

## Interaction between Rice Husk Biochar and Three Species of Fungus on Growth and Yield of Shallot (*Allium ascalonicum* L.)

Padil Wijaya<sup>1</sup>, Elkawakib Syam'un<sup>2\*</sup>, Syatrianty A. Syaiful<sup>3</sup>

<sup>1</sup>Agrotechnology Master Program, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, Indonesia.

<sup>2,3</sup>Agrotechnology Study Program, Departement of Agronomy, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, Indonesia.

**ABSTRACT:** This study aimed to evaluate the application of rice husk biochar and three types of fungi on shallot growth and yield components of shallot. The research was carried out as a Split Plot Design. As the main plot, rice husk biochar with three levels, namely 0 t ha<sup>-1</sup>, 2 t ha<sup>-1</sup>, and 4 t ha<sup>-1</sup>. As subplots, three types of fungus with four levels, namely without fungus/control, *Trichoderma asperellum*, *Beauveria bassiana*, and *Metarhizium anisopliae*. The results showed that there was an interaction between of rice husk biochar 4 t ha<sup>-1</sup> with three types of fungi namely *Trichoderma asperellum* which gave the best results for N content (2.35%) and P content (0.57%) in shallot leaves. Application of rice husk biochar 4 t ha<sup>-1</sup> gave the best results on bulb diameter (35.66 mm). The application of three types of fungi, namely *Trichoderma asperellum*, gave the best results for plant height (43.47 cm), number of leaves (7.30 strands), bulb diameter (35.33 mm), dry bulb weight (21.23 g), yield per hectare (11.56 tons), and the lowest percentage of fusarium wilt disease (1.36%), and *Beauveria bassiana* with the lowest percentage of *Spodoptera exigua* attacks (5.33%). These results indicate that applied rice husk biochar and three types of fungi can increase the growth and yield of shallot plants and suppress *Spodoptera exigua* attack and the incidence of Fusarium wilt disease in shallots.

**KEYWORDS:** *Beauveria bassiana*; *Metarhizium anisopliae*; Rice husk biochar; shallot; *Trichoderma asperellum*

**Published Online:**  
22 May 2023

**Corresponding Author:**  
Elkawakib Syam'un

### INTRODUCTION

Shallot is one of the horticultural commodities which is quite popular in Indonesian society. Shallots are usually much needed as a spice in cooking and traditional medicine. The demand and need for shallots continue to increase from year to year in line with the increase in population, the diversity of needs that is increasing as well as the high purchasing power of the people. Shallot consumption by the household sector in 2021 reached 790.63 thousand tons, an increase of 8.33% (60.81 thousand tons) from 2020, which was 729.82 thousand tons, with an average consumption of shallots reaching 0.56 ounce per capita per week. National shallot production in 2019 was 1,580,247 tons, with a productivity of 9.93 t ha<sup>-1</sup>. Whereas in 2020, production reached 1,815,445 tons with a productivity of 9.71 t ha<sup>-1</sup> (Central Statistics Agency, 2021). This shows that there has been an increase in shallot production, but productivity has not shown a significant increase.

A decrease in soil fertility generally causes a decrease in shallot productivity. Now, cultivation of shallots with high yields generally rely heavily on inorganic fertilizers. However, it turns out that inorganic fertilizers cause many problems of environmental damage (Hawayanti and Palmasari, 2018) and the productivity of agricultural land decreases (Supartha et al., 2012). The long-term and excessive use of chemical pesticides is a huge burden for society and the environment. which also increases pest resistance (Wang et al., 2021). One of the problems in shallot cultivation is the excessive use of inorganic fertilizers and pesticides. As a result, apart from polluting the environment, it will also damage the soil's physical, chemical, and biological fertility, ultimately reducing agricultural land productivity. The extinction of natural microbes that decompose in the soil makes plants vulnerable to pests and diseases that affect the production and productivity of shallots and are not environmentally friendly.

Efforts can be made to overcome these problems by using organic matter in the form of rice husk biochar. Adding organic material in the form of rice husk biochar is an alternative to reducing the use of inorganic fertilizers. Rice husk biochar is an agricultural waste utilization that can increase soil fertility. Using rice husk biochar is one of the main parts of agricultural waste

## Padil Wijaya et al, Interaction between Rice Husk Biochar and Three Species of Fungus on Growth and Yield of Shallot (*Allium ascalonicum* L.)

management, and biochar can increase soil fertility and shallot production (Pakpahan et al., 2020). Rice husk biochar can function as a soil conditioner because it can absorb and retain nutrients and increase soil fertility (Karam et al., 2022). Moisture readiness and nutrient retention capacity are essential for optimal plant growth. Abukari (2019) found that adding 4 t ha<sup>-1</sup> rice husk biochar to the soil increased its water-holding capacity. Rice husk biochar can store large amounts of water due to its high number of micro and macro pores, which has practical and capillary solid strength. Mesopores are also crucial in terms of the transport of solutions within the soil.

In addition, pests and diseases are also a problem in reducing agricultural yield. The main pests in shallot cultivation are *Spodoptera exigua* pest and fusarium wilt disease. *Spodoptera exigua* caused damage to shallot plants around 44.72% to 81.90% before harvest and damage to shallot bulbs reaching 10-12% after harvest (Soumia et al., 2020) and caused a yield decrease of up to 70% (Nilamsari et al., 2021). Meanwhile, fusarium wilt causes shallot yield losses of more than 50% (Supyani et al., 2021). Therefore, it is necessary to control to prevent greater losses.

