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Evaluation of Beneficial Fungus on Production, Pest, and Disease Incidence in Shallot

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ABSTRACT: The use of beneficial fungi has been widely proven to impact growth and positively reduce pest and plant disease attacks. This study aimed to evaluate the application of three species of beneficial fungi to production, Fusarium wilt incidence, and *Spodoptera exigua* attack. The data were analyzed for variance and further tested using the Tukey test. The results showed an interaction between the three types of fungi with the spraying concentration at the lowest incidence of Fusarium wilt, namely *Trichoderma asperellum* treatment with a 14 g/L⁻¹ (0.00%). Applying *Beauveria bassiana* gave the highest observed chlorophyll index (21.47) and the lowest attack percentage of *Spodoptera exigua* (3.17%). Applying *Trichoderma asperellum*, *Beauveria bassiana*, and *Metarhizium anisopliae* separately, at a concentration of 14 g L⁻¹ gave the best effect on chlorophyll index (21.59), bulb diameter (32.17 mm), fresh bulb weight (15.21 g), dry bulb weight (14.71 g), and production per hectare (16.35 t). These results show that applying three fungi species can reduce pest and disease attacks, stimulate growth, and increase shallot production.

KEYWORDS: *Beauveria bassiana*, *Metarhizium anisopliae*, Shallot, *Trichoderma asperellum*

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INTRODUCTION

Shallots are a type of plant that is popular among Indonesians. In addition to being consumed on a household scale, shallots are an important raw material for culinary industries (Dewi and Sutrisna 2016). Shallots can also be used as medicine because they contain several bioactive compounds, that can improve heart health and prevent cancer and diabetes, while anti-inflammatories can help fight obesity and prevent or treat allergies (Hikmah and Pasail, 2021; Sun et al., 2019).

Shallot production in Indonesia will reach 2 million tons in 2021. This number has increased by 10.42% from 2020, which amount to 1.82 million tons. Shallot production continues to increase with an average of 8% per year (Central Bureau of Statistic, 2022). On the other hand, the price of shallots is very volatile and is a significant contributor to inflation (Nurhayati et al., 2022). Shallot production increases with the increase in harvested area, not because it increases crop productivity. Pest and disease attacks are one of the causes of shallot plants not having high productivity. Pests and diseases that cannot be controlled optimally can result in relatively significant losses, either in the form of loss of yields or decreased quality, which directly reduces farmers' income (Sembiring & Prasetya, 2021).

There are two important plant pests in shallot plants, namely *Spodoptera exigua*, which can cause a decrease in shallot production or yield loss of 32-42%, and fusarium wilt, which can cause a yield loss of 65.49% or more in shallot cultivation in Indonesia (Kusumawati et al., 2022; Hadiwiyono, 2020). The control efforts that farmers often carry out are the use of pesticides. However, increased pesticide use can impact ecosystem instability, residues in crops and processed materials, environmental pollution, and even death in humans (Dhiani et al., 2019). So, it is crucial to do environmentally friendly shallot cultivation.

Applying *Trichoderma asperellum*, *Beauveria bassiana*, and *Metarhizium anisopliae* can potentially control the pests and pathogens of shallots. *T. asperellum* plays a role in controlling Fusarium wilt, while *B. bassiana* and *M. anisopliae* both can infect *S. exigua*. The results of research conducted by Antari et al. (2020) found that using *T. asperellum* inhibited the growth of *Fusarium oxysporum* f.sp. capsici by 100% and increase plant growth. Furthermore, the application of *B. bassiana* affected the attack intensity of *S. exigua* on local Palu shallot plants with the lowest attack intensity (2.02%) in the treatment of 10 g L⁻¹ of water with an interval of 5 days (Razak et al., 2016). The application of *M. anisopliae* mixed with the insecticide Abamectin was recorded to have a high mortality rate in *S. exigua* (Hasyim et al., 2016). Utilization of *B. bassiana* and *M. anisopliae* can also trigger physiological

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mechanisms that promote nutrient absorption, also increase tolerance to abiotic stresses and drought (Petlamul & Prasertsan 2012; Bamisile et al. 2018). Based on the description above, it is necessary to conduct research by comparing the effect of the three types of fungi separately on shallot production, *S. exigua* attack, and incidence of Fusarium wilt disease on shallots.

