Jurnal Perlindungan Tanaman Indonesia, Vol. 26, No. 1, 2022: 57–66
DOI: 10.22146/jpti.70673
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

The Potential of *Rhizophagus intraradices* and *Trichoderma asperellum* to Induce Shallot Resistance against Twisted Disease

Hertina Artanti[1)], Tri Joko[1)], Susamto Somowiyarjo[1)], & Suryanti[1)]*

[1)]Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada
Jln. Flora No. 1 Bulaksumur, Sleman, Yogyakarta 55281 Indonesia

**Corresponding author. E-mail: suryanti.faperta@ugm.ac.id*

Received November 24, 2021; revised February 9, 2022; accepted July 29, 2022

ABSTRACT

Twisted disease caused by *Fusarium* spp. is one of the primary diseases on shallots with potency to cause enormous losses by causing stunting and bulb rot. One alternative disease control is the induction of plant resistance since the seed stage. The aim of this study was to determine the content of salicylic acid, jasmonic acid, and phenolic compounds of shallot bulb seeds coated with biological control agents as a resistance response to twisted disease. The shallot cultivar used was Crok kuning. The treatments used in this study were the type of biological control agents, including *Rhizophagus intraradices* and *Trichoderma asperellum,* combined with and seed coating application time (one month before planting and simultaneously at planting). Biological control agents in the form of powder formulation applied as seed coating material and seeds were stored for one month before planting. The results showed that application of biological agents delayed the occurrence of the twisted disease symptoms. The salicylic acid content from plant treated with *R. intraradices* at the time of planting was slightly higher than the control. The jasmonic acid content in *T. asperellum* plants treated at planting was higher than then control. Total phenolic content from plants treated with *T. asperellum* at planting time was higher than the control. In general, application of biological control agent as seed coat did not result in significant increase in salicylic acid, jasmonic acid nor the phenolic compounds, compared to the pathogen infected control.

Keywords: Crok kuning; *Fusarium*; jasmonic acid; phenol content; salicylic acid

INTRODUCTION

Twisted disease is one of the primary diseases of shallot (*Allium cepa* L. var. *ascalonicum*). Lestiyani *et al.* (2016) reported that this disease could be caused by several *Fusarium* species, namely *F. oxysporum F. solani*, and *F. acutatum*. This pathogen can spread through seeds, soil, or water. Symptoms begin with yellowing on the leaf's tip that then spread to the base, leaves will then wither, twist, and dry up, followed by rotting of the tuber disc and death of the plant (Fitriani *et al.*, 2020). Asaad *et al.* (2020) reported that this disease could reduce shallot production by more than 50%. Twisted disease is a soil-borne disease where its pathogen can be seed-borne and survive during storage, making this disease even more challenging to control. Disease management among growers primarily rely on the use of synthetic fungicides.

Excessive use of synthetic fungicides has the risk of pathogen resistance and residues both on produce and within the environment. Several preventive measures can be taken to suppress the development of twisted disease, including the application of agricultural lime before planting, the use of resistant varieties, and seed treatment. Seed treatment such as seed coating using synthetic pesticides is a preventive measures commonly implemented by growers. In addition, to synthetic pesticides, alternative coating materials are used including kaolin, ash, limestone, and biological agents.

Some biological control agents have been widely used for seed treatments, including *Trichoderma* sp. and Arbuscular Mycorrhizal Fungi (AMF). *Trichoderma* sp. is a biocontrol agent that can activate plant systemic resistance rapidly. Upon root colonization, the plant produces phytohormones such as salicylic acid,

jasmonic acid, ethylene, abscisic acid, auxin, and gibberellins (Morán-Diez *et al*., 2021).

Trichoderma asperellum Samuels Lieckfeldt & Nirenberg is a biological control agent that has the potential to control twisted disease. Seed treatment can accelerate root colonization during early stages of plant growth. As a result of root colonization by *T. asperellum*, there have been reported changes to plant metabolism. A study by Méndez *et al.* (2020) revealed that *T. asperellum* BCC1 has potential as a biocontrol agent because it is antagonistic to *Sclerotium cepivorum* and induces systemic defenses in *Allium cepa* L. mediated by jasmonic acid and ethylene pathway. Inoculation of *T. asperellum* can increase total phenolic content in shallots (Ortega-García *et al*., 2015). Shallot seed coating using *T. harzianum* was more effective in reducing incidence of damping-off and was able to increase seedling resistance against *F. oxysporum* and *F. solani* compared to spraying (Dabire *et al.,* 2016).

