disease in shallots (Abo-Elyousr *et al*., 2014). The objective of this study was to determine the effects of AMF and *Trichoderma* sp. application on shallot growth and disease suppression in the field.

MATERIALS AND METHODS

Field study was conducted at Gotakan Village, Panjatan, Kulon Progo; while other work was done at the glass house and Laboratory of Plant Pathology, Faculty of Agriculture, Universitas Gadjah Mada during January–November 2019. Field study used a Complete Randomized Block Design (RCBD) with three blocks and three treatments, which were AMF, *Trichoderma* sp., and control. Each block was in one row and 15 shallot plants were taken as samples for observation. Crok Kuning shallot variety was planted with spacing 10 cm in row and 20 cm between rows. Ten grams of AMF (*Glomus* spp.) in zeolite formulation were applied into each planting hole before planting; whereas 20 g for *Trichoderma* sp. in caolin formulation was applied one day before planting. Before applying biological agents, cow manure at a dose of 1 t/ha was applied in the soil.

Disease Severity and Plant Growth Observation

Plant height and number of leaves were observed every week. The shallot bulbs were harvested at seven weeks after planting. The variables observed were: fresh and dry weight of shoot, root length, and bulbs weight. Disease severity was observed as soon as the symptom appeared and it was repeated weekly. Fusarium basal rot and purple blotch were observed using the following formula (Ismiyatuningsih *et al.*, 2016):

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

DS = Disease severity
n = number of plants or leaves infected
v = disease score
N = number of observed plants or leaves
Z = highest score used

Purple blotch disease was scored using scoring used by Putrasemedja *et al.* (2012) with slight modifications :

Score 0 = Healthy plants (no symptoms)
Score 1 = the infected tissues are 0–10% of the leaf
Score 2 = the infected tissues are 11–20% of the leaf
Score 3 = the infected tissues are 21–40% of the leaf
Score 4 = the infected tissues are 41–60% of the leaf
Score 5 = the infected tissues are 61–100% of the leaf

The score used for fusarium basal rot were (Nugroho *et al.*, 2015):

Score 0 = No symptoms
Score 1 = Some leaves turn yellow but not dry
Score 2 = Some leaves are dry but not withered
Score 3 = Plants are wither but not yet rotten
Score 4 = Roots are rotten
Score 5 = Plants die

The shallot crops were harvested at seven weeks after planting. The variables observed were fresh and dry weight of shallot shoot and and bulbs, as well as the root length. In addition, the shallot bulbs were further tested for their resistances to *Fusarium solani*. Harvested shallot bulbs were inoculated by spraying the pathogen conidial suspension with concentration of 10^6 conidia/ml. *Fusarium solani* used in this disease resistance test was a collection of Plant Disease Laboratory, Faculty of Agriculture, Universitas Gadjah Mada. Fusarium basal rot disease is caused by 3 species of fusarium: *Fusarium oxysporum, Fusarium solani*, and *Fusarium acutatum* (Lestiyani, 2015).

Pathogen-inoculated shallot bulbs were then incubated in a sterile container at room temperature for 7 days. Disease incidence was determined by counting the percentage of bulbs with necrotic symptom:

$$\text{Infection percentage} = \frac{\sum \text{bulb with necrosis}}{\sum \text{total bulb incubated}} \times 100\%$$

RESULTS AND DISCUSSION

Disease Severity and Plant Growth

Disease severity of fusarium basal rot was started to appear at the first week after shallots were planted. Shallots treated with AMF and *Trichoderma* sp. had lower disease severity of fusarium basal rot (Figure 1).

At seven weeks after planting the severity of fusarium basal rot in shallot treated with AMF and *Trichoderma* sp. was 0.89% and 1.78%, compared with control which 4% (Figure 1). Lower disease severity of fusarium basal rot in shallots treated with AMF implied interaction between AMF and the shallot roots. Mycorrhizal fungi provide an effective alternative method of disease control especially for the pathogens which affect the below ground of the plant. Zeng (2006) stated that interactions between pathogens, AMF, and host plant are able to reduce disease severity of soil-borne pathogens. This research proved that lower disease severity of fusarium basal rot at shallots treated with AMF implied interaction between AMF and shallot roots. Previous research showed that AMF suppress the progress of

