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The Growth and Yield of Shallot (*Allium cepa* L. Aggregatum group) in Application of Beneficial Microorganisms

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ABSTRACT
Shallot (*Allium cepa* L. Aggregatum group) is one of the most widely utilized vegetables in Indonesia. Some technologies have been adapted to improve its productivity, e.g. the application of beneficial microorganisms. Mycorrhizal fungi, *Trichoderma* sp., and *Bacillus thuringiensis* as well as the combination of these microorganisms were applied on shallot cultivations. The experiment aims to investigate the effect of those microorganisms on the growth and yield of shallot. The experiment was arranged in a Randomized Complete Block Design with two treatment factors: (1) two shallot cultivars, Biru Lancor and Crok Kuning and (2) six beneficial microorganism treatments, i.e., control, mycorrhizae, *Trichoderma* sp., *Bacillus thuringiensis*, mycorrhizae and *Trichoderma* sp., combination, and those three microorganism combination. The objective was to study the effect of those microorganism application and their combination on the improvement of growth and yield of Crok Kuning and Biru Lancor cultivars. The observation was conducted on infection percentage of mycorrhizae, total population of *Trichoderma* sp., leaf area, leaf area index, net assimilation rate, crop growth rate, harvest index, and plant yield. The data obtained were subjected to analysis of variance (ANOVA) and continued with Duncan's Multiple Range Test (DMRT) at 5% significance level. The results showed the leaf area of shallot was improved as affected by the mycorrhizal fungi application. The effectiveness and implication of mycorrhizal fungi on shallot growth would decrease if the application was combined with other microorganisms. However, this application had not been able to increase component yield and yield of Biru Lancor and Crok Kuning.

Keywords: *Bacillus thuringiensis*, microbial interactions, mycorrhizal fungi, *Trichoderma* sp.

INTRODUCTION

Shallot (*Allium cepa* L. Aggregatum group) is one of the most widely utilized *Allium* by Indonesian people as a spice in dishes and an herbal medicine (Pangestuti & Sulistyaningsih, 2011). National production of shallot increasing by 5.43% per year starting from 2004 to 2014 (Direktorat Jenderal Hortikultura, 2016). However, the average of national productivity of shallot was low (10.22 ton ha^{-1} during 2012–2014) compared to the potential yield of shallot (Direktorat Jenderal Hortikultura, 2016). The productivity value was less than 50% from the potential yield of some shallots cultivars. Crok Kuning, a local cultivar originated from Bantul Yogyakarta has a potential yield of 24–26 ton ha^{-1} (Anonymous, 2016a), whereas Biru Cultivar a, local cultivar originated from Probolinggo has a potential yield of 12.47–14.08 ton ha^{-1} (Anonymous, 2016b). The low shallot productivity can be improved through genetic modification such as hybrid shallot cultivars (Sulistyaningsih *et al*., 2002) and agronomy technology such as the application of beneficial microorganism (Akhwan *et al*., 2012; Darsan *et al*., 2016; Tuhuteru *et al*., 2016).

Some beneficial microorganism can enhance the plant productivity through several mechanisms by fixing nitrogen, dissolving phosphate, producing siderophores, secreting phytohormones, and controlling pests and diseases. Nowadays, the most popular beneficial microorganisms used by farmers in shallot

cultivation are mycorrhizal fungi (Akhwan *et al*., 2012), *Trichoderma* sp. (Darsan *et al*., 2016), and *Bacillus thuringiensis*.

Mycorrhizal fungi are fungi that form a symbiotic association with the roots of a vascular hostplant (Brundrett, 2004). Previous researchers demonstrated that growth of shallot depends on the presence of mycorrhizal fungi especially in saline soil (Akhwan *et al*., 2012). Mycorrhizal fungi combine with guano applied at rhizosphere under salinity stress could enhance the root, Net assimilation rate, dry weight of shallot (Akhwan *et al*., 2012). Mycorrhiza applied at peat soil could enhance plant height, root volume, phosphorus uptake, and bulb dry weight (Suryani *et al*., 2017). *Trichoderma* sp. is a filamentous imperfect fungus which is commonly used as a biocontrol for some of the crop diseases and a biofertilizer (Lorito *et al*., 2010). Application of *Trichoderma* sp. in shallot bulb at sandy soil could increase plant height, leaf area index, net assimilation rate, crop growth rate, nitrate reductase activities, total chlorophyll and fresh bulb weight (Darsan *et al*., 2016) and improving seedling of onion (Debire *et al*., 2016). *Bacillus thuringiensis* is more often used as a bio-insecticide to eliminate and control pests (Chattopadhyay *et al*., 2004). Qi *et al*. (2016) reported that application of *Bacillus thuringiensis* could enhance shoot dry weight of tomato seed. Wilson *et al*. (2006) reported that *Bacillus thuringiensis* were able to produce siderophore.