Arbuscular mycorrhizal fungi (AMF) is an alternative biological control agents in suppressing soil-borne pathogens. In addition to control plant disease, AMF can enhance plant growth and nutrient uptake, especially phosphorus. Plant-AMF interactions can enhance plant defense through changes in secondary metabolic pathways leading to increase tolerance against biotic and abiotic stresses (Kaur & Suseela, 2020). The application of *R. irregularis* as a coating material for chickpeas (*Cicer arietinum* L.) has reported to increase plant biomass, nutrient absorption and nutrient concentrations within plants that later have impact on increasing productivity (Rocha *et al.,* 2019). Shallots inoculated with AMF and *Trichoderma* sp. had low *Fusarium* wilt disease severity of 0.89% and 1.78%, respectively, at seven weeks after planting. Low disease severity on plants also demonstrated interaction between biocontrol agents and plant host roots (Afiefah *et al*., 2020). Induced resistance using *Rhizophagus irregularis* is known to affect the transcription of pathogenesis-related protein (PR) genes thereby increasing systemic acquired resistance (SAR), as well as increasing the regulation of enzymes involved in jasmonic acid biosynthesis in mycorrhiza-treated cotton (Zhang *et al.,* 2018).

The interaction between biological agents and host plants can result in plant defense responses against pathogens (Wardhika *et al*., 2014). Induction of plant resistance using biological control agents induced systemic resistance by producing various defense compounds. The purpose of this study was to determine the content of salicylic acid, jasmonic acid, and phenolic compounds in shallots coated with biological agents as an indicator of resistance response against twisted disease.

MATERIALS AND METHODS

This research was conducted from August until November 2021 in the Greenhouse and Laboratory of Plant Pathology, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada. The experiment was performed using a Completely Randomized Design (CRD). The treatment consisted of five treatment that were combination of biological agents (*Rhizophagus intraradices* N.C. Schenck & G.S. Sm. (synonym *Glomus intraradices*) and *Trichoderma asperellum*), pathogen, and application time (one month before planting and simultaneously as planting). The shallot variety used in this research was Crok kuning. The planting medium used was a mixture of soil taken from a shallot plantations in Gotakan Village, Panjatan District, Kulon Progo Regency, mixed with goat manure with a 2:1 ratio. Soil from shallot plantation was used to imitate growing medium from field conditions.

Seed Coating Application with Biocontrol Agents

Rhizophagus intraradices and *T. asperellum* were mixed with kaolin powder then used as a coating material for the shallot bulbs seed. *R. intraradices* and *T. asperellum* were also mixed with kaolin powder and used as the mixture before. The dose of *R. intraradices* used was 5 g/kg AMF with a spore density of 12.19 spores/g and mixed with 5 g/kg kaolin, while *T. asperellum* used was 5 g/kg seed with a spore density of 4.6×10^6 cfu/g. Seed coating was done by coating the surface of the shallot bulb with the biological agents' mixture until the entire surface of the bulb was covered.

The experiment, was conducted in a completely randomized design (CRD) with five treatments and three replications in each treatment. The combination of treatments for the application of biological agents was: A1C1 = seed coating with *R. intraradices* at the planting time; A1C2 = seed coating with *R. intraradices*

one month before planting; A1C1= seed coating with *T. asperellum* at the planting time; A1C2 = seed coating with *T. asperellum* one month before planting; Control = without seed coating.

Shallot Planting

Shallot planting was done in a 35 × 29 × 12 cm plastic trays, filled with planting media up to 2/3 of the tray. Before planting, approximately 1/3 part of the bottom end of shallot bulbs was first cut and planted using spacing of 10 × 10 cm. Six shallot bulbs were planted per tray.

Pathogen Inoculation

Fusarium solani inoculation was performed 14 days after planting (DAP) by applying 10 mL of spore suspension (1.6×10^6 spores/ml) per plant (Wijoyo *et al.*, 2019). Roots were wounded using a sterile scalpel and spore suspension was then poured onto the roots. Shallot bulbs that were not coated with biological control agents and inoculated with *F. solani* were used as control. Responses related to induction of plant resistance were observed three weeks after pathogen inoculation, included salicylic acid, jasmonic acid, phenolic content, and calculated Area Under Disease Progress Curve (AUDPC).

Analysis of Salicylic Acid and Jasmonic Acid Content

Analysis of salicylic acid and jasmonic acid content was done at the Laboratory of Agrochemical Residue, Bogor. The analysis was carried out based on methods from Tenhaken and Rubel (1997), which used 1 g of fresh leaves taken from each replicate homogenized with 3 ml of a mixture of methanol and acetone (1:1, v/v), then centrifuged at 5,000 rpm for 10 minutes. The supernatant was discarded, and pellet was extracted using 1 mL of a mixture of methanol and acetone (1:1, v/v) and centrifuged at 5,000 rpm for 10 minutes. The supernatant was then dried using a freeze dryer. The dried residue was suspended using 30% methanol and centrifuged at 5,000 rpm for 10 minutes. The supernatant was analyzed using High-Performance Liquid Chromatography (HPLC). The mobile phase used a solution of methanol-sodium acetate buffer 50 mM pH 4.5 (30:70 in 500 mL), homogenized for 10 minutes using a magnetic stirrer with a flow rate of 0.6 mL/minute. Before use, the sample and mobile phase solution was filtered using a 0.45 m RC cellulose acetate filter membrane. The wavelength used was 280 nm with VP-ODS Ultrasphere column type, UV detector at λ 280 nm.