One of the efforts to control this pest is using endophytic fungi as biological control agents in the form of *Trichoderma asperellum*, *Beauveria bassiana*, and *Metarhizium anisopliae*. In addition, it playing a role in dissolving phosphorus elements bound in the soil and can stimulate plant growth (Wang et al., 2021). *Trichoderma asperellum* also acts as an antagonistic biological control agent against fusarium wilt because of its ability to produce secondary bioactive metabolites, Stracquadanio et al. (2020). The application of *Trichoderma asperellum* increased shallot crop yields by 32.36% in the dry bulbs weight component compared to the control (Septania et al., 2022). Antari et al. (2020) found that the application of *Trichoderma asperellum* was able to produce 100% inhibition of the growth of *Fusarium oxysporum* f.sp. *capsica*. The *Beauveria bassiana* acts as an entomopathogen (it lives as a parasite in insects) so that it can control the pest *Spodoptera exigua*. *Beauveria bassiana* can produce various toxic substances in the form of secondary metabolites that act as parasites and kill insect hosts, Wang et al. (2021). Razak et al. (2016) found that the application of *Beauveria bassiana* can suppress the attack of *Spodoptera exigua* on local Palu shallot plants with the lowest attack intensity (2.02%) at a dose of 10 g L<sup>-1</sup>. Then, testing the effectiveness of using *Beauveria bassiana* and *Metarhizium anisopliae* applied to *Spodoptera exigua* can reduce the percentage of attacks by 14.28% and 11.25%, respectively (Pangestiningasih, 2011). This study aims to evaluate the application of rice husk biochar and three types of fungi (*Trichoderma asperellum*, *Beauveria bassiana*, and *Metarhizium anisopliae*) on the growth and yield of shallots.

## MATERIALS AND METHODS

### Location and experimental design

The research was conducted at the Teaching Farm, Faculty of Agriculture, Hasanuddin University, Makassar, with the coordinates of 5° 7'40.07"S 119° 28'48.94"E at an altitude of 9 m above sea level. This research was conducted from July to November 2022. The research was carried out as a Split Plot Design. As the main plot, rice husk biochar with three levels, namely without biochar/control (b0) = 0 t ha<sup>-1</sup>, b1 = 2 t ha<sup>-1</sup>, and b2 = 4 t ha<sup>-1</sup>. As subplots, three types of fungus with four levels, namely without fungus/control = m0, m1 = *Trichoderma asperellum*, m2 = *Beauveria bassiana*, and m3 = *Metarhizium anisopliae*. There were 12 treatment combinations with three replications to obtain 36 experimental plot units.

### Biochar preparation

Rice husk biochar was prepared by putting rice husk waste into the combustion furnace. The combustion process was carried out in a pyrolysis furnace (incomplete combustion where oxygen in the air does not participate in the combustion reaction). Rice husk burning was carried out in a tightly closed furnace to reduce oxygen availability during combustion at 300°C for 3-4 hours. After the material is entirely black, the fire under the stove is extinguished and allowed to stand until the temperature of the biochar decreases. Furthermore, the biochar is removed from the combustion furnace and ready to be applied according to the treatment dose.

### Fungus preparation

Fungus isolates of *Trichoderma asperellum*, *Beauveria bassiana*, and *Metarhizium anisopliae* were propagated according to the method used by Siahaan et al. (2021). Fungus isolates were cultured on potato dextrose agar (PDA) media in a petri dish. After 14 days after inoculation (DAI), the fungus isolates were propagated on rice media to produce optimal amounts of conidia. Rice grain is a growing medium used to reproduce fungus isolates. As a candidate for growing media, rice was previously washed using water until clean. Then, the rice is soaked in water for two hours. After soaking, put it in a plastic bag and tie it. The rice medium was sterilized in an autoclave at 121°C for 30 minutes and then cooled. Each plastic bag containing the rice medium was inoculated with a fungus suspension on 100 g of the media.

### Preparation land and planting

The research area is cleaned of all rubbish and dirt. Land preparation is done by using a hand tractor. After that, make beds measuring 100 cm x 200 cm. Then do the installation of mulch and make planting holes with a spacing of 15 cm x 15 cm. The pre-planting herbicide was then sprayed with the active ingredient oxyfluorfen 240 g/L. The shallot seedlings were transferred to the previously prepared beds 45 days after sowing with one plant in each hole. Rice husk biochar and *Trichoderma asperellum* were applied before

## Padil Wijaya et al, Interaction between Rice Husk Biochar and Three Species of Fungus on Growth and Yield of Shallot (*Allium ascalonicum* L.)

transplanting by sprinkling in each planting hole. Then, *Beauveria bassiana* and *Metarhizium anisopliae* were applied 14 days after transplanting (DAT) by spraying on the plant parts. Fertilization is done by giving half of the fertilization dose recommended by Hermanto et al. (2017). The pre-planting fertilizer used was elemental phosphate,  $P_2O_5$ , at a dose of 62.5 kg/ha, and the application was carried out seven days before planting. After transplanting, 60 kg/ha of  $K_2O$  and 90 kg/ha of N were applied at 15, 30, and 45 days of age. Harvesting takes place when the plants enter the harvest season and meet the harvest criteria.

### Observation parameters and data analysis

The parameters observed in this study were plant height (cm), number of leaves (strands), bulbs diameter (mm), dry bulb weight (g), yield per hectare (tons), N and P content of shallot leaves (%), the percentage of *Spodoptera exigua* attacks (%), and the incidence of fusarium wilt (%).