The applications of mycorrhiza, *Trichoderma* sp., and *Bacillus thuringiensis*, as well as the combination among those microorganisms on shallot cultivation, have never reported yet. The objective of this research was to study the effect of the application of mycorrhizal fungi, *Trichoderma* sp., and *Bacillus thuringiensis* as well as the combination among those microorganisms on the improvement of growth and yield of Crok Kuning and Biru Lancor cultivars.

MATERIALS AND METHODS

The field experiment was conducted at Ngantak, Parangtritis, Kretek, Bantul Regency, D.I. Yogyakarta located at S 8° 0' 23.400" and E 110° 17' 52.800" with the altitude of 9.2 m above sea level, and flat contour witha slope of 0.5% from July to September 2015. Geographically, the experimental plot was arranged in Randomized Complete Block Design comprised of two treatment factors, namely shallot cultivars, and type of beneficial microorganisms. The first factor consisted of two cultivars, namely Biru Lancor and Crok Kuning; while the second factor consisted of six microorganism treatments, i.e., control, mycorrhiza, *Trichoderma* sp., *Bacillus thuringiensis*, mycorrhiza-*Trichoderma* sp. combination, and the combination of the three microorganisms. Three blocks were used as replications. All treatments were planted at the same time on a plot size of 2 m x 10 m with the spacing of 20 cm × 20 cm.

Trichoderma sp. was cultured in Laboratory of Biology, Department of Forestry and Plantation, D.I. Yogyakarta. Mycorrhiza was mixed in a zeolite, and *Bacillus thuringiensis* were cultivated in Laboratory of Agricultural Microbiology, Universitas Gadjah Mada. Mycorrhiza was applied at dose 2.5 g.plant^{-1} placed under bulb in the planting holes (Sumiati & Gunawan, 2006). *Trichoderma* sp.was applied at a dose of 625 kg.ha^{-1}, was applied within rows when the age of plant was 2 weeks after planting (WAP). *Bacillus thuringiensis* was sprayed at a dose of 1.5 mL.L^{-1} and a volume rate of 175 L.ha^{-1} twice a week. Fertilizers were used at during planting, 2 WAP and 6 WAP with a total dose of Nitrogen 250 kg.ha^{-1}, phosphor 220 kg.ha^{-1}, Potassium 250 kg.ha^{-1} and Sulphur 65 kg.ha^{-1}. Organic matters, 2 ton of coconut dusk was applied at the same time of shallot bulbs planting. *Spodoptera exigua* was controlled using synthetic pesticide with cypermethrin and profenofosas an active ingredient. Stemphylium leaf blight and purple blotch were controlled using synthetic pesticide with carbendazim and azoxystrobin as an active ingredient.

The percentage of mycorrhizal infection was observed at 6 WAP using methods as followed: 25 roots (each 1 cm length) was rinsed several times with tap water, kept submerged for 24 hours in 10% KOH solution, and acidified in 2% HCl solution for an hour. After rinsing, the root materials were stained with Acid Fuchsine solution. The stained root specimens were placed in a glass slide and examined by light microscope. Estimation of infection percentage of mycorrhiza was assessed following the method of McGonigle *et al*. (1990). A total colony of *Trichoderma* sp. was observed before and after shallot planted. It was observed using methods as follows: 1 g of composite soil around shallot plant diluted at 3–10, 4–10, and 5–10 in sterilized water. A volume of 0.1 ml dilution was placed on *Trichoderma* Selective Medium (TSM). In TSM for 1 L contained Calcium Nitrate (1 g), Potassium nitrate (0.26 g), Magnesium sulfate heptahydrate (0.26 g), Monopotassium phosphate (0.12 g), Calcium chloride dihydrate (1 g), Citric acid (0.05 g), Sucrose (2 g), Agar (20 g), Chlortetracycline (0.05 g) and captan (0.04 g).

The data were observed on growth parameters such as leaf area (cm^2) at 3, 6 and 9 weeks after planting (WAP), net assimilation rate ($g.(dm^2)^{-1}.week^{-1}$), crop growth rate ($kg.(m^2)^{-1}.week^{-1}$) at 3–6 WAP and 6–9 WAP, and yield components such as number of bulb per cluster, bulb diameter (cm), bulb weight (g), bulb fresh weight per cluster (g), and harvest index at 9 WAP.

The data obtained were subjected to analysis of variance (ANOVA) and mean comparisons were determined through Duncan's Multiple Range Test (DMRT) at 5% significance level.

RESULTS AND DISCUSSION

The key for symbiotic between mycorrhiza and host plant was the infection capability of the mycorrhiza (Smith *et al*., 2011). It was proven by percentage of mycorrhizal infection on the host plant. The high percentage of mycorrhizal infection on the root indicated the high mycorrhiza development in the plant. Plant with a high percentage of Mycorrhiza infection showed high phosphorus uptake, high root coverage, high shoot development, and high productivity (Smith *et al*., 2011).