Analysis of Total Phenolic Content

Analysis of total phenolic content was done at the Laboratory of Food Technology and Agricultural Products Analysis, Faculty of Agricultural Technology, Universitas Gadjah Mada. As much as 1 mL of the sample solution was diluted to a total volume of 100 mL, then 1 mL was taken and diluted again to a total volume of 10 mL to obtain total dilution of 1000x (fp = 1000x). The diluted solution of 1 mL was sampled, and then 5 mL of 2% Na_2CO_3 was added, and left for 10 minutes. Folin-Ciocalteu solution of 0.5 mL was added, then vortexed and left for 30 minutes (Senter *et al.*, 1989; Plumer, 1971 as cited in Susilowati *et al.*, 2014). The absorbance was measured at a wavelength of 750 nm. The phenolic concentration was calculated based on the standard curve obtained from the pure phenol solution of 10–50 ppm as follows:

$$\% \text{ Phenol} = \frac{\text{x. fp. } 100\%}{\text{mg sample}}$$

Determination of Twisted Disease Intensity and Area Under Disease Progress Curve (AUDPC)

Twisted disease intensity was observed once a week and calculated using the following formula (Wibowo *et al.*, 2010):

$$\text{Disease Intensity} = \frac{\Sigma(ni \times vi)}{Z \times N} \times 100\%$$

n = number of infected plants having the same score; v = severity score; Z = maximum rating scale number; N = total number of plants observed.

Twisted disease symptom severity scores were categorized as follow: 0 = Symptomless; 1 = Leaf yellowing appears; 2 = The yellowing leaf area developing and leaves began to wilt; 3 = The wilt leaf developing, above half of the leaves yellowed and wilted; 4 = The tuber began to rot; 5 = The plant dies.

The AUDPC value was determined using the formula from (Cooke *et al.*, 2006) as follows:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

AUDPC = the area under the disease progress curve; n = total number of observations; Yi = assessment of disease intensity at the i^{th} observation; ti = time at the i^{th} observation.

RESULTS AND DISCUSSION

Salicylic Acid (SA) Content in Shallot Leaves

The salicylic acid content from *R. intraradices* treatment applied simultaneously at planting time slightly increased compared to the control (Figure 1). AMF is considered as a plant pathogen at early stage of initiation, thereby triggering plants to activate defense signals associated with biotrophs pathogens. In the early stages of colonization, AMF triggers salicylic acid production and has strong effect on early stages of AMF formation (Kaur & Suseela, 2020). These results were consistent with research by Poveda *et al.* (2019), which explained that AMF is biotrophic fungi that make them sensitive to the defense response associated with salicylic acid. This resistance response prevents the fungus from entering the vascular system. During the early interaction between fungi and plants, symbiosis process occurs by suppressing plant defense response associated with salicylic acid, followed by an increase in defense of the jasmonic acid pathway until an appropriate interaction is formed. The alter-nation of salicylic acid to jasmonic acid-dependent defense responses occurred after colonization is established in roots.

In this study, biological control agents were not able to significantly increase the salicylic acid content. This probably have to due to the length of the sampling period, which was conducted at three weeks after pathogen inoculation. According to Yuan *et al.* (2019), salicylic acid content in cucumber leaf was detected and significantly increased at 96 hours after inoculation of *T. longibrachiatum* H9.

Jasmonic Acid (JA) Content in Shallot Leaves

The jasmonic acid content in *T. asperellum* treatment applied simultaneously at planting was relatively higher than control (Figure 2). *Trichoderma asperellum* is known to mediate induce systemic resistance (ISR) by jasmonic acid signaling pathway. Jasmonic acid is an essential phytohormone in regulating plant resistance system triggered by a beneficial microorganism such as *T. asperellum*. Pieterse *et al.* (2014) reported that jasmonic acid regulates the induction of plants' systemic resistance triggered by beneficial microbes such as *Pseudomonas*, *Bacillus*, *Trichoderma*, and AMF. In PGPF, several elicitors with defense activating properties have been identified such as xylanases and cellulases together with proteins and peptides with more specific defense eliciting functions such as Sm1 from *T. virens*.

Trichoderma asperellum produces hydrophobin class 1 (*TasHyd1*), which is associated with its attachment to the root surface, protection of hyphal tips against plant defense compounds, and later associated with plant defense responses and induction of plant resistance. In addition, aspartyl protease enzymes identified in *T. harzianum* and *T. asperellum* were

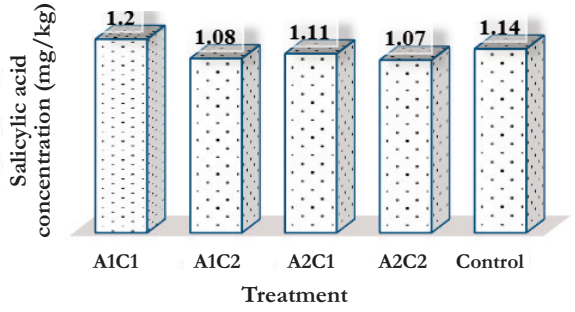

Figure 1. The salicylic acid content from shallot leaves treated with seed coating using biological agents three weeks after the inoculation of *Fusarium solani* (A1C1 = seed coating with *Rhizophagus intraradices* at the same time as planting; A1C2 = seed coating with *R. intraradices* at one month before planting; A2C1 = seed coating with *Trichoderma asperellum* at the same time as planting; A2C2 =seed coating with *T. asperellum* at one month before planting; Control = without seed coating)