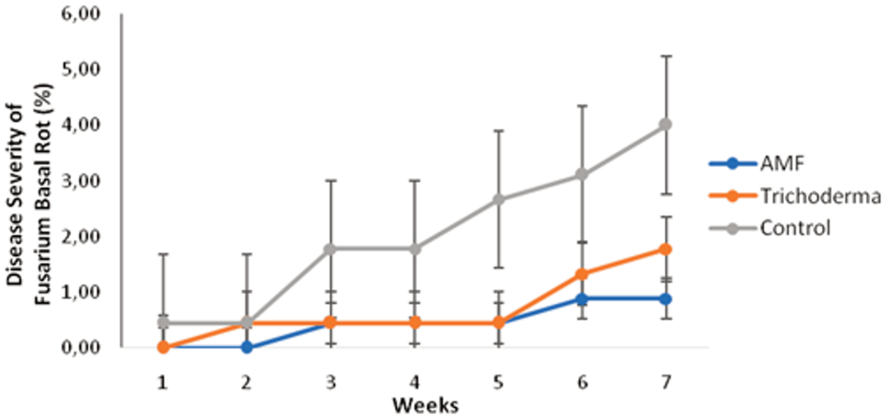

Figure 1. Disease severity of fusarium basal rot in all treatments during seven weeks of observation with vertical bars representing standard errors

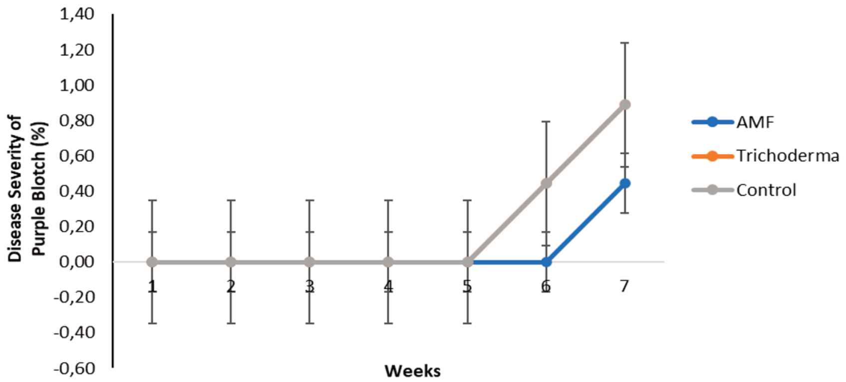

Figure 2. Disease severity of purple blotch in all treatments during seven weeks of observation with vertical bars representing standard errors

fusarium wilt in shallots with the longest disease incubation period in 21 days and decreasing disease incidence up to 40 % (Fitriani *et al.,* 2019).

The mycorrhizal symbiosis involves several mechanisms in controlling plant disease such as creating mechanical barrier hindering the pathogen penetration and subsequent spread; thickening of cell wall through lignification and production of other polysaccharides which in turn hinder the entry of root pathogen; producing antibiotics and toxins that inhibit the pathogen; compensating the nutrient absorption in roots (Zeng, 2006).

The benefits of *Trichoderma* sp. have been reported in controlling fusarium basal rot in onion. The antagonistic mechanisms of *Trichoderma* against fungal pathogen can be divided into three types, which are antibiosis, parasitism, and competition for space and nutrition (Baker & Cook, 1982). *Trichoderma* can produce secondary metabolites, such as polyketides, alkyl pyrones, isonitriles, alametichin, tricholin, peptaibols, 6-penthyl- α-pyrone, massoilactone, viridin, gliovirin, glisoprenins, and heptelidic acids (Raaijmakers *et al.,* 2009). Several *Trichoderma* spp. produce iron chelating siderophores which hinder growth of other fungi (Ghosh *et al.,* 2017). *Trichoderma* can also parasites other fungi and causes vacuolar enlargement and lysis of the host cells. *Trichoderma* penetrates host cell walls by excreting degrading enzymes, such as protease, chitinase, and glukanase (Harjono & Widyastuti, 2001).

In this study, purple blotch disease was started to appear at five weeks after planting. The disease severity was very low. At seven weeks after planting, the disease severity in the control was only 0.89%. It may caused by the relative humidity is low. The field is located in lowland at altitude 7 masl (*Badan Pusat Statistik Kabupaten Kulon Progo*, 2019).

Alternaria porri on onion occured following a long period of relative humidity (>90%) or dew deposition and temperatures ranges between 20º–25º C (Gupta & Pathak, 1986; Evert & Lacy, 1996). High humidity was not the only factor caused disease infection, but it has to be supported by the presence of thin film water on the leaf surface at least for 4 hours since the attachment of the conidia on the leaves (Hadisutrisno *et al.,* 1996).