Disease incidence of Fusarium wilt was calculated with a formula according to Triwindodo and Tanjung (2020) as follows:

$$DI = \frac{n}{N} \times 100\% \quad (1)$$

Note:

n = number of infected plants

N = total number of plants

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

$$PA = \frac{a}{b} \times 100\% \quad (2)$$

Note:

a = number of attacked plants

b = total number of plants

Observational data were analyzed using analysis of variance (ANOVA). If the results have a significant effect, a Least Significant Difference (LSD) further test is carried out with  $\alpha$  0.05. All data analysis processes were carried out using Microsoft Excel 2019 software.

## RESULTS

### Plant height (cm)

The treatment of rice husk biochar had no significant effect on plant height. However, the treatment of three types of fungi had a significant effect on plant height at 21 DAT, 28 DAT, 32 DAT, and 42 DAT (Table 1). *Trichoderma asperellum* treatment had the highest average plant height at 42 DAT (43.47 cm) which was significantly different from the other treatments. Meanwhile, the treatment without fungi had the lowest average plant height at 42 DAT (40.51 cm).

**Table 1. Plant height at 21, 28, 35, and 42 DAT (cm).**

Treatment	Average number of leaves			
	21 DAT	28 DAT	35 DAT	42 DAT
Rice husk biochar				
0 t ha <sup>-1</sup>	4.21	4.88	5.60	6.78
2 t ha <sup>-1</sup>	4.25	5.03	5.71	6.72
4 t ha <sup>-1</sup>	4.53	5.33	6.24	7.25
LSD 0.05%	Ns	ns	ns	ns
Fungus species				
Control	4.23	4.90 <sup>b</sup>	5.80 <sup>b</sup>	6.71 <sup>b</sup>
<i>Trichoderma asperellum</i>	4.47	<b>5.23<sup>a</sup></b>	<b>6.03<sup>a</sup></b>	<b>7.30<sup>a</sup></b>
<i>Beauveria bassiana</i>	4.33	5.14 <sup>a</sup>	5.81 <sup>b</sup>	6.88 <sup>b</sup>
<i>Metarhizium anisopliae</i>	4.29	5.04 <sup>ab</sup>	5.76 <sup>b</sup>	6.77 <sup>b</sup>
LSD 0.05%	ns	0.21	0.20	0.39

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

### Average number of leaves

The treatment of rice husk biochar had no significant effect on the number of leaves, but the treatment of the three types of fungi had a significant effect on the number of leaves at 28 DAT, 35 DAT, and 42 DAT (Table 2). *Trichoderma asperellum* treatment had the highest average number of leaves at 42 DAT (7.30 strands) which was significantly different from the other treatments. Meanwhile, the treatment without fungi had the lowest average number of leaves at 42 DAT (6.71 strands).

**Table 2. Average number of leaves at 21, 28, 35, and 42 DAT.**

Treatment	Bulb diameter (mm)	Dry bulb weight (g)	Yield per hectare (t)
Rice husk biochar			
0 t ha <sup>-1</sup>	33.68 <sup>a</sup>	18.03	9.82
2 t ha <sup>-1</sup>	33.97 <sup>a</sup>	18.59	10.12
4 t ha <sup>-1</sup>	<b>35.66<sup>p</sup></b>	21.19	11.53
LSD 0.05%	1.39	tn	tn
Fungus species			
Control	33.61 <sup>b</sup>	17.50 <sup>c</sup>	9.53 <sup>c</sup>
<i>Trichoderma asperellum</i>	<b>35.33<sup>a</sup></b>	<b>21.23<sup>a</sup></b>	<b>11.56<sup>a</sup></b>
<i>Beauveria bassiana</i>	34.66 <sup>ab</sup>	19.47 <sup>ab</sup>	10.60 <sup>ab</sup>
<i>Metarhizium anisopliae</i>	34.15 <sup>ab</sup>	18.87 <sup>bc</sup>	10.27 <sup>bc</sup>
LSD 0.05%	1.23	1.82	0.99

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

### **Bulb diameter, dry bulb weight, and yield per hectare**

Treatment of rice husk biochar had no significant effect on dry bulb weight and yield per hectare, but had a significant effect on bulb diameter. While the treatment of the three types of fungi significantly affected bulb diameter, dry bulb weight, and yield per hectare (Table 3). Treatment of rice husk biochar 4 t ha<sup>-1</sup> had the largest average bulb diameter (35.66 mm) which was significantly different from the treatment of 2 t ha<sup>-1</sup> (33.97 mm) and control (33.68 mm). While the treatment of three types of fungi, namely *Trichoderma asperellum* treatment, had the largest average bulb diameter (35.33 mm), not significantly different from the treatment of *Beauveria bassiana* (34.66 mm) and *Metarhizium anisopliae* (34.15 mm), but significantly different from the control (33.61 mm). Then, *Trichoderma asperellum* treatment had the highest average dry bulb weight (21.23 g), which was not significantly different from *Beauveria bassiana* treatment, but significantly different from *Metarhizium anisopliae* treatment and the treatment without fungi. In comparison, the treatment without fungi had the lowest average dry bulb weight (17.50 g). Furthermore, *Trichoderma asperellum* treatment had the highest average yield per hectare (11.56 tons) which was not significantly different from *Beauveria bassiana* treatment, but significantly different from *Metarhizium anisopliae* treatment and the treatment without fungi. In contrast, the treatment without fungi had the lowest average yield per hectare (9.53 tons).