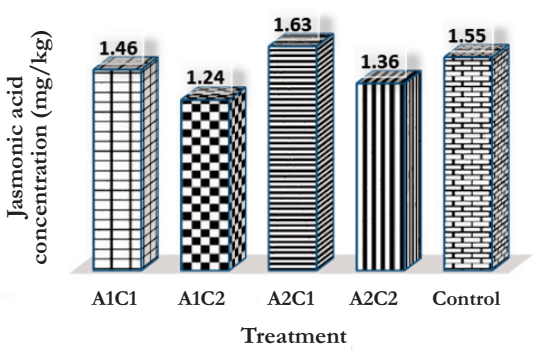

Figure 2. The jasmonic acid concentration in shallot leaves treated with seed coating using biological agents three weeks after the inoculation of *Fusarium solani* (A1C1 = seed coating with *Rhizophagus intraradices* at the same time as planting; A1C2 = seed coating with *R. intraradices* at one month before planting; A2C1 = seed coating with *Trichoderma asperellum* at the same time as planting; A2C2 = seedcoating with *T. asperellum* at one month before planting; Control = without seed coating)

involved in mycoparasitism, increased plant resistance, and induction of secondary metabolites such as phytoalexins (Ent *et al.*, 2009). Nawrocka and Małolepsza (2013) reported that *Trichoderma* colonization associated with the induction of salicylic acid, jasmonic acid, and ethylene pathways in the same plant, which might indicate the presence of an alternate mechanism of *Trichoderma*-induced resistance. In addition, it may also imply complicate signaling network connecting SAR and ISR pathways of defense responses, depending on the plant species, *Trichoderma* strain, and the target pathogen.

Total Phenolic Content in Shallots Leaves

The total phenolic content of each treatment did not show significant differences (Figure 3). The application of biological control agent did not trigger plants to respond pathogen attack by producing higher phenolic compounds that can inhibit pathogen growth.

The colonization phase of biological agents determines the level of phenolic compounds in induced plants. The same results in all treatments were presumably because the biological agents were in the early colonization phase; hence, the phenol accumulation did not show any difference between treatments. Yao *et al.* (2007) reported that differences in the developmental phase of AMF colonization caused differences in the level of phenolic compounds.

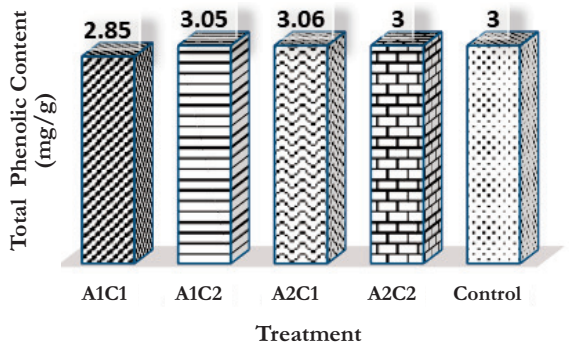

Figure 3. The total phenolic compound concentration in shallot leaves treated with seed coating using biological agents three weeks after the inoculation of *Fusarium solani* (A1C1 = seed coating with *Rhizophagus intraradices* at the same time as planting; A1C2 = seed coating with *R. intraradices* at one month before planting; A2C1 = seed coating with *Trichoderma asperellum* at the same time as planting; A2C2 = seed coating with *T. asperellum* at one month before planting; Control = without seed coating)

A significant increase occurred in the final phase of colonization when appressoria formation took place in the roots. In the early colonization phase, when arbuscular, vesicles, and spores were formed, the content of phenolic compounds were low.

In this study, the application of *T. asperellum* did not increase phenolic compound. These results were different from results of Ortega-García *et al.* (2015) that showed increase of phenolic compounds as a result of the interaction between onion roots and *T. asperellum*. Such difference in the result was may be due to inadequate root colonization by *T. asperellum*. Different *Trichoderma* isolates and onion variety influenced the synthesis of phenolic compounds.

Effect of Biological Agents Coating Application on Twisted Disease Intensity

Symptoms occurred during the first week after pathogen inoculation. The early symptoms observed were leaf yellowing, turning pale green, curling and twisting, then wilting and drying out (Figure 4). The tuber was undeveloped and rot, then the plant dies. This symptom was similar to the twisted disease symptom reported by Lestiyani *et al.* (2016). Symptoms of wilting and yellowing are presumably secondary symptoms caused by the disruption of the water translocation from the roots to the rest of the plant. A decrease in chloroplasts causes the change in leaf color to yellow due to fusaric acid produced by *Fusarium* sp., a metabolite compound toxic to plants and not specific to the host only (Agrios, 2005).

