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SHALLOT RESISTANCE IN INTEGRATION WITH BIOLOGICAL AGENTS TO WILT DISEASE (*FUSARIUM OXYSPORUM* F.SP. *CEPAE*)

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SUMMARY

Shallot (*Allium cepa* L.) is a root crop widely grown and used as a food ingredient and spice in food preparation. Shallot plants have green leaves that grow upright and bulbs that form and develop underground. Wilt disease (*Fusarium oxysporum* f.sp. *cepae*) is an affecting disease that often attacks shallot crops. The presented research sought to determine the influence and interaction between cultivars and biological agents in controlling wilt disease and their correlation with the shallot's growth components. This research transpired from June to October 2023 at the experimental garden, Universitas Samudra, Aceh, Indonesia. The experiment comprised a randomized complete block design (RCBD) with two factors and four replications. The first factor included three shallot cultivars, i.e., Bima Brebes, Tajuk, and Batu Ijo. The second factor was the provision of *Trichoderma*, namely, *T. viride* and *T. harzianum,* and no *Trichoderma* (control). The shallot cultivar Batu Ijo, interacting with *T. harzianum,* showed the best growth characteristics in plant height against the wilt disease, and *T. harzianum* also slowed down the *F. oxysporum* infection rate in shallot plants.

Keywords: Shallot (*A. cepa* L.), cultivars, growth characteristics, *Fusarium oxysporum* f.sp. *cepae*, *Trichoderma* species, wilt disease

Key findings: Shallot (*A. cepa* L.) cultivars, in integration with *Trichoderma* species, showed the best response for growth and development characteristics by revealing tolerance to the wilt disease (*Fusarium oxysporum* f.sp. *cepae*).

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INTRODUCTION

Shallot (*Allium cepa* L.) is a bulbous plant belonging to the onion family (Alliaceae). This plant has tubers serving as food ingredients and spices in various food preparations. Shallots have a sweeter and slightly spicy taste compared to garlic. Shallot is a spice often used in diverse dishes worldwide to provide a distinctive aroma and flavor. Based on the Central Statistics Agency data, domestic shallot production has increased from 1.81 million tons in 2020 to 2.01 million tons in 2021. However, in the Aceh Province, Indonesia, shallot production has decreased from 11,240 tons in 2020 to 10,130 tons in 2021 (Statistics Indonesia, 2022).

Shallot plants usually grow from seeds and parent bulbs. Once planted, the tuber will develop the roots and the leaves that grow upwards. The leaves obtain energy through photosynthesis, providing nutrients for tuber growth. Macronutrients, such as nitrogen (N), can also promote leaf growth. Past studies have proven the close relationship between chlorophyll and nitrogen content (Putri et al., 2021). The reason is that N is a building block for chlorophyll and protein molecule development, which also influences chloroplast formation and chlorophyll accumulation in them. Optimal nitrogen application can promote plant growth, protein synthesis, and chlorophyll production, which causes the leaf color to become dark green (Buda et al., 2018).

Shallots need favorable growing conditions, such as sufficient sunlight, loose and fertile soil, and suitable humidity. Harvested shallot bulbs can serve directly as food and have an extended storage period. Proper postharvest conditions are crucial for onion bulbs' visual quality, storability, and physicochemical composition. For instance, onion curing has considerably improved the anthocyanin and flavonol contents (Oku et al., 2019). The harvesting time (age of the onion) has also appeared to affect the enzyme activity and sugar accumulation in onion bulbs (Gateri et al., 2018).

The shallot demand increases with time; however, its enhancement has no linkage with a significant increase in onion production. Plant diseases caused by various pathogens are the hindrances mainly faced in shallot cultivation. Wilt disease caused by *Fusarium oxysporum* is one of the critical diseases often attacking shallot plants. Nowadays, wilt disease is the deadliest pest in shallot cultivation, and it is generally difficult to manage and control (Deden and Umiyati, 2017). In shallot wilt disease, plants develop false stems and leaves that grow longer and twisted (Djamaluddin et al., 2022).