**Table 3. Bulb diameter, dry bulbs weight, and yield per hectare.**

Treatment	Plant height (cm)			
	21 DAT	28 DAT	35 DAT	42 DAT
Rice husk biochar				
0 t ha <sup>-1</sup>	23.03	32.03	37.40	40.58
2 t ha <sup>-1</sup>	22.50	31.76	37.36	41.12
4 t ha <sup>-1</sup>	23.76	34.67	41.26	43.51
LSD 0.05%	ns	ns	ns	ns
Fungus species				
Control	21.84 <sup>b</sup>	31.02 <sup>b</sup>	37.38 <sup>b</sup>	40.51 <sup>b</sup>
<i>Trichoderma asperellum</i>	<b>24.68<sup>a</sup></b>	<b>34.49<sup>a</sup></b>	<b>40.02<sup>a</sup></b>	<b>43.47<sup>a</sup></b>
<i>Beauveria bassiana</i>	23.60 <sup>ab</sup>	33.31 <sup>ab</sup>	39.17 <sup>ab</sup>	41.51 <sup>b</sup>
<i>Metarhizium anisopliae</i>	22.26 <sup>b</sup>	32.46 <sup>ab</sup>	38.12 <sup>ab</sup>	41.45 <sup>b</sup>
LSD 0.05%	1.97	2.39	1.94	1.95

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

### **Leaf nitrogen content (%)**

The observations on the average N content of shallot leaves and analysis of variance showed a very significant interaction between the treatment of rice husk biochar and three types of fungi on the N content of shallot leaves (Table 4). The treatment of rice husk biochar 4 t ha<sup>-1</sup> with *Trichoderma asperellum* had the highest average N content (2.35%), while the lowest in the treatment without rice husk biochar and without fungi (1.36%).

# **Padil Wijaya et al, Interaction between Rice Husk Biochar and Three Species of Fungus on Growth and Yield of Shallot (*Allium ascalonicum* L.)**

**Table 4. Leaf nitrogen content (%).**

Rice husk biochar	Control	<i>Trichoderma asperellum</i>	<i>Beauveria bassiana</i>	<i>Metarhizium anisopliae</i>	LSD
					0.05%
0 t ha <sup>-1</sup>	1.36 <sup>c</sup> <sub>q</sub>	1.80 <sup>a</sup> <sub>r</sub>	1.57 <sup>b</sup> <sub>r</sub>	1.45 <sup>c</sup> <sub>r</sub>	0.09
2 t ha <sup>-1</sup>	1.37 <sup>c</sup> <sub>q</sub>	2.17 <sup>a</sup> <sub>q</sub>	1.87 <sup>b</sup> <sub>q</sub>	1.78 <sup>b</sup> <sub>q</sub>	
4 t ha <sup>-1</sup>	2.05 <sup>b</sup> <sub>p</sub>	<b>2.35<sup>a</sup><sub>p</sub></b>	2.12 <sup>b</sup> <sub>p</sub>	2.07 <sup>b</sup> <sub>p</sub>	
LSD 0.05%	0.10				

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

## **Leaf phosphorus content (%)**

The observations on the average P content of shallot leaves and analysis of variance showed a significant interaction between the treatment of rice husk biochar and three types of fungi on the P content of shallot leaves (Table 5). The treatment of rice husk biochar 4 t ha<sup>-1</sup> with *Trichoderma asperellum* had the highest average P content (0.57%), while the lowest was in the treatment without and 2 t ha<sup>-1</sup> rice husk biochar with without fungi (0.24%).

**Table 5. Leaf phosphorus content (%).**

Rice husk biochar	Control	<i>Trichoderma asperellum</i>	<i>Beauveria bassiana</i>	<i>Metarhizium anisopliae</i>	LSD
					0.05%
0 t ha <sup>-1</sup>	0.24 <sup>c</sup> <sub>q</sub>	0.41 <sup>a</sup> <sub>q</sub>	0.34 <sup>b</sup> <sub>q</sub>	0.35 <sup>b</sup> <sub>q</sub>	0.05
2 t ha <sup>-1</sup>	0.24 <sup>c</sup> <sub>q</sub>	0.43 <sup>a</sup> <sub>q</sub>	0.36 <sup>b</sup> <sub>q</sub>	0.34 <sup>b</sup> <sub>q</sub>	
4 t ha <sup>-1</sup>	0.34 <sup>c</sup> <sub>p</sub>	<b>0.57<sup>a</sup><sub>p</sub></b>	0.44 <sup>b</sup> <sub>p</sub>	0.38 <sup>c</sup> <sub>q</sub>	
LSD 0.05%	0.05				

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

## ***Spodoptera exigua* attack percentage (%)**

The rice husk biochar treatment had no significant effect on the percentage of *Spodoptera exigua* attacks. Meanwhile, treatment using three types of fungi significantly affected the percentage of *Spodoptera exigua* attacks on shallot (Table 6). *Beauveria bassiana* treatment had the lowest average attack percentage of *Spodoptera exigua* (5.33%) which was not significantly different from *Metarhizium anisopliae* treatment, but significantly different from *Trichoderma asperellum* treatment and without fungi. While the treatment without fungi had the highest average percentage of *Spodoptera exigua* attacks (9.64%).