Observation of twisted disease intensity on shallot plants treated with biological agent coating showed that the coating could delay the appearance of the twisted disease symptoms (Figure 5). Symptoms began to appear a week after pathogen inoculation in the control treatment. In plants treated with *T. asperellum*, symptoms appeared in the second week after pathogen inoculation while in the treatment of AMF, symptoms appeared in the fourth week after pathogen inoculation.

The incubation period of pathogens in AMF-treated plants was longer than in other treatments. This was possibly related to salicylic acid production. In the early stages of colonization, biological agents are recognized as pathogens by plants. The plants respond by activating defense compounds through the salicylic acid pathway results in salicylic acid

Figure 4. Shallot plants showing symptoms of twisted disease one week after *Fusarium solani* inoculation on control treatment (yellow arrow)

accumulation. Zubek *et al.* (2015) reported increased salicylic acid content and activation of salicylic acid-dependent signaling pathways were found in the early stages of development of *R. irregularis* in roots. In addition, competition between biological agents and pathogens can prolong the incubation period of pathogens. Competition may occur between the need for nutrients and space for biological agents to colonize roots with *Fusarium* sp. to infect plants. According to Coronado *et al.* (2013), root colonization by *R. intraradices* can protect root tissue from pathogens by competing in same growing space. *R. intraradices* was able to act as a bioprotectant when >90% roots were colonized. Competition for space with other microbes occurs when *R. intraradices* colonization in the root system is high. The success of mycorrhizal fungal colonization can be seen in the ability of mycorrhizal fungi to suppress pathogens and delay the appearance of symptoms up to three weeks after pathogen inoculation. These results were consistent with Talanca (2010), who explained that AMF used more carbohydrates before being excreted in the form of root exudates and causes pathogens not to grow.

The application of biological agents acted as a bioprotectant that inhibited *F. solani* infection led to longer incubation period in the treated plants than in control plants. The longer incubation period of the pathogen was probably related to the antagonistic ability of biological agents. Himaya *et al.* (2021) reported that AMF colonization establishes beneficial interactions for plants and can reduced damage caused by soil borne pathogen and nematodes. These antagonist mechanisms include reducing the site of infection for pathogen through space competition, alteration in root morphology, changes in the composition of root exudate and advancement of crop development, and ultimately plant immune structure stimulation. Marzuki and Assad (2021) reported that the application of *Trichoderma* sp. at various doses could suppress the development of twisted disease up to 25 days after planting. In addition, Fitriani *et al.* (2020) also reported that the treatment of a single application of arbuscular mycorrhizal fungi on shallots inoculated with *Fusarium oxysporum* f.sp. *cepae* had the most prolonged incubation period compared to other treatments.

Even though the application of biocontrol agents prolonged the incubation period of twisted disease, the treatments could not effectively suppress the disease. The reduction of the disease was only found in the treatment of *T. asperellum* one month before planting (A2C2) (Figure 5). Based on the AUDPC value, this treatment was only able to suppress the twisted disease by 12.33% (Figure 6).

Relatively lower AUDPC values were found in the treatment of *T. asperellum* one month before planting (A2C2) with the value of 327. This treatment was able to suppress the development of *F. solani*. The ability of *Trichoderma* to suppress pathogens

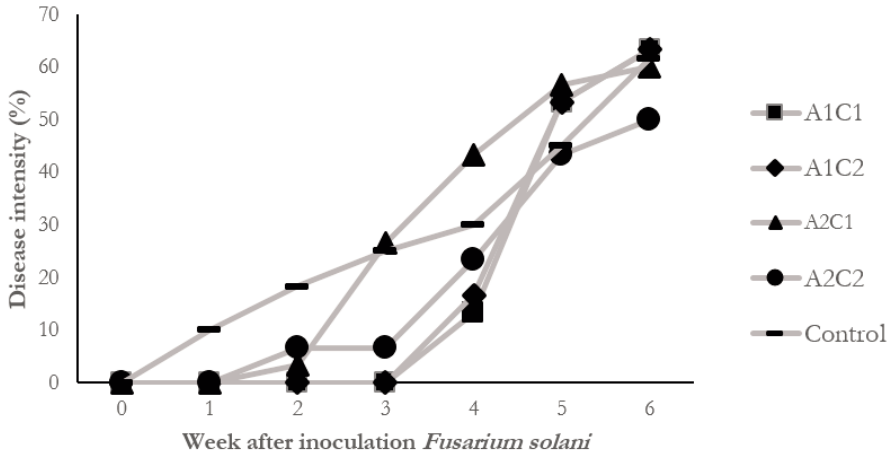

Figure 5. The development of disease intensity and the time of appearance of twisted disease symptoms on shallot plants coated with biological agents (A1C1 = seed coating with *Rhizophagus intraradices* at the same time as planting; A1C2 = seed coating with *R. intraradices* at one month before planting; A2C1 = seed coating with *Trichoderma asperellum* at the same time as planting; A2C2 = seed coating with *T. asperellum* at one month before planting; Control = without seed coating)