MATERIALS AND METHODS

Location and Experimental Design

The research was conducted at the laboratory of Plant Diseases, Department of Plant Pests and Diseases, Faculty of Agriculture, Hasanuddin University. Then, field research was carried out at the Teaching Farm, Faculty of Agriculture, Hasanuddin University, Makassar, with coordinates 5° 7'40.07" s 119°28'48.94" E at an altitude of 9 m above sea level. This research was carried out from August to November 2022. This research was carried out in the form of a split plot design experiment, which consisted of the main plots, namely the type of fungus (C). Subplots were concentrations of fungus (K).

Table 1. Combination between type of fungus and concentration

Treatments Code	Combinations
C1K0	<i>T. asperellum</i> 0 g L ⁻¹
C1K1	<i>T. asperellum</i> 7 g L ⁻¹
C1K2	<i>T. asperellum</i> 14 g L ⁻¹
C2K0	<i>B. bassiana</i> 0 g L ⁻¹
C2K1	<i>B. bassiana</i> 7 g L ⁻¹
C2K2	<i>B. bassiana</i> 14 g L ⁻¹
C3K0	<i>M. anisopliae</i> 0 g L ⁻¹
C3K1	<i>M. anisopliae</i> 7 g L ⁻¹
C3K2	<i>M. anisopliae</i> 14 g L ⁻¹

Fungus Propagation

Fungus isolates *T. asperellum* (isolated from cocoa plant in Soppeng, South Sulawesi), *B. bassiana* (isolated from corn plant in Soppeng, South Sulawesi), and *M. anisopliae* (isolated from rubber tree in Banyuwangi, West Java). According to Siahaan et al. (2021). In petri dishes, fungus isolates were grown on potato dextrose agar (PDA). Rice as a growing medium was previously washed using water. After cleaning, it was then soaked in water for two hours. After soaking, put in a plastic bag. Next, the rice medium was sterilized in an autoclave at 121°C for 30 minutes and then cooled. After 14 days since inoculation, a quarter of the media that has grown by fungus is put into 100 g of rice that has been previously sterilized. After that, leave it for two weeks at room temperature.

Field Experiment

Field research included sowing, making beds with a size of 100 cm × 100 cm, and replanting shallots after 40 days after sowing (DAS) in each hole of the bed filled with one plant with a spacing of 15 cm × 10 cm. Maserati is the shallot variety used because it is one of the varieties whose seeds have been mass-produced. Then, application of the fungi *T. asperellum*, *B. bassiana*, and *M. anisopliae* by spraying on the plants after 14 days after planting with a vulnerability of spraying seven days, and spraying stop one week before harvest. Watering is done twice a day. Weeds are controlled physically by uprooting them from around the plantation. Fertilization was done at the age of 15, 30 and 45 days, with NPK (16:16:16) as much as 750 kg/ha and Urea 130.4 kg/ha.

Observation Parameters

The parameters observed in this study were plant height (cm), number of leaves, stomatal opening area (µm²), leaf chlorophyll index, bulb diameter (mm), fresh bulb weight (g), dry bulb weight (g), bulb production per hectares (tons), disease incidence (%) and pest attack percentage (%). Disease incidence of Fusarium wilt was calculated with a formula according to Triwindodo and Tanjung (2020) as follows:

$$DI = \frac{n}{N} \times 100\%$$

Note:

n = number of infected plants

N = total number of plants

While the percentage of *S. exigua* attack was calculated with a formula according to Supartha et al. (2022) as follows:

$$PA = \frac{a}{b} \times 100\%$$

Note:

a = number of attacked plants

b = total number of plants

RESULTS

Vegetative Growth

Treatment of the three types of fungi *T. asperellum*, *B. bassiana*, and *M. anisopliae* with spraying concentrations did not affect shallot plant height at 20 days after planting (DAT), 40 DAT, and 60 DAT observations (Figure 1). Applying *B. bassiana* treatment at a concentration of 14 g L⁻¹ (c2k2) produced the highest plant height at 20 DAT (32.67 cm), 40 DAT (44.39 cm), and 60 DAT (46.62 cm). Whereas without fungal treatment (c1k0), the lowest plant height was observed at 20 DAT (29.99 cm), 40 DAT (37.27 cm), and 60 DAT (42.83 cm).

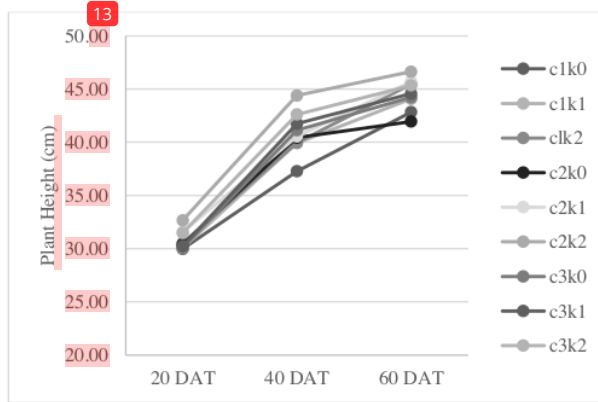


Figure 1. Plant height at 20, 40, and 60 DAT

Three types of fungi *T. asperellum*, *B. bassiana*, and *M. anisopliae*, with spraying concentrations, did not affect the number of leaves at 20 DAT, 40 DAT, and 60 DAT observations (Figure 2). Applying *B. bassiana* with a concentration of 7 g L⁻¹ (c2k1) in the 20 DAT observation produced the highest average of number of leaves (4.57), the treatment of the fungus *B. bassiana* with a concentration of 14 g L⁻¹ (c2k2) produced the highest number of leaves at 40 DAT observations (6.10) and 60 DAT observations (6.86). While the *T. asperellum* treatment at a concentration of 14 g L⁻¹ (c1k2) produced the lowest number of leaves at 20 DAT observations (3.76), without treatment (c2k0) produced the lowest number of leaves at 40 DAT (5.24) and 60 DAT (6.00).

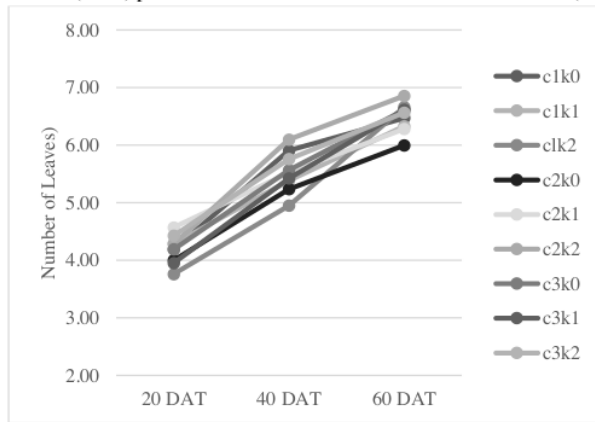


Figure 2. Number of leaves at 20, 40, and 60 DAT

Physiology Traits

The application of the three types of fungi *T. asperellum*, *B. bassiana*, and *M. anisopliae* and dosage did not have a statistical effect on the area of stomata opening. However, it did affect the chlorophyll index (Table 2). The highest stomatal opening area was in the *B. bassiana* treatment (c2) (155.24 μm²) and the lowest in the *T. asperellum* treatment (c1) (107.28 μm²). The highest chlorophyll index parameter was recorded in the *B. bassiana* treatment (c2) (21.47), which was not significantly different from the *M. anisopliae* treatment (c3) (16.91) but significantly different from the *T. asperellum* treatment (c1) (20.56). Likewise, the treatment with the highest chlorophyll index concentration of 14 g L⁻¹ (k2) (21.59) was significantly different from the 0 g L⁻¹ treatment (k0) (16.41) but not significantly different from the 7 g L⁻¹ treatment (k1) (20.93).