**Table 6. *Spodoptera exigua* attack percentage (%).**

Rice husk biochar	Control	<i>Trichoderma asperellum</i>	<i>Beauveria bassiana</i>	<i>Metarhizium anisopliae</i>	Mean
0 t ha <sup>-1</sup>	10.20	9.52	5.44	6.12	7.82
2 t ha <sup>-1</sup>	9.52	9.86	5.44	5.78	7.65
4 t ha <sup>-1</sup>	9.18	9.18	5.10	5.44	7.23
Mean	9.64 <sup>b</sup>	9.52 <sup>b</sup>	<b>5.33<sup>a</sup></b>	5.78 <sup>a</sup>	
LSD 0.05%	0.52				

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

## **Fusarium wilt incidence (%)**

The rice husk biochar treatment had no significant effect on fusarium wilt. Meanwhile, treatment using three types of fungi significantly affected the incidence of fusarium wilt (Table 7). *Trichoderma asperellum* treatment had the lowest average percentage of fusarium wilt disease (1.36%), which was significantly different from the other treatments. In comparison, the treatment without fungi had the highest average percentage of fusarium wilt disease (5.10%).



Table 7. Fusarium wilt incidence (%).

Rice husk biochar	Control	<i>Trichoderma asperellum</i>	<i>Beauveria bassiana</i>	<i>Metarhizium anisopliae</i>	Mean
0 t ha <sup>-1</sup>	5.44	1.70	4.76	4.42	4.08
2 t ha <sup>-1</sup>	5.10	1.36	4.42	5.10	4.00
4 t ha <sup>-1</sup>	4.76	1.02	4.42	4.42	3.66
Mean	5.10 <sup>b</sup>	<b>1.36<sup>a</sup></b>	4.54 <sup>b</sup>	4.65 <sup>b</sup>	
LSD 0.05%	0.62				

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

## DISCUSSION

The results showed that the application of rice husk biochar 4 t ha<sup>-1</sup> with *Trichoderma asperellum* had a significant effect on bulb diameter (35.66 mm), and there was a very significant interaction with N (2.35%) and P (0.57%) content in leaf shallot. This proves that the combined treatment of rice husk biochar with *Trichoderma asperellum* can increase the physiological response of shallots. The increase in the N content in the leaves is due to biochar being able to maintain the stability of the N element in the soil so that it is not leached and remains available for plants. This is to what was stated by Nguyen et al. (2017), that biochar has a high-water holding capacity so that the N nutrient is maintained and not leached, making it more available to plants. In addition, biochar can increase soil moisture and pH, thereby stimulating N mineralization and nitrification processes which cause plant uptake to increase. The same thing was conveyed by Karam et al. (2022) found that rice husk biochar can function as a soil conditioner because it can absorb and retain nutrients so that it can increase soil fertility. The study's results by Kim et al. (2016) also found that applying rice husk biochar can improve the physicochemical properties of the soil and increase plant growth. Abukari (2019) stated that the addition of 4 t ha<sup>-1</sup> rice husk biochar to the soil could increase the water-holding capacity due to the high number of micro and macro pores so that it has a functional and capillary solid strength. Moisture readiness and nutrient retention capacity are essential for optimal plant growth. This is consistent with the findings of Pakpahan et al. (2020) that the application of rice husk biochar can increase soil fertility so that shallots can produce well.

The application of *Trichoderma asperellum* into the soil also plays a role in plant growth, closely related to the P content in the leaves. One of the roles of *Trichoderma asperellum* is its ability to dissolve phosphorus that are still bound in the soil (Wang et al., 2021). The presence of phosphorus -solubilizing fungi in the soil can accelerate the provision of nutrients for plants to increase plant growth and production. Phosphorus solubilizing fungi can secrete phosphatase enzymes which play a role in the hydrolysis of organic P to inorganic P (George et al., 2002; Sriwantoko et al., 2020) and phosphorus functions in stimulate root growth to form an excellent root system for plants (Triadiawarman et al., 2022). The study's results by Tchameni et al. (2017) suggested that the administration of *Trichoderma asperellum* could increase chlorophyll, P uptake, and acid phosphatase activity. Therefore, the application of rice husk biochar and *Trichoderma asperellum* play a role in the physiological response of plants, especially to the high N and P content in shallots. This also proves that the administration of rice husk biochar with *Trichoderma asperellum* has a significant effect on bulb diameter due to the benefits of biochar as a provider of N elements (Nguyen et al., 2017), so it is suitable for increasing plant growth (Kim et al., 2016) which is supported by the role of element P in forming a great root system (Triadiawarman et al., 2022). The availability and absorption of these promising nutrients also have an excellent effect on bulb diameter.

The treatment of *Trichoderma asperellum* alone also had a significant effect on plant height (43.47 cm), number of leaves (7.30 strands), dry bulb weight (21.23 g), and yield per hectare (11.56 tons). These results indicate that the application of *Trichoderma asperellum* increased growth response and high yield. Tchameni et al. 2017 reported that adding *Trichoderma asperellum* increased the height and number of plant leaves. This result is in line with research by Antari et al. (2020), that the addition of *Trichoderma asperellum* gave better results on the parameters of plant height and the number of leaves compared to those without *Trichoderma asperellum*. In addition, the application of *Trichoderma asperellum* increased shallot crop yields by 32.36% in dry bulb weight components compared to the control (Septania et al., 2022). This is due to the role of *Trichoderma asperellum*, which can dissolve phosphorus that are still bound in the soil so that they become available. These nutrients can be absorbed optimally by plants because *Trichoderma asperellum* can stimulate root growth so that a root system is formed good. The ability of *Trichoderma asperellum* is related to the availability and absorption of these nutrients, so it is closely related to the increase in plant height, number of leaves, dry bulb weight, and yield per hectare. The results of Scudeletti et al. (2021) reported that the use of *Trichoderma asperellum* increased the rate of photosynthesis and water use efficiency and increased the development of roots and stems.