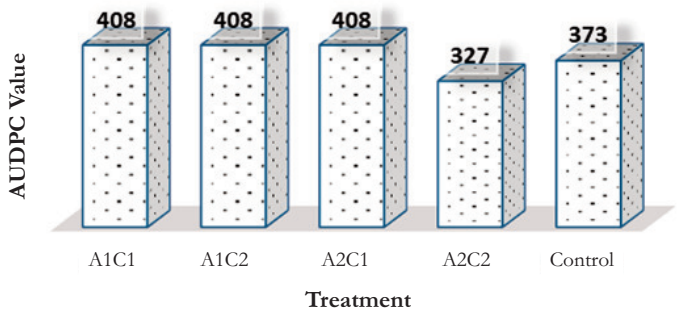

Figure 6. Area Under Disease Progress Curve (AUDPC) value of twisted disease intensity six weeks after the inoculation of *Fusarium solani* (A1C1 = seed coating with *Rhizophagus intraradices* at the same time as planting; A1C2 = seed coating with *R. intraradices* at one month before planting; A2C1 = seed coating with *Trichoderma asperellum* at the same time as planting; A2C2 = seed coating with *T. asperellum* at one month before planting; Control = without seed coating)

was probably due to several mechanisms, such as mycoparasitism. According to Zin and Badaluddin (2020), *Trichoderma* sp. can penetrate host cell walls by forming a hook-shaped structure for penetration. *Trichoderma* sp. hyphae grow along the host hyphae and secretes cell-wall degrading enzymes and secondary metabolites during the penetration process. This activity could suppress the growth of pathogens and even kill the pathogens. Ismail *et al.* (2020) reported that shallot treated with the combination of mulch with *T. asperellum* and compost with *T. asperellum* showed lower disease incidence than untreated shallot, which were 50%, 43%, and 70%, respectively. *T. asperellum* penetrated roots and suppress of *Fusarium oxysporum* f.sp. *cepae* by direct and indirect mechanism. Direct mechanism is based on the ability of *Trichoderma* to parasitize and produce antibiotics while indirect mechanisms are based on *Trichoderma* ability to trigger induce systemic resistance (ISR) and inhibit pathogen.

In this study, seed coating treatment using *R. intraradices* and *T. asperellum* generally did not result in significant increase of plant defense compounds such as salicylic acid, jasmonic acid and total phenolic content in shallots. However, seed coating treatment using *R. intraradices* and *T. asperellum* was able to delay the appearance of twisted disease symptom respectively 4 and 2 weeks after the inoculation of *F. solani*.

CONCLUSION

Seed coating using *R. intraradices* or *T. asperellum* did not significantly increase the salicylic and jasmonic acid production compared to the pathogen inoculated control. Application *R. intraradices* and *T. asperellum* by seed coating also did not result in significant reduction of twisted disease intensity and AUDPC value, but these biological control agents acted as bioprotectants that delayed the appearance twisted disease symptoms.

ACKNOWLEDGEMENT

This research was financially supported by the Faculty of Agriculture, Universitas Gadjah Mada, through the Excellence Research Grant (No. 1558/PN/PT/2020) as a part of the first author's Master thesis project. The author would like to thank the Research and Development Agency, Ministry of Agriculture, for the opportunity to be a graduate student under the Study Program of Phytopathology, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada.

LITERATURE CITED

Agrios, G. N. (2005). *Plant Pathology* 5th Edition. California, United States: Elsevier Academic Press.

Afiefah, C. N., Suryanti, Joko, T., & Somowiyarjo, S. (2020). Beneficial Effects of Arbuscular Mycorrhizal Fungi and *Trichoderma* on Diseased Shallot. *Jurnal Perlindungan Tanaman Indonesia*, *24*(1), 105–114. https://doi.org/10.22146/jpti.53517

Asaad, M., Rusdi, & Agussalim. (2020). Kajian Pengendalian Penyakit Layu Bawang Merah dengan Biopestisida di Sulawesi Tenggara [The Study of Shallot Wilt Disease with Biopesticide in South Sulawesi]. *Jurnal Pengkajian dan Pengembangan Teknologi Pertanian*, *23*(2), 199–211. Retrieved from https://ejurnal.litbang.pertanian.go.id/index.php/jpengkajian/article/download/9059/9285

Cooke, B. M., Jones, D. G., & Kaye. B. (2006). *The Epidemiology of Plant Diseases* 2nd Edition. Netherlands: Springer. Retrieved from https://www.agrifs.ir/sites/default/files/TheEpidemiologyofPlantDiseases(BB.M.Cooke)(781402045790)(Springer-2006).pdf

Coronado, R. A. F., Moreno, M. G. C., Ayala, R. D. R., Sanchez, M. A. A., & Mendoza, I. E. M. (2013). Induced Protection by *Rhizophagus intraradices* against *Fusarium* Wilt of Tomato. *Interciencia*, *38*(1), 48–53. Retrieved from https://www.interciencia.net/wp-content/uploads/2017/12/048-c-MALDONADO-6.pdf