Table 2. Stomatal opening area and chlorophyll index

Treatment	Stomatal Opening Area (μm^2)	Chlorophyll Index
Fungus Species		
<i>T. asperellum</i>	107.28	20.56 ^b
<i>B. bassiana</i>	155.24	21.47 ^b
<i>M. anisopliae</i>	123.25	16.91 ^a
Tukey 0.05	ns	2.02
Fungus Concentration		
0 g L ⁻¹	112.26	16.41 ^a
7 g L ⁻¹	138.94	20.93 ^b
14 g L ⁻¹	134.57	21.59 ^b
Tukey 0.05	ns	1.79

Note: means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey multiple comparison test.

Bulb Traits and Production

The application of three types of fungi, *T. asperellum*, *B. bassiana*, and *M. anisopliae* did not affect bulb diameter, fresh bulb weight, dry bulb weight, and production per hectare (Table 3). The 14 g L⁻¹ (k2) concentration treatment produced the highest diameter (32.17 mm), which was not significantly different from the 7 g L⁻¹ concentration (k1) (30.72 mm) and the 0 g L⁻¹ concentration (k0) (29.02 mm). The highest fresh bulb weight was at the treatment concentration of 14 g L⁻¹ (k2) (15.21 g), which was not significantly different from the concentration of 7 g L⁻¹ (k1) (13.91 g) and the concentration of 0 g L⁻¹ (k0) (12.15 g). The highest bulb dry weight parameter was at a concentration of 7 g L⁻¹ (k1) (14.71 g), which was not significantly different from a concentration of 7 g L⁻¹ (k1) (13.27 g) and a concentration of 0 g L⁻¹ (k0) (11.24 g). Likewise, the highest production of shallots per hectare at the treatment concentration of 14 g L⁻¹ (k2) (16.35 t ha⁻¹), which was not significantly different from the concentration of 7 g L⁻¹ (k1) (14.74 t ha⁻¹) and the concentration of 0 g L⁻¹ (k0) (12.49 t ha⁻¹).

Table 3. Bulb diameter, fresh bulb weight, dry bulb weight, production per hectare

Treatment	Bulb Diameter (mm)	Fresh Bulb Weight (g)	Dry Bulb Weight (g)	Production per Hectare (t)
Fungus Species				
<i>T. asperellum</i>	29.65	12.68	12.02	13.35
<i>B. bassiana</i>	31.21	14.37	13.77	15.30
<i>M. anisopliae</i>	31.06	14.21	13.43	14.93
Tukey 0.05	ns	ns	ns	ns
Fungus Concentration				
0 g L ⁻¹	29.02 ^a	12.15 ^a	11.24 ^a	12.49 ^a
7 g L ⁻¹	30.72 ^{ab}	13.91 ^{ab}	13.27 ^{ab}	14.74 ^{ab}
14 g L ⁻¹	32.17 ^b	15.21 ^b	14.71 ^b	16.35 ^b
Tukey 0.05	2.95	2.68	2.53	2.81

Note: Means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey multiple comparison test.

Pest and Disease Observation

Three types of fungi *T. asperellum*, *B. bassiana* and *M. anisopliae* affected the percentage of *S. exigua* attacks on shallots (Figure 4B). In contrast, the concentration treatment did not affect the percentage of *S. exigua* attacks on shallots (Table 4). The lowest percentage of *S. exigua* attack was in the *B. bassiana* (c2) treatment (3.17%), which was not significantly different from the *T. asperellum* (c1) treatment (4.76%) but significantly different from the *M. anisopliae* (c3) treatment (3.49%).