The treatment of *Trichoderma asperellum* significantly affected the lowest percentage of fusarium wilt (1.36%). Besides dissolving phosphorus and stimulating root growth, *Trichoderma asperellum* also functions as a biological controller (biocontrol) that is antagonistic to fusarium wilt. The low percentage of attack is associated with the success of *Trichoderma asperellum* as an

## Padil Wijaya et al, Interaction between Rice Husk Biochar and Three Species of Fungus on Growth and Yield of Shallot (*Allium ascalonicum* L.)

antagonist against endophytic (living in plant tissue) diseases. According to Harman et al. (2004); Ismail et al. (2020), *Trichoderma asperellum* is a non-pathogenic fungi that lives and colonizes endophytic plant roots to protect plants from disease. Apart from roots, *Trichoderma asperellum* is also present as an endophyte found in plants' stem and leaf tissue (Bailey et al., 2009; Rosmana et al., 2018; Ratnawati et al., 2020). The results of Herrera-Téllez et al. (2019), that the treatment of *Trichoderma asperellum* showed low symptoms of *Fusarium oxysporum* disease compared to plants without treatment. Then, Stracquadanio et al. (2020) stated that *Trichoderma asperellum* is an antagonist in biological control because of its ability to produce secondary bioactive metabolites that can be used to control plant diseases. The antagonistic properties are based on the activation of mechanisms that can work synergistically either directly, namely competition for space and nutrients, promotion of growth, and induction of plant defenses. Meanwhile, indirectly, namely mycoparasitism and the production of active metabolites and lytic enzymes (Köhl et al., 2019).

The percentage of *Spodoptera exigua* attacks in the *Beauveria bassiana* treatment significantly affected the lowest percentage of *Spodoptera exigua* attacks (5.33%). This indicates the success of *Beauveria bassiana* as an entomopathogen (which lives as a parasite in insects) so that it can control *Spodoptera exigua* pests. Wang et al. (2021) reported that *Beauveria bassiana* can produce various types of toxic substances in the form of secondary metabolites such as *beauvericin*, *bassianin*, *bassianolide*, *beauverolides*, *tenellin*, *oosporein*, and oxalic acid. This toxic substance acts as a parasite and kills insects that are the target of *Beauveria bassiana*. According to Chu et al. (2017) that indications of symptoms in host insects when infected with *Beauveria bassiana* include severe dehydration, abnormal behavior, lack of coordination, convulsions, eating disorders, and metabolic disturbances, which eventually lead to insect death. The dead host produces many conidia (Feng et al., 2015). These conidia will be carried by air and then infect other hosts: the cycle will continue to become a chain of infection that will continue (Pedrini, 2018). The infection cycle not only depends on successful epidermis penetration but also requires in vivo dimorphic transitions, namely the transformation of conidia into hyphae (Wang et al., 2017). Test the effectiveness of using *Beauveria bassiana* and *Metarhizium anisopliae* applied to *Spodoptera exigua* can reduce the percentage of attacks by 14.28% and 11.25% (Pangestiningih, 2011). Razak et al. (2016) reported that the application of *Beauveria bassiana* could suppress *Spodoptera exigua* attacks on local Palu shallot plants with the lowest attack intensity (2.02%). This is in line with research by Hasyim et al. (2016) that the use of *Beauveria bassiana* was able to kill *Spodoptera exigua* above 90%.

## CONCLUSIONS

There was an interaction between of rice husk biochar 4 t ha<sup>-1</sup> with three types of fungi namely *Trichoderma asperellum* which gave the best results for N content (2.35%) and P content (0.57%) in shallot leaves. Application of rice husk biochar 4 t ha<sup>-1</sup> gave the best results on bulb diameter (35.66 mm). The application of three types of fungi, namely *Trichoderma asperellum*, gave the best results for plant height (43.47 cm), number of leaves (7.30 strands), bulb diameter (35.33 mm), dry bulb weight (21.23 g), yield per hectare (11.56 tons), and the lowest percentage of fusarium wilt disease (1.36%), and *Beauveria bassiana* with the lowest percentage of *Spodoptera exigua* attacks (5.33%). Based on these findings, it can be concluded that using rice husk biochar and three types of fungi can increase the growth and yield of shallots and suppress *Spodoptera exigua* attack and the incidence of fusarium wilt in shallots.

## ACKNOWLEDGEMENTS

The researcher would like to thank Lembaga Penelitian dan Pengabdian Masyarakat (LPPM), Hasanuddin University, with Penelitian Dasar Universitas Hasanuddin (PDU) scheme, for their financial support in conducting this study.