At seven weeks after planting disease severity of purple blotch was observed on shallots treated with *Trichoderma* sp. and control (0.89%), while shallots treated with AMF showed 0.44% (Figure 2). The interaction between AMF and host plants has been reported to inhibit proliferation of necrotrophic pathogens and decrease disease symptoms, such as *Alternaria solani* and *Botrytis cinerea* on tomatoes (Pozo*et al.,* 2010; de la Noval *et al.,* 2007) and *Magnaporthegrisea* on rice (Campos-Soriano *et al.,* 2012). Interaction between AMF and host plant may lead to systemic protection including protection of above-ground organs as the fungi can activate local and systemic resistance (Xavier & Boyetchko, 2004). Decrease in disease severity may also due to lignification of plant cell wall as a result of AMF colonization. Lignification is considered an important mechanism for disease resistance and it may contribute to reducing proliferation in vascular tissues in root plant that use AMF (Kapulnik & Douds, 2000). AMF-inoculated plants had increased disease resistance possibly due to morphological alterations, such as thickening of the cell wall by lignification (Al-Raddad, 1987). Thickening of the cell wall through lignification and production of other penetration and growth of pathogens like *Fusarium oxysporum*, *Phomaterrestris* and *Meloidogyne incognita* have been demonstrated (Bagyaraj, 2014).

Purple blotch disease is a foliar disease. In this study, purple blotch disease severity in shallots treated with *Trichoderma* sp. were similar with control. In this case, *Trichoderma* sp. was not able to induce plant resistant to purple blotch disease. Another study also reported that *Trichoderma* sp. was not able to induce plant resistance. Bahramisharif & Rose (2018) reported that the application of *T. harzianum* and oak-bark compost in planting medium increased disease severity of tomato late blight. The application of *T. harzianum* combined with oak-bark compost negatively affected the root growth and resulted in significantly higher disease severity in both whole plant and detached assays. In contrast, application of *B. subtilis* subsp. *subtilis* combined with oak-bark compost effectively reduced the disease severity.

The Effects of Trichoderma *and AMF on the Growth and Yield of Shallot*

Application of *Trichoderma* sp. resulted more higher plant than AMF and control at five week after planting (Figure 3). *Trichoderma* sp. and AMF application tended to increase plant height and number of leaves than control between at five and four weeks. Number of leaves was different among all treatments (Figure 4). Application of AMF resulted in more number of leaves than *Trichoderma* sp. and control at four weeks after planting. This was probably due to the fungal effects on shallot roots and the increased in nutrition absorption.

The ability of *Trichoderma* to increase plant growth was reported in other studies. Doni *et al.* (2014) reported that *Trichoderma* spp. were able to

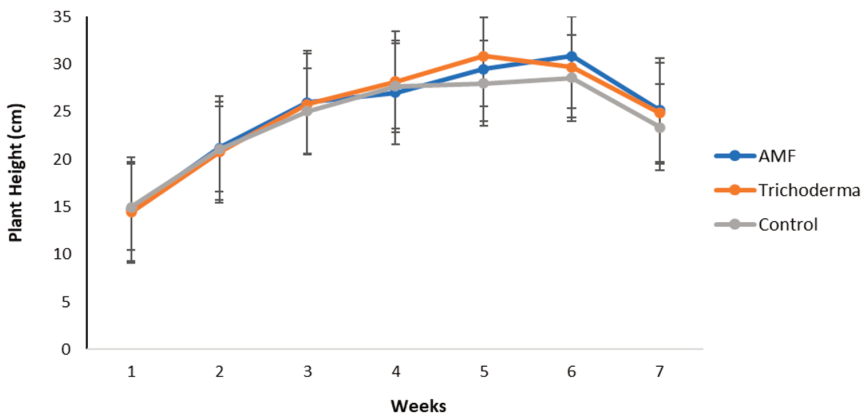

Figure 3. Plant height in all treatments during seven weeks of observation with vertical bars representing standard errors