In the present era, wilt disease management still relies on fungicides. Continuous chemical applications result in environmental degradation, causing disease resistance to frequently used fungicides and toxic residual effects on humans (Syarifuddin et al., 2021). The biological agent, Trichoderma, has emerged as highly effective in controlling the wilt disease and pivotal in suppressing its further development and incidence. The antagonistic fungi *T. harzianum*'s isolation from various plant rhizospheres reached assessment for their effects on several diseases.

T. harzianum can reproduce massively and survive adverse conditions through nutrient and space competition with fungal pathogens (Sood et al., 2020). Besides *T. harzianum*, Turkan et al. (2023) hypothesized that the *T. viride* strain tested with antifungal activity, verifying it on one of the plant pathogenic fungi, *Fusarium culmorum* Sacc. A reported use of *Trichoderma spp*. as a biocontrol agent has reduced Fusarium wilt disease in tomatoes and suggested the mechanism underlying the biocontrol through *Trichoderma spp*. (Li et al., 2018) and its administration on production factors of tomato plants (Sopialena, 2018).

The existence of plant-disturbing organisms (PDO) is an obstacle to cultivating shallot plants. Applying environment-friendly biological agents has produced shallots that are safe for consumption. The presented research sought to determine the influence and interaction between shallot cultivars and biological agents in controlling wilt disease (*Fusarium oxysporum*) and their association with the growth parameters of shallot genotypes.

MATERIALS AND METHODS

Study sites and materials

This study commenced from June to October 2023 at the Samudra University, Aceh, Indonesia, with an altitude of ±25 masl. The materials used in this research were the seeds of three shallot cultivars, Bima Brebes, Tajuk, and Batu Ijo, isolates of *F. oxysporum*, *T. viride*, and *T. harzianum*, sterile water, distilled water, Spritus, 96% alcohol, aluminum foil, cotton, tissue, plastic wrap, PDA media, topsoil, and gloves. The experiment employed a randomized complete block design (RCBD) with two factors and four replications. The first factor comprised three shallot cultivars, namely, Bima Brebes (V1), Tajuk (V2), and Batu Ijo (V3), while the second factor was administering Trichoderma (T): control (T0), *Trichoderma viride* (T1), and *Trichoderma harzianum* (T2).

Preparation of planting medium and materials

The planting medium consisted of a mixture of topsoil, sand, and compost in a 1:1:1 ratio; then, placed into a polybag with a diameter of 40 cm until filled. Before planting, sprinkle the planting medium with water to create a superior environment for planting the seeds. The seeds of all shallot cultivars (Bima Brebes, Tajuk, and Batu Ijo) first underwent cleaning with the ends cut off; then, the seeds were pushed into the planting medium with the cut ends facing upwards.

F. oxysporum and biological agents application

F. oxysporum isolates rejuvenation in petri dishes at room temperature (25 °C–77 °C) took seven days. Then, culturing *F. oxysporum* on rice media followed, with the rice washed and placed in plastic for sterilization using an autoclave for 30 min at a temperature of 121 °C. Next, the removed rice proceeded air-drying at 60 °C, then inoculated with F. oxysporum and incubated for seven days. After seven days, the density measurement of F. oxysporum spores on rice media used a haemocytometer. If the density exceeds 104, dilution followed to obtain a spore density of 104 cfu/g and applied as much as 30 g by planting into the medium (Purwati et al., 2008).

Applying biological agents (*T. viride* and *T. harzianum*) ensued for seven days after planting the shallot seeds. The *T. viride* and *T. harzianum* inoculations into sterilized corn media received incubation for 14 days. After 14 days, the spore density calculations of *T. viride* and *T. harzianum* on corn media transpired, with 30 g applied with a spore density of 104 cfu/g.

Data recorded

Plant height (cm) measurements began after seven weeks of inoculation (WAI) of biological agents using a ruler. The height came from the base of the lower stem to the tip of the tallest leaf. The leaves per plant bore counting at the seven WAI of biological agents. The bulbs on the shallot plants also reached counting after eight WAI. The recorded bulb weight (g) engaged the analytical balance after harvesting and cutting off the leaves.