## REFERENCES

1. Abukari, A. (2019). Influence of RHB on water holding capacity of soil in the Savannah ecological zone of Ghana. *Turkish J. Agric – Food Sci. Technol.* 7: 888–891.
2. Antari, N.M., Darmayasa, I.B.G., and Hardini, J. (2020). Effectiveness of *Trichoderma Asperellum* TKD with mediator of manure to control fusarium disease on red pepper plant (*Capsicum annum* L.). *Simbiosis*. 8: 63-71.
3. Bailey, B.A., Strem, M.D., and Wood, D. (2009). *Trichoderma* species form endophytic associations within *Theobroma cacao* trichomes. *Mycol. Res.* 113: 1365-1376.
4. Central Statistics Agency. (2021). National Shallot Plant Production. [Online]. Available at [https://www.bps.go.id/site.\(https://www.bps.go.id/indicator/55/61/1/produksi-tanaman-sayuran.html](https://www.bps.go.id/site.(https://www.bps.go.id/indicator/55/61/1/produksi-tanaman-sayuran.html).
5. Chu, Z.J., Sun, H.H., Zhu, X.G., Ying, S.H., and Feng, M.G. (2017). Discovery of a new intravacuolar protein required for the autophagy, development and virulence of *Beauveria bassiana*. *Environ. Microbiol.* 19: 2806–2818.
6. Feng, P., Shang, Y., Cen, K., and Wang, C. (2015). Fungal biosynthesis of the bibenzoquinone oosporein to evade insect immunity. *Proc. Natl. Acad. Sci. U.S.A.* 112: 11365–11370.
7. George, T.S., Gregory, P.J., Wood, M., Read, D., and Buresh, R.J. (2002). Phosphatase activity and organic acids in the rhizosphere of potential agroforestry species and maize. *Soil Biol. Biochem.* 34: 1487-1494.

## Padil Wijaya et al, Interaction between Rice Husk Biochar and Three Species of Fungus on Growth and Yield of Shallot (*Allium ascalonicum* L.)

8. Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., and Lorito, M. (2004). *Trichoderma* species – opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol.* 22: 43-56.
9. Hasyim, A., Setiawati, W., Jayanti, H., Hasan, N., and Syakir, M. (2017). Identification and pathogenicity of entomopathogenic fungi for controlling the beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae). *Adv. Agric. Bot.* 9: 34-46.
10. Hawayanti, E. and Palmasari, B. (2018). Increasing the production of shallots (*Allium ascalonicum* L.) through fertilizing livestock waste on tidal land. *Klorofil: Jurnal Penelitian Ilmu-Ilmu Pertanian.* 13: 114-122.
11. Hermanto, C., Maharijaya, A., Hayati, I.W., Rosliani, R., Setyawati, A., Husni, I., Sari, M., Wibawa T., Sunarto, B., Kurdi, Adin, A., Julietha, D., Suad, D., Efendi, M., Hariyanto, Nggaro, Y.Y.M., Anggraeni, F., Waludin, J., Sumarno, A., Subardi, and Setiani, R. (2017). Shallot Cultivation Guidelines Using Seeds. Jakarta: Directorate of Vegetables and Medicinal Plants.
12. Herrera-Téllez, V.I., Cruz-Olmedo, A.K., Plasencia, J., Gavilanes-Ruiz, M., Arce-Cervantes, O., Hernández-León, S., and Saucedo-García, M. (2019). The protective effect of *Trichoderma asperellum* on tomato plants against *Fusarium oxysporum* and *Botrytis cinerea* diseases involves inhibition of reactive oxygen species production. *Int. J. Mol. Sci.* 20: 1-13.
13. Ismail, N., Rosmana, A., Sjam, S., and Ratnawati, R. (2020). Shallot basal bulb rot management through integration of *Trichoderma asperellum*, composted plant residues and natural mulch. *J. Pure. Appl. Microbiol.* 14: 1779-1788.
14. Karam, D.S., Nagabovanalli, P., Rajoo, K.S., Ishak, C.F., Abdu, A., Rosli, Z., Muharam, M., and Zulperi, D. (2022). An overview on the preparation of rice husk biochar, factors affecting its properties, and its agriculture application. *J. Saudi. Soc. Agric. Sci.* 21: 149-159.
15. Kim, H.S., Kim, K.R., Yang, J.E., Ok, Y.S., Owens, G., Nehls, T., Wessolek, G., and Kim, K.H. (2016). Effect of biochar on reclaimed tidal land soil properties and maize (*Zea mays* L.) response. *Chemosphere.* 142: 153-159.
16. Köhl, J., Kolnaar, R., and Ravensberg, W.J. (2019). Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. *Front. Plant. Sci.* 10: 845.
17. Nguyen, T.T.N., Xu, C.Y., Tahmasbian, I., Che, R., Xu, Z., Zhou, X., Wallace, H.M., and Bai, S.H. (2017). Effects of Biochar on Soil Available Inorganic Nitrogen: A Review and Meta- Analysis. *Geoderma.* 288: 79–96.
18. Nilamsari, E.I., Nugroho, L.H., and Sukirno, S. (2021). Effectiveness of N-Hexane and ethanol extract of giant calotrope (*Calotropis gigantea* L.) leaves as insecticide against shallot pest *Spodoptera exigua* (Hübner): *Proceedings of the 7th International Conference on Biological Science (ICBS 2021)* (pp. 284-289). Atlantis Press.
19. Pakpahan, T.E., Hidayatullah, T., and Mardiana, E. (2020). Application of biochar and manure to shallot cultivation in inceptisol soil at the Medan Agricultural Development Polytechnic experimental garden. *Jurnal Agricola Ekstensia.* 14: 49-53.
20. Pangestiningih, Y. (2011). Test the effectiveness of several entomopathogenic fungi and botanical insecticides against *Spodoptera exigua* Hubn. on shallot plants (*Allium ascalonicum* L.). *Jurnal Ilmu Pertanian KULTIVAR.* 5: 90-91.
21. Pedrini, N. (2018). Molecular interactions between entomopathogenic fungi (Hypocreales) and their insect host: perspectives from stressful cuticle and hemolymph battlefields and the potential of dual RNA sequencing for future studies. *Fungal Biol.* 122: 538–545.
22. Razak, N.A., Nasir, B., and Khasanah, N. (2016). Effectiveness of *Beauveria bassiana* Vuill at the control *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae) on shallot plant local Palu (*Allium wakegi*). *e-J. Agrotekbis.* 4: 565-570.
23. Rosmana, A., Sjam, S., Asman, A., Jayanti, N.J., Satriana, S., Padang, A.T., and Hakkar, A.A. (2018). Systemic deployment of *Trichoderma asperellum* in *Theobroma cacao* regulates co-occurring dominant fungal endophytes colonization. *J. Pure. Appl. Microbiol.* 12: 1071-1084.
24. Scudeletti, D., Crusciol, C.A.C., Bossolani, J.W., Moretti, L.G., Momesso, L., Tubana, B.S., Castro, S.G.Q.d., Oliveira, E.F.D., and Hungria, M. (2021). *Trichoderma asperellum* inoculation as a tool for attenuating drought stress in sugarcane. *Front. Plant. Sci.* 12: 1-13.
25. Septania, V.P., Saidah., and Basri, Z. (2022). Growth and yield of shallot (*Allium ascalonicum* L.) on the combination of *Trichoderma asperellum* and manure. *Jurnal Agrotech.* 12: 1-9.
26. Siahaan, P., Wongkar, J., Wowiling, S., and Mangais, R. (2021). Pathogenicity of *Beauveria bassiana* (Bals.) Viull. isolated from several host species against the green ladybug, *Nezara viridula* L. (Hemiptera: Pentatomidae). *Jurnal Ilmiah Sains.* 21: 26-33.
27. Soumia, P.S., Karuppaiah, V., Mahajan, V., and Singh, M. (2020). Beet Armyworm *Spodoptera exigua*: Emerging Threat to Onion Production. *Natl. Acad. Sci. Lett.* 43: 473-476.
28. Sriwantoko, S., Syam'un, E., and Ulfa, F. (2020). Growth of red onion plant (*Allium ascalonicum* L.) in an application by phosphate solubilizing microbes and goat dung compost. *Adv. Environ. Biol.* 14: 23-30.