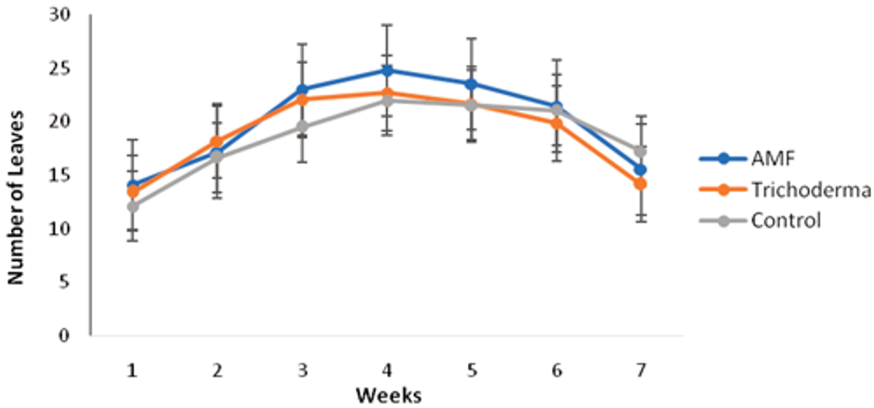

Figure 4. Number of leaves in all treatments during seven weeks of observation with vertical bars representing standard errors

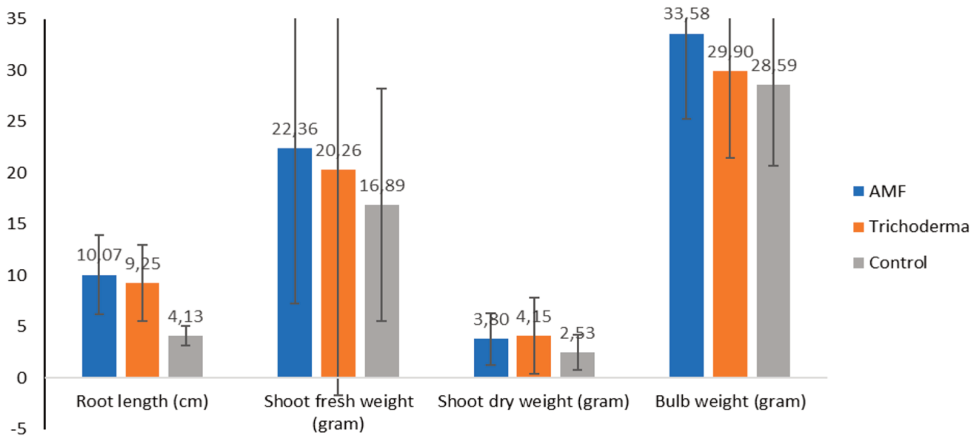

Figure 5. Root length, shoot fresh and dry weight, bulb weight in all treatments after seven weeks of observation with vertical bars representing standard errors

enhance rice growth components including plant height, and also leaf number, tiller number, root length and root fresh weight. The application of *Trichoderma* as shallot seed treatment increased plant height, the leaf area index, extensive root, net assimilation rate (NAR), plant growth rate, Nitrate Reductase Activities (NRA), total chlorophyll, and fresh bulb weight (Darsan *et al.,* 2016).

Several studies have also reported that AMF were able to increase plant height and number of leaves (Shuab *et al.* 2014). Balliu *et al.* (2015) found that AMF increased N, P, K, Ca absorption, leaf area and tomato plant growth. The symbiosis of AMF with root of their host plant allows mycorrhizae to obtain essential nutrients in exchange of N, P, K, Ca, Zn, & S for plant. Arbuscular mycorrhizal fungi produce arbuscular structures which help increase inorganic minerals, carbon compounds, and phosphor exchanges (Li *et al.,* 2016; Prasad *et al.,* 2017). Therefore, they significantly boost the phosphorus concentration in both root and shoot systems (Al-Hmoud & Al-Momany, 2017). Under phosphorus limited condition, AMF root association improves phosphorus supply (Bucher, 2007).

This research showed that the AMF application tended to result in higher shoot fresh and dry weight, bulb weight, and root length then those in control (Figure 5). Widi *et al.,* (2010) also found an increase of tuber weight of shallot after AMF application. In addition, AMF was also reported to increase shallot bulb diameter, bulb weight, and bulb fresh weight per cluster (Hidayat *et al.,* 2018). Mycorrhizal fungi colonization causes more effective macro nutrient absorption which increases photosynthetic products and hence results in biomass accumulation (Begum *et al.,* 2019).

AMF colonization is widely believed to stimulate nutrient uptake in plants. It is evident that inoculation of AMF can enhance the concentration of various micro-nutrients and micro-nutrients significantly, which leads to increase photosynthate production and hence increase biomass accumulation (Chen *et al.,* 2017; Mitra *et al.,* 2019). Evelin *et al.* (2012) stated that AMF improve the uptake of almost all essential nutrients and contrarily decrease the uptake of Na and Cl, leading to growth stimulation. Arbuscular mycorrhizal fungi facilitate uptake of soil nutrients, especially of N & P, which can effectively promote the growth of host plant (Smith *et al.,* 2011).