Table 4. Percentage of *S. exigua* attack (%)

Treatment	<i>T. asperellum</i>	<i>B. bassiana</i>	<i>M. anisopliae</i>
0 g L ⁻¹	5.23	3.81	5.23
7 g L ⁻¹	3.34	2.86	3.33
14 g L ⁻¹	3.81	3.34	3.34
Mean	4.76 ^a	3.17 ^b	3.49 ^b
Tukey 0.05		0.84	

Note: Means followed by the same letter are not significantly different for $p \leq 0.05$ according to Tukey multiple comparison test.

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The treatment of three types of fungi *T. asperellum*, *B. bassiana*, and *M. anisopliae*, with different concentrations, affected the incidence of fusarium wilt. The lowest incidence of fusarium wilt was in the *T. asperellum* treatment at a concentration of 14 g L⁻¹ (k2) (0.00%), while the highest incidence was in the *T. asperellum* treatment at a concentration of 0 g L⁻¹ (k0) (3.80%) (Figure 3).

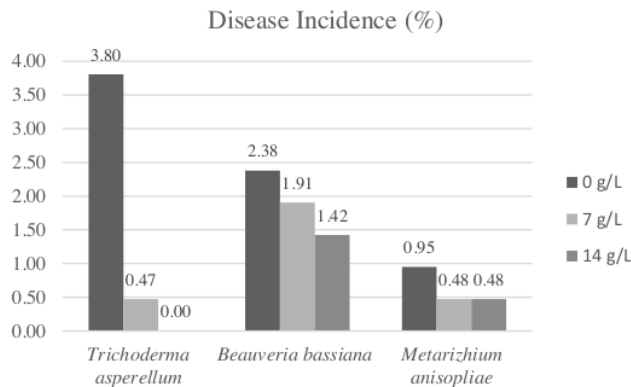


Figure 3. Fusarium wilt incidence

DISCUSSION

Applying *T. asperellum*, *B. bassiana*, and *M. anisopliae* separately and at different doses did not affect shallot plant growth. There was no significant difference in the height and number of leaves of plants not sprayed with fungi or those applied with fungi. The same thing was also found in the observation of the number of leaves, namely the application of *B. bassiana* recorded the highest average number of leaves. Research conducted by Ezpinosa et al. (2019) also found no significant effect between the growth of *Allium schoenoprasum* L. plants inoculated with *B. bassiana* and those that were not inoculated.

Physiologically, the width of the opening of the stomata was not affected by the spraying of the fungus. According to Driesen et al. (2020), guard cells directly regulate stomata opening. These guard cells are susceptible to the external conditions of the plant, including light, carbon dioxide, temperature, and humidity. On the other hand, an increase in chlorophyll index occurred in plants sprayed with the fungus *B. bassiana*. However, in general, the application of the fungi *B. bassiana*, *T. asperellum*, and *M. anisopliae* separately, at a dose of 14 g L⁻¹ (k2), was also proven to increase the chlorophyll index of shallots. Research conducted by Saraghi et al. (2021) found that soaking chili seeds with the fungus *B. bassiana* could increase chlorophyll b and chlorophyll in chili leaves. There is a close correlation between the colonization treatment and the chlorophyll content of chili leaves; the higher the colonization percentage of chili leaves, the higher the leaf chlorophyll content.

Application of the fungi *B. bassiana*, *T. asperellum*, and *M. anisopliae* separately, at a dose of 14 g/L (k2), increased tuber diameter, fresh tuber weight, dry tuber weight, and production per hectare. Increased growth, especially in plant tubers, is closely related to the ability of the plant itself to form assimilates. High chlorophyll levels will linearly increase the capacity of plants to photosynthesize. Chlorophyll is an important pigment that supports the process of photosynthesis (Li et al., 2023). Several studies have proven the positive effect of the application of fungi on plant growth. Mantzoukas et al. (2021) and Mantzoukas et al. (2022) found that *B. bassiana* inoculation influenced root growth in *Vitis vinifera* which was also inoculated with *B. bassiana* and was able to colonize melon and strawberry plants which had an impact on increasing the number of flowers and fruits formed. The study also proved that the fungus *B. bassiana* has two functions at once: an entomopathogen and a plant growth promoter.