For wilt disease incidence (DI), measuring commenced 2-5 WAI by looking at the symptoms appearing on the plant and then calculated using the following formula (Kaeni et al., 2014).

$$\mathrm{DI} = \frac{\text{Number of diseased plants}}{\text{Number of healthy plants}} \times 100\%$$

For wilt disease severity (DS), conducting measurements every 2-5 WAI comprised counting the number of symptomatic leaves and matching them with the scale of *F. oxysporum* disease attacks using the following formula (Hekmawati et al., 2018).

$$DI = \frac{\Sigma(n \times v)}{\Sigma(N \times V)} \times 100\%$$

Whereas:
n: number of plants attacked
v: score in each attack category (0–5)
N: number of plants observed
V: score for the highest attack category

Symptoms of damage to the shallot plant leaves were also visible, with a disease score used to measure severity, including score 0 (no symptoms/healthy), score 1 (0%–10% symptoms of leaf twisting attack), score 2 (10%–30% leaf twisting symptoms), score 3 (31%–75% leaf twisting symptoms), score 4 (> 75% leaf twisting symptoms), and score 5 (75%–100% of plants die) (Hadiwiyono et al., 2020).

Statistical analysis

All the recorded data underwent analysis of variance (ANOVA) using Statistical Analysis System (SAS) version 9.4, with the means further compared and separated with a Duncan's Multiple Range Test (DMRT) (Hanafiah, 2016).

RESULTS AND DISCUSSION

***F. oxysporum* symptoms on shallot**

The initial symptoms of the wilt disease from *F. oxysporum* on the shallot plants had yellow leaf tips and non-erect leaf growth distinctions, and some leaves appeared pale (Figure 1). Further disease exploitation caused the leaves to turn yellow and die one by one, and by the final phase, all the leaves were gone, and the tubers rotted. Miftahurrohmah and Wahyuni (2022) reported that at the initial symptoms of *F. oxysporum*, the shallot leaves did not grow upright but twisted starting from the base of the false stem, with several plants stunted. The leaves became pale green, yellowish, and wilted, with the tubers rotting.

Pathological wilting of plants has generally referred to vessel plugging and systemic toxicity. The xylem vascular wilt pathogen causes a fatal disease in crop plants. The proliferation of this pathogen in the xylem brings massive disruption of water and mineral transport, resulting in the infected plants' wilting and dying. After reaching the xylem vascular tissue, it multiplies rapidly, spreading vertically in the xylem sap and horizontally between the blood vessels and into the surrounding tissue. Plants resistant to these pathogens are complex (Andersen et al., 2018).

Figure 1. Initial symptoms of the wilt disease caused by *F. oxysporum* on shallot plants: (a) healthy plants (b) unhealthy plants.

Plants have different responses to disease attacks. However, resistant plants' most effective defense response is forming physico-chemical barriers in the xylem tissue. Structural barriers, namely, tyloses and gel, limit the vertical spreading within the blood vessel lumen (Kashyap et al., 2021). The systemic toxicity theory hypothesizes that toxin(s) produced by *F. oxysporum* is the chief cause of plant wilt via membrane injury and water leakage (Wang et al., 2014). However, the plants suffer from water stress and pathogen infections; an inherent water imbalance mechanism occurs, resulting in leaf rolling (Kadioglu et al., 2012; Sun et al., 2017).

Plant height and leaves per plant

Observations on the plant height and number of leaves per plant recording ensued seven weeks after inoculation with the biological agent in shallot plants (Table 1). Based on the results, the highest plant height resulted in the cultivar Batu Ijo from all the treatments of the Trichoderma. Cultivar Batu Ijo showed the foremost growth compared with two other shallot cultivars (Bima Brebes and Tajuk). Cultivar Batu Ijo also showed superior morphological characteristics with a larger leaf area and bulbs than the other cultivars. Shi et al. (2020) also stated that each cultivar has a different growth capacity under controlled environmental conditions and bore chief influences from genetic and morphological factors, for example, in the elite rice cultivar 9,311.

Plant morphology responds to light intensity that influences the growth according to plant height. On the other hand, Keller (2020) stated that genotypes with a high leaf area affect the assimilate's translocation to plant parts. The recently sourced leaf initially exports its assimilate to the growing shoot tips and blooming leaves. As the new leaves grow from the shoot tip, old leaves move further away from the shoot tip and begin to export assimilates toward the base of the shoot. Assimilation transport is a dynamic process that can be adaptive to the developmental status of the vine and variations and constraints caused by the environment. In contrast, insufficient ATP production reduces carbon assimilation and plant growth under low light intensity. Suwannarut et al. (2023) also reported that low irradiance causes a decrease in stomatal conductance, photosynthesis, and plant growth rate.