Dabire, T. G., Bonzi, I., Somda, & Legreve, A. (2016). Evaluation of the Potential of *Trichoderma harzianum* as a Plant Growth Promoter and Biocontrol Agent against *Fusarium* Damping-off in Onion in Burkina Faso. *Asian Journal of Plant Pathology*, *10*(4), 49–60. https://doi.org/10.17311/ajppaj.2016.49.60

Ent, A. V. D., Wees, S. C. M. V., & Pieterse, C. M. J. (2009). Jasmonate Signaling in Plant Interactions with Resistance-inducing Beneficial Microbes. *Phytochemistry*, *70*(13–14), 1581–1588. https://doi.org/10.1016/j.phytochem.2009.06.009

Fitriani, M. L., Wiyono, S., & Sinaga, M. S. (2019). Potensi Kolonisasi Mikoriza Arbuskular dan Cendawan Endofit dan Kemampuannya dalam Pengendalian Layu Fusarium pada Bawang Merah [Colonization Potential of Arbuscular Mycorrhiza and Endophytic Fungi and Its Effectiveness in Control Fusarium Wilt on Shallot]. *Jurnal Fitopatologi Indonesia*, *15*(6), 228–238. https://doi.org/10.14692/ jfi.15.6.228-238

Himaya, S. M. M. S., Sivasubramaniam, N., & Afreen, S. M. M. S. (2021). A Review on Role of Mycorrhizal Fungi in Plant Disease Management. *Sri Lankan Journal of Technology*, *1*(2), 41–50. Retrieved from https://seu.ac.lk/sljot/publication/vIn2/sljot2.pdf

Ismail, N., Rosmana, A., Sjam, S., & Ratnawati. (2020). Shallot Basal Bulb Rot Management through Integration of *Trichoderma asperellum,* Composted Plant Residues and Natural Mulch. *Journal of Pure and Applied Microbiology*, *14*(3), 1779–1788. https://doi.org/10.22207/JPAM.14.3.16

Kaur, S., & Suseela, V. (2020). Unraveling Arbuscular Mycorrhiza-Induced Changes in Plant Primary

and Secondary Metabolome. *Metabolites*, *10*(8), 335. https://doi.org/10.3390/metabo10080335

Lestiyani, A., Wibowo, A., Subandiyah, S., Gambley, C., Ito, S., & Harper, S. (2016). Identification of *Fusarium* spp., the Causal Agent of Twisted Disease of Shallot. *Acta Horticulturae*, *1128*, 155–160. https://doi.org/10.17660/ActaHortic. 2016. 1128.22

Marzuki, M. I., & Assad, W. (2021). Pengaruh Aplikasi *Trichoderma* sp. terhadap Hasil dan Penekanan Penyakit Moler pada Tanaman Bawang Merah di Lahan Kering pada Musim Penghujan [Effect of the *Trichoderma* sp. Application on Yield and Moler's Disease Suppression of Shallot on Dry Lands in the Rainy Season]. *Jurnal Pengkajian dan Pengembangan Teknologi Pertanian*, *24*(1), 1–11. Retrieved from https://repository.pertanian.go.id/handle/123456789/13544

Morán-Diez, M. E., Martínez de Alba, Á. E., Rubio, M. B., Hermosa, R., & Monte, E. (2021). *Trichoderma* and the Plant Heritable Priming Responses. *Journal of Fungi, 7*(4), 318. https://doi.org/10.3390/jof7040318

Nawrocka, J., & Małolepsza, U. (2013). Diversity in Plant Systemic Resistance Induced by *Trichoderma*. *Biological Control*, *67*(2), 149–156. https://doi.org/10.1016/j.biocontrol.2013.07.005

Ortega-García, J. G., Montes-Belmont, R., Rodríguez-Monroy, M., Ramírez-Trujillo, J. A., Suárez-Rodríguez, R., & Sepúlevda-Jiménez, G. (2015). Effect of *Trichoderma asperellum* Applications and Mineral Fertilization on Growth Promotion and the Content of Phenolic Compounds and Flavonoid in Onions. *Scientia Horticulturae*, *195*, 8–16. https://doi.org/10.1016/j.scienta.2015.08.027

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., van Wees, S. C. M., & Bakker, P. A. H. M. (2014). Induced Systemic Resistance by Beneficial Microbes. *Annual Review of Phytopathology*, *52*(1), 347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Poveda, J., Hermosa, R., Monte, E., & Nicolás, C. (2019). *Trichoderma harzianum* Favours the Access of Arbuscular Mycorrhizal Fungi to Non-host *Brassicaceae* Roots and Increases Plant Productivity. *Scientific Reports*, *9*(1), 11650. https://doi.org/10.1038/s41598-019-48269-z

Rocha, I., Duarte, I., Ma, Y., Alonso, P. S., Latr, A., Vosátka, M., Freitas, H., & Oliveira, R. S. (2019). Seed Coating with Arbuscular Mycorrhizal Fungi for Improved Field Production of Chickpea. *Agronomy*, *9*(8), 471. https://doi.org/10.3390/agronomy9080471