**Padil Wijaya et al, Interaction between Rice Husk Biochar and Three Species of Fungus on Growth and Yield of Shallot (*Allium ascalonicum* L.)**

29. Stracquadanio, C., Quiles, J.M., Meca, G., and Cacciola, S.O. (2020). Antifungal activity of bioactive metabolites produced by *Trichoderma asperellum* and *Trichoderma atroviride* in liquid medium. *J. Fungi*. 6: 263.
30. Supartha, I.N.Y., Wijana, G., and Andyana, G.M. (2012). Application of organic fertilizer types to rice plants in organic farming systems. *E-Jurnal Agroteknologi Tropika*. 1: 98-106.
31. Supartha, I.W., Susila, I.W., Sumiarta, I.K., Rauf, A., Cruz, L.B.D., Yudha, I.K.W., Utama, I.W.E.K., and Wiradana, P.A. (2022). Preference, population development, and molecular characteristics of *Spodoptera exigua* (Lepidoptera: Noctuidae) on shallot cultivars: A field trial scale. *Biodiversitas*. 23: 783-792.
32. Supyani., Poromarto, S.H., Supriyadi., Permatasari, F.I., Putri, D.H., Putri, D.T., and Hadiwiyono. (2021). Disease intensity of moler and yield losses of Shallot cv. Bima caused by *Fusarium oxysporum f.sp. cepae* in Brebes Central Java: *IOP Conference Series: Earth and Environmental Science. The 8th International Conference on Sustainable Agriculture and Environment* (p. 012049). Surakarta, Indonesia: IOP Publishing.
33. Tchameni, S.N., Sameza, M.L., O'donovan, A., Fokom, R., Ngonkeu, E.L.M., Nana, L.W., Etoa, F.X., and Nwaga, D. (2017). Antagonism of *Trichoderma asperellum* against *Phytophthora megakarya* and its potential to promote cacao growth and induce biochemical defence. *Mycology*. 8: 84–92.
34. Triadiawarman, D., Aryanto, D., and Krisbiyantoro, J. (2022). The role of macro nutrients on the growth and yield of shallots (*Allium cepa* L.). *Jurnal Agrifor: Jurnal Ilmu Pertanian dan Kehutanan*. 21: 27-32.
35. Triwidodo, H. and Tanjung, M.H. (2020). Shallot (*Allium ascalonicum*) pests and its control measures in Brebes, Central Java. *Agrovigor*. 13: 149–154.
36. Wang, H., Peng, H., Li, W., Cheng, P., and Gong, M. (2021). The toxins of *Beauveria bassiana* and strategies to improve their virulence to insects. *Front. Microbiol.* 12: 1-11.
37. Wang, H., Zhang, R., Duan, Y., Jiang, W., Chen, X., Shen, X., Yin, C., and Mao, Z. (2021). The endophytic strain *Trichoderma asperellum* 6S-2: an efficient biocontrol agent against apple replant disease in China and a potential plant-growth-promoting fungus. *J. Fungi*. 7: 1050.
38. Wang, J., Ying, S.H., Hu, Y., and Feng, M.G. (2017). Vital role for the J-domain protein Mdj1 in asexual development, multiple stress tolerance, and virulence of *Beauveria bassiana*. *Appl. Microbiol. Biotechnol.* 101: 185–195.