The number of leaves showed a nonsignificant difference between the treatments (Table 1). It refers to the three shallot cultivars having the same characteristics regarding the number of leaves produced; however, the leaf area of the cultivar Batu Ijo has thicker and broader characteristics. Applying biological agents did not influence an increase in leaf number.

Table 1. Plant height and leaves per plant at seven weeks after inoculating shallot.

Treatments	Plant height (cm)	Leaves plant^{-1}
V1T0	33.10 bc	25.67
V1T1	28.00 c	17.33
V1T2	38.75 b	24.67
V2T0	29.00 bc	17.67
V2T1	34.65 bc	19.67
V2T2	34.80 b	20.00
V3T0	41.60 a	26.33
V3T1	43.30 a	22.67
V3T2	45.00 a	23.00

Note: Numbers followed by different letters in the same column are significantly different according to DMRT at 5%. V1T0 = Bima Brebes without *Trichoderma*; V1T1 = Bima Brebes with *Trichoderma viride*; V1T2 = Bima Brebes with *Trichoderma harzianum*; V2T0 = Tajuk without *Trichoderma*; V2T1 = Tajuk with *Trichoderma viride*; V2T2 = Tajuk with *Trichoderma harzianum*; V3T0 = Batu Ijo without *Trichoderma*; V3T1 = Batu Ijo with *Trichoderma viride*; V3T2 = Batu Ijo with *Trichoderma harzianum.*

Commonly, biological control agents may not affect non-target organisms. Unfortunately, *Trichoderma spp*., shown as antagonistic strains, do not usually target pathogenic organisms but other microorganisms (Ros et al., 2017). In addition, the plants inoculated with Trichoderma showed decreased cell membrane permeability and malondialdehyde (MDA) content in the leaves.

Additionally, MDA is one of the cell membrane's final lipid peroxidation products. It causes membrane lipid peroxidation, damages the structure and function of biomembranes, and changes the membrane's permeability, which affects a series of physiological and biochemical reactions in the cells. Therefore, MDA content is a widely used indicator of plant membrane damage. Hence, high MDA levels will permanently change the proteins and nucleic acids (Morales and Munne-Bosch, 2019). The higher the membrane permeability and MDA content, the greater the extent of cell membrane damage. The findings of Liu et al. (2014) revealed that *T. harzianum* induced the generation of phytoalexin and lignin in eggplants and enhanced the activities of PAL, PPO, POD, and SOD, which eventually increased the eggplant's resistance to fusarium wilt.

Bulb number and weight

Observations on the number and weight of shallot bulbs recorded after eight weeks from Trichoderma inoculation appear in Table 2. Based on the results, the cultivar Bima Brebes without Trichoderma treatment had the most bulbs, which was not significantly different from Bima Brebes with Trichoderma treatment at the eight WAI compared with other treatments. It is because biological control was the best possible solution, mainly to improve it further to produce pesticide-free shallots meeting global market requirements, instead of chemical control by fungicides such as mancozeb, propineb, and difenoconazole (Priya et al., 2015). Given that fungicides are expensive, frequent use of such fungicides can result in emerging resistant strains of pathogens, and the harvested shallot bulbs may contain pesticide residues. Therefore, shallot plants require biological agents that enhance tuber growth.

Furthermore, *Trichoderma spp*. inoculation of shallot plants encouraged vigorous plant growth with a high number and increased bulb size compared with the control treatment. The significant increase in shallot plant growth and tubers' yield can come from the benefits of *Trichoderma spp*. inoculation. Woo et al. (2014) stated that *Trichoderma spp*. is the most common biocontrol agent against a broad spectrum of pathogens infecting the roots, shoots, and postharvest products. Several researchers reported that seed treatment succeeded in ensuring colonization of *Trichoderma spp*. in the roots, which benefits plants (Xue et al., 2017). Inoculation

Table 2. Bulb number and weight at eight weeks after inoculation in shallot.