Furthermore, the results of research by Liao et al. (2014) and Gonzalez-Perez et al. (2022) found that *M. anisopliae* had a positive impact on corn plants, marked by an increase in cob biomass and several other parameters. As well as providing a growth-promoting effect on tomato, corn, and Arabidopsis plants and increasing plant fresh weight so that it can be used as a growth-promoting microorganism and a biocontrol agent. Furthermore, Scudeletti et al. (2021) and Illescas et al. (2022) found that sugarcane inoculated with *T. asperellum* under dry stress conditions had better photosynthetic rates, stomatal conductance and water use efficiency compared to plants without inoculation. Moreover, *T. asperellum* can increase wheat crop yields in water-deficit conditions.

The lowest percentage of *S. exigua* attacks was recorded in the application of *B. bassiana*. The lowest *S. exigua* attack is undoubtedly influenced by the success of *B. bassiana* as an entomopathogen that can cause death in these pests. Razak et al. (2016) found that applying *B. bassiana* affected the intensity of *S. exigua* attack on local Palu shallot plants with the lowest intensity (2.02%). Furthermore, Wang et al. (2021) suggested that *B. bassiana* produces various poisons, secondary metabolites such as beauvericin, bassianin, bassianolide, beauverolides, tenellin, oosporein, and oxalic acid. This toxin helps *B. bassiana* to parasitize and kill its target insects. *B. bassiana* and *M. anisopliae* also have good potential for developing biological control agents

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for shallot pests against *S. exigua*, *B. bassiana* and *Verticillium lecanii* can kill up to 90% of *S. exigua* and up to 98% of *M. anisopliae* (Hasyim et al. 2017).

The lowest incidence of fusarium wilt in shallots was in the *T. asperellum* treatment of 14 g/L. Applying *T. asperellum* with 25 g doses can suppress fusarium wilt compared to no application. This is because *T. asperellum* is antagonistic to *F. oxysporum*. The results of research conducted by Antari et al. (2020), the highest percentage of fusarium wilt (100%) was in red chili plants in the treatment without *T. asperellum*, whereas the plants given *T. asperellum* did not show any symptoms of fusarium 23. Furthermore, the research results by Herrera-Tellez et al. (2019) found that tomato plants inoculated with *T. asperellum* showed less severe disease symptoms caused by *F. oxysporum* than plants that were not given *T. asperellum*. Furthermore, the research results of Stracquadiano Stracquadiano et al. (2020) found that *T. asperellum* is an antagonist in plant biological control and its ability to produce secondary bioactive metabolites that can be used for plant disease management. In addition, *T. asperellum* is one of the isolates that can inhibit the growth of *F. oxysporum* on *Allium cepa* var. *Ascalonicum* (Karim et al., 2022). Based on the research results, applying three functions can reduce pest and disease attacks, stimulate growth and increase shallot production.

CONCLUSIONS

The results showed an interaction between the three types of fungi with the spraying concentration at the lowest incidence of Fusarium wilt, namely *Trichoderma asperellum* treatment with a 14 g/L⁻¹ (0.00%). Applying *Beauveria bassiana* gave the highest observed chlorophyll index (21.47) and the lowest attack percentage of *Spodoptera exigua* (3.17%). Applying *Trichoderma asperellum*, *Beauveria bassiana*, and *Metarhizium anisopliae* separately, at a concentration of 14 g L⁻¹ gave the best effect on chlorophyll index (21.59), bulb diameter (32.17 mm), fresh bulb weight (15.21 g), dry bulb weight (14.71 g), and production per hectare (16.35 t). These results show that applying three fungi species can reduce pest and disease attacks, stimulate growth, and increase shallot production.

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