Senter, S. D., Robertson, J. A., & Meredith, F. I. (1989). Phenolic Compounds of the Mesocarp of Cresthaven Peaches during Storage and Ripening. *Journal of Food Science, 54*(5), 1259–1268. https://doi.org/10.1111/j.1365-2621.1989.tb05968.x

Susilowati, T., Kawiji, & Ariviani, S. (2014). Kapasitas Antioksidan dan Kadar Kurkuminoid Ekstrak Rimpang Temulawak (*Curcuma xanthorrhiza*) Menggunakan Pelarut Air dengan Variasi Proporsi Pelarut dan Metode Pemanasan [Antioxidant Capacity and Curcuminoid Content of Temulawak Rhizome Extract (*Curcuma xanthorrhiza*) Using Water Solvent with Variation of Solvent Proportion and Heating Method]. *Biofarmasi*, *12*(2), 83–89. Retrieved from https://smujo.id/jnpb/article/view/2195

Talanca, H. (2010). Status Cendawan Mikoriza Vesikular-Arbuskular (MVA) pada Tanaman. *Prosiding Pekan Serealia Nasional 2010*, 353–357. Retrieved from https://balitsereal.litbang.pertanian.go.id/wp-content/uploads/2016/12/p45.pdf

Tenhaken, R., & Rubel, C. (1997). Salicylic Acid is Needed in Hypersensitive Cell Death in Soybean but Does Not Act as a Catalase Inhibitor. *Plant Physiology*, *115*(1), 291–298. https://doi.org/10.1104/pp.115.1.291

Wardhika, C. M., Suryanti, & Joko, T. (2014). Eksplorasi Bakteri yang Berpotensi sebagai Agens Pengendali Hayati *Fusarium solani* dan *Meloidogyne incognita* pada Lada [Exploration of Bacteria as Biological Control Agents of *Fusarium solani* and *Meloidogyne incognita* on Pepper]. *Jurnal Perlindungan Tanaman Indonesia*, *18*(2), 89–94. Retrieved from https://jurnal.ugm.ac.id/jpti/article/view/15608/

Wibowo, A., Joko, T., Subandiyah, S., Mariska, I., Supriyati, Y., Suryadi, Y., & Roostika, I. (2010). Peningkatan Ketahanan Tanaman Pisang Kepok Kuning terhadap Penyakit Darah melalui Variasi Somaklonal dan Simbiosis Endofitik [Increase Resistance of Kepok Kuning Banana against Blood Disease through Somaclonal Variation and Endophytic Symbiosis]. *Jurnal Perlindungan Tanaman Indonesia*, *16*(1), 15–21. Retrieved from https://jurnal.ugm.ac.id/jpti/article/view/11738

Wijoyo, R. B., Sulistyaningsih, E., & Wibowo, A. (2020). Growth, Yield and Resistance Responses of Three Cultivars on True Seed Shallots to Twisted Diseases with Salicylic Acid Application. *Caraka Tani: Journal of Sustainable Agriculture 35*(1), 1–11. https://doi.org/10.20961/carakatani.v35il.30174

Yao, Q., Zhu, H. H., & Zeng, R. S. (2007). Role of Phenolic Compounds in Plant Defense: Induced by Arbuscular Mycorrhizal Fungi. *Allelopathy Journal*, *20*(1), 1–14. Retrieved from https://www.researchgate.net/publication/286204952

Yuan, M., Huang, Y., Ge, W., Jia, Z., Song, S., Zhang, L., & Huang, Y. (2019). Involvement of Jasmonic Acid, Ethylene and Salicylic Acid Signaling Pathways behind the Systemic Resistance Induced by *Trichoderma longibrachiatum* H9 in Cucumber. *BMC Genomics*, *20*(1), 144. https://doi.org/10.1186/s12864-019-5513-8

Zin, N. A., & Badaluddin, N. A. (2020). Biological Functions of *Trichoderma* spp. for Agriculture Applications. *Annals of Agricultural Sciences*, *65*(2), 168–178. https://doi.org/10.1016/j.aoas.2020.09.003

Zhang, Q., Gao, X., Ren, Y., Ding, X., Qiu, J., Li, N., Zeng, F., & Chu, Z. (2018). Improvement of *Verticillium* Wilt Resistance by Applying Arbuscular Mycorrhizal Fungi to a Cotton Variety with High Symbiotic Efficiency under Field Conditions. *International Journal of Molecular Sciences*, *19*(1), 241. https://doi.org/10.3390/ijms19010241

Zubek, S., Rola, K., Szewezyk, A., Majewska, M. L., & Turnau, K. (2015). Enhanced Concentrations of Elements and Secondary Metabolites in *Viola tricolor* L. Induced by Arbuscular Mycorrhizal Fungi. *Plant and Soil, 390*(1–2), 129–142. https://doi.org/10.1007/s11104-015-2388-6