Treatments	Bulb number	Bulb weight (g)
V1T0	9.50 a	4.30 c
V1T1	5.50 c	6.95 bc
V1T2	8.50 ab	5.36 c
V2T0	6.75 bc	5.47 c
V2T1	6.00 c	6.75 bc
V2T2	5.25 c	11.03 ab
V3T0	7.00 bc	6.51 bc
V3T1	5.50 c	10.98 ab
V3T2	5.00 c	12.79 a

Note: Numbers followed by different letters in the same column are significantly different according to DMRT at 5%. V1T0 = Bima Brebes without *Trichoderma*; V1T1 = Bima Brebes with *Trichoderma viride*; V1T2 = Bima Brebes with *Trichoderma harzianum*; V2T0 = Tajuk without *Trichoderma*; V2T1 = Tajuk with *Trichoderma viride*; V2T2 = Tajuk with *Trichoderma harzianum*; V3T0 = Batu Ijo without *Trichoderma*; V3T1 = Batu Ijo with *Trichoderma viride*; V3T2 = Batu Ijo with *Trichoderma harzianum.*

of shallots with T. asperellum isolate promoted bulb growth and modulated the number of antioxidant compounds found with reduced fertilizer use (Ortega-García et al., 2015), and alleviate symptoms of the copper stress conditions (Tellez-Vargas et al., 2017).

Wilt disease incidence

The recorded disease incidence of *F. oxysporum* on the shallot plants occurred when the plants showed the first symptoms of attack at the two to five WAI (Table 3). Based on the data, the first disease symptom appeared at two WAI in the cultivar Bima Brebes with the control treatment of Trichoderma. At three WAI, the incidence of *F. oxysporum* on shallot plants increased and showed significant differences between the treatments. Cultivar Bima Brebes treated with *T. viride* displayed early symptoms of *F. oxysporum* wilt infection, followed by the cultivars Bima Brebes and Tajuk with the Trichoderma control treatment, and Tajuk with *T. viride* and *T. harzianum*. At four WAI, shallot cultivar Batu Ijo began to show the symptoms of *F. oxysporum* wilt infection with all the treatments of Trichoderma and, finally, at six WAI, all the treatments demonstrated with *F. oxysporum* infection.

On the other hand, the treatment with *T. harzianum* showed a slower pattern of *F. oxysporum* incidence compared with other treatments. It may be because *T. harzianum* has high adaptability to form a symbiotic relationship with plant roots and has better mycoparasitic abilities than other biological agents. These results agreed with the opinion of Panchalingam et al. (2022), who stated that Trichoderma sp. can produce antagonistic mechanisms in the form of competition for growing habitats and mycoparasites by seizing the nutrients and space near the plant's rhizosphere, consume oxygen in the air, and weaken the growth of plant pathogenic fungi. According to Mohiddin et al. (2021), the growth rate of Trichoderma was much faster than that of plant pathogenic fungi. Therefore, it can effectively inhibit plant pathogenic fungi growth.

Unfortunately, *Trichoderma spp*. appearing as antagonistic strains do not usually target pathogenic organisms and other microorganisms (Ros et al., 2017; Marlin et al., 2018). Several researchers have observed the unintended consequences of *Trichoderma spp*. on soil microbial populations. According to Halifu et al. (2019), cell wall secretion degrading enzymes, such as cellulase, xylanase, and glucanases by Trichoderma species, impair the microbial cells' functions, such as nutrient absorption in the rhizosphere. As a result, the disruption of the microbial community structure occurs, which is consistent with the findings of Ros et al. (2017). However, the genus *Trichoderma spp*. has some advantages over soil microorganisms, according to Cai et al. (2014). These fungi can also enhance plant growth by releasing hormone-like compounds that boost root development and plant growth.

Wilt disease severity

In shallot plants, the observations on the disease severity of *F. oxysporum* were evident when the plants showed symptoms of yellowing leaves, which also matched with the scoring of the severity of *F. oxysporum* (Table 4). Based on the results, the disease severity of *F. oxysporum* in the second WAI to the fifth WAI increased, marked by the spread of *F. oxysporum* attacks on the base of shallot leaves. The high intensity of rainfall at the research location at two to four WAI was the reason that caused the rate of Fusarium wilt infection to accelerate, as supported by environmental factors with high humidity. Following Oktavia et al. (2017), the high rainfall, humidity, and warm temperatures are ideal for enhancing plant diseases, and rainwater splashing sped up the spread of the disease from one plant to another.