The application of *Trichoderma* sp. also tended to increase the shoot fresh and dry weight and the root length (Figure 5). The direct beneficial effects of *Trichoderma* sp. on plants are promoting and improving plant root growth and structure, improving seed vigor and growth, and decomposing, recycling, and utilizing soil nutrients (Harman, 2011; Howell *et al.,* 2000; Shoresh *et al.,* 2010). According to Levy *et al.* (2004) *Trichoderma* directly affects plant growth by producing plant growth-regulating hormones. *Trichoderma* can also break down organic matter in soils, so it is easily being absorbed by the plants. Inoculation of *Trichoderma asperellum* produced better leaf greenness, stomata opening width, number of roots, plant height, number of plant leaves, fresh bulb weight per plant (Setyaningrum *et al.,* 2019). Sutarman *et al.* (2018) also found that *Trichoderma* formulated in spent substrate of oyster mushroom increased the height and the number of leaves on shallot.

Effects of Arbuscular Mycorrhiza Fungi and Tri*choderma Application on Disease Resistances of Shallot Bulb*

The result showed that AMF and *Trichoderma* sp. treatments showed lower infection of *F. solani* than control. The lowest disease infection was observed in AMF treatment (Figure 6). *Fusarium solani* infection caused necrotic symptoms on shallot basal plate resulting in death and brown color of plant tissue. Shallots treated with AMF and *Trichoderma* sp. resulted in lower infection compared to the control. This will be beneficial for shallot bulb used for planting material which may carry seed-borne pathogen, such as *F.solani.* This result was consistent with Niemira *et al.* (1996) that reported a suppression of postharvest damages caused by *Fusarium sambucinum* on potato minitubers, in AMF inoculated plants.

Sari (2016) stated that one of mechanisms of AMF in suppressing the disease development is by inducing natural plant protection mechanisms, such as the accumulation of salicylic acid in leaves, cell wall lignification, narrower stomata openings, and increase of nutrient absorption. Salicylic acid is a compound which signals plant protective mechanism (Pozo & Azcon-Aguilar, 2007). AMF can also indirectly induce lignification that acts as barrier on cell walls which inhibits pathogen penetration (Sari, 2016). Lignin is believed to disrupt hydraulic

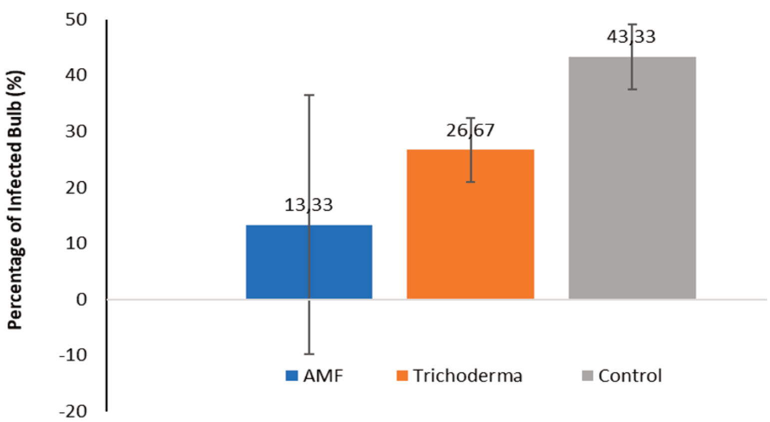

Figure 6. Percentage of infected shallot bulb in all treatments with vertical bars representing standard errors

enzymatic processes, pathogen penetration mechanism, also water and molecule exchange between plants and pathogen (Nicholson & Hammerschmidt, 1992). Lignin are able to [i]improve disease resistance and enhance the mechanical strength by cell wall thickening (Yang *et al.,* 2018). Mycorrhiza can also stimulate plant resistance-related enzymes, such as polyphenol oxidase and peroxidase (Al-Askar & Rashad, 2010).

CONCLUSION

The application of AMF and *Trichoderma* sp. tended suppress the development of fusarium basal rot and increase shallot bulb weight. Only the application of AMF tended suppress the development of purple blotch diseases. The application of AMF and *Trichoderma* sp. also tended to result in better plant growth and the yield. The antagonist also increased root length, fresh weight, and bulb weight. In addition, the application of AMF and *Trichoderma* sp. was able to increase shallot bulb resistances against infection of *F. solani.*

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