Most plant diseases sustained effects from rainy conditions, high soil moisture, and air humidity (Velásquez et al., 2018; Maharijaya et al., 2023; Maulidha et al., 2024). In particular, rain and extreme humidity highly promoted pathogen virulence that infects aerial tissues. Pathogens with high humidity,

Table 3. Wilt disease incidence caused by *F. oxysporum* on shallot plants at 2–5 weeks after inoculation.

Treatments	Disease incidence at weeks (%)			
	2	3	4	5
V1T0	50	75 a	100 a	100 a
V1T1	0	100 a	100 a	100 a
V1T2	0	0 b	100 a	100 a
V2T0	0	75 a	100 a	100 a
V2T1	0	75 a	100 a	100 a
V2T2	0	50 a	100 a	100 a
V3T0	0	0 b	25 b	50 ab
V3T1	0	0 b	25 b	50 ab
V3T2	0	0 b	25 b	25 b

Note: Numbers followed by different letters in the same column are significantly different according to DMRT at 5%. V1T0 = Bima Brebes without *Trichoderma*; V1T1 = Bima Brebes with *Trichoderma viride*; V1T2 = Bima Brebes with *Trichoderma harzianum*; V2T0 = Tajuk without *Trichoderma*; V2T1 = Tajuk with *Trichoderma viride*; V2T2 = Tajuk with *Trichoderma harzianum*; V3T0 = Batu Ijo without *Trichoderm*a; V3T1 = Batu Ijo with *Trichoderma viride*; V3T2 = Batu Ijo with *Trichoderma harzianum.*

Table 4. Wilt disease severity caused by *F. oxysporum* on shallot plants at 2–5 weeks after inoculation.

Treatments	Disease severity at weeks (%)			
	2	3	4	5
V1T0	10	20 a	20 a	20 a
V1T1	0	20 a	30 a	20 a
V1T2	0	0 b	20 a	0 b
V2T0	0	15 a	25 a	15 a
V2T1	0	15 a	30 a	15 a
V2T2	0	10 ab	20 a	10 ab
V3T0	0	0 b	0 b	0 b
V3T1	0	0 b	5 b	0 b
V3T2	0	0 b	5 b	0 b

Note: Numbers followed by different letters in the same column are significantly different according to DMRT at 5%. V1T0 = Bima Brebes without *Trichoderma*; V1T1 = Bima Brebes with *Trichoderma viride*; V1T2 = Bima Brebes with *Trichoderma harzianum*; V2T0 = Tajuk without *Trichoderma*; V2T1 = Tajuk with *Trichoderma viride*; V2T2 = Tajuk with *Trichoderma harzianum*; V3T0 = Batu Ijo without *Trichoderma;* V3T1 = Batu Ijo with *Trichoderma viride*; V3T2 = Batu Ijo with *Trichoderma harzianum.*

especially pathogenic fungi (*Fusarium spp*.), harm and indirectly affect crop yields (Waheed et al., 2023). Lahlali et al. (2022) stated that plant fungal pathogens imposed a chief burden on crop production worldwide. These setbacks are measurable directly through yield losses and indirectly through social, environmental, and economic costs. Like other species, fungal pathogens need exposure to specific environmental conditions to thrive. As a result of the tolerance, fungal pathogens can find their ecological position, which determines the geographic areas and seasons where these pathogens can flourish (Chauchan, 2020).

CONCLUSIONS

The shallot cultivar Batu Ijo has the best plant height characteristic with tolerance to the wilt disease (*Fusarium oxysporum* f.sp. cepae). However, the biological agents of Trichoderma have no significant effects on enhancing the number of leaves. Applying *T. harzianum* to shallot plants slowed down the rate of *F. oxysporum* infection. Rain intensity influences the severity of wilt disease caused by *F. oxysporum*. The study highly recommends cultivating the Batu Ijo variety to reduce the rate of fusarium infection in endemic areas, with planting to proceed after the rainy season.

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