A single application of mycorrhiza would increase the percentage of mycorrhizal infection in the application of mycorrhiza and a total population of *Trichoderma* sp. in the application of *Trichoderma* sp., respectively (Table 1). According to Shuab *et al*. (2014), the inoculation of mycorrhizal fungi on onion would improve the percentage of mycorrhizal infection. Klein & Eveleigh (1998) categorized *Trichoderma* sp. into a saprophytic group. The amendment of organic matter at a dose of 2 $ton.ha^{-1}$ on the beginning of the experiment was able to assure the longevity of *Trichoderma* sp.as shown in a total population of *Trichoderma* sp., at harvest time (Table 1).

Table 1 presented the decrement of the percentage of mycorrhiza infection when mycorrhiza applied with *Trichoderma* sp. and/or *Bacillus thuringiensis*. It was known that *Trichoderma* sp. was able to produce gliotoxin and viridine as antibiotics and secrete enzyme ß-1,3-glucanase, protease, and chitinase that attack the hyphae (resulting in exolytic) (Viterbo *et al*., 2002). The microorganism-plant competition on nutrition in utilizing root exudate and volatile compounds produced by microorganisms and on growing habitat were the main factors contributing to the decrease of mycorrhizal fungi capability in infecting hostplant (Fracchia *et al*., 2004).

The application of Mycorrhiza greatly increased the leaf area at 6 WAP (Table 2). According to Deressa and Schenk (2008), the increment of plant growth caused by increment of P uptake in Onion applied with Mycorrhiza. Phosphor plays a role in cell division, elongation, and enlargement (Karanova *et al*., 2006). An increment of nutrient uptake was possible through two pathways, which were by root epidermis pathway and by external hyphae of mycorrhizal fungi (Smith *et al*., 2011). This was in accordance with research done by Bolandnazar *et al.* (2007) which shown that application of mycorrhiza under three irrigation intervals could increase leaf area index of onion (*Allium cepa* L. Aggregatum group).

The application of combination between mycorrhiza

Table 1. Percentage of mycorrhizal infection (%) of Biru Lancor and Crok Kuning at 3 WAP and a total population of Trichoderma sp. (cfu g^{-1}) at the before application and harvest time

Treatment	Percentage of Mycorrhizal infection (%)	Total population of Trichoderma sp. (cfu.g^{-1})	
		Before application	Harvest Time
Cultivar			
Biru Lancor	54.89 p	4.50 x 103	3.58 x 107p
Crok Kuning	56.78 p	4.50 x 103	3.56 x 107p
Microorganism			
Control	35.66 c	4.50 x 103	5.10 x 104 b
Trichoderma sp.	34.33 c	4.50 x 103	1.79 x 108 a
Mycorrhiza	89.00 a	4.50 x 103	1.68 x 104 b
Bacillus thuringiensis	28.66 c	4.50 x 103	5.10 x 103 b
Mycorrhiza–Trichoderma sp.	75.33 b	4.50 x 103	2.56 x 107 a
Combination of three microorganisms	72.00 b	4.50 x 103	1.57 x 107 a
CV (%)	15.79		25.76*
Interaction	(-)		(-)

Note: Mean values in a column followed by same letters indicated no significant difference by DMRT at α = 5%; (-) indicated an no interaction between cultivar and microorganism, *: analysis was performed on ArcsineTransformation.

Table 2. Leaf area (cm^2) of Biru Lancor and Crok Kuning at 3, 6, and 9 WAP

Treatment	Leaf Area (cm^2)		
	3 WAP	6 WAP	9 WAP
Cultivar			
Biru Lancor	105 q	423 q	143 q
Crok Kuning	145 p	654 p	196 p
Microorganism			
Control	133 a	484 b	161 a
Trichoderma sp.	126 a	576 ab	151 a
Mycorrhiza	96 a	719 a	148 a
Bacillus thuringiensis	141 a	580 ab	203 a
Mycorrhiza–Trichoderma sp.	108 a	431 b	201 a
Combination of three microorganisms	144 a	531 b	158 a
CV (%)	26.12	24.82	30.41
Interaction	(-)	(-)	(-)

Note: Mean values in a column followed by same letters indicated no significant difference by DMRT at α = 5%; (-) indicated no interaction between cultivar and microorganism.

and *Trichoderma* sp. and/or *Bacillus thuringiensis* decreased the leaf area compared to the single application of mycorrhiza. The decrease of leaf area was closely related to the percentage of mycorrhiza infection that would also be decreased when Mycorrhiza was combined with *Trichoderma* sp. and/or B. *thuringiensis* (Table 1). The decrement of the percentage of mycorrhiza infection also affected P uptake that played role in cell division, elongation, and enlargement (Karanova *et al*., 2006).

Net assimilation rate in *Trichoderma* sp. application at week 6-9 week after planting and crop growth rate in mix of three microorganisms at week 6–9 weak after planting had negative value. It is shown that shallot has assimilation rate lower than respiration rate. Net assimilation rate and crop growth rate will decrease during the senescence, where leaf area is decreasing at the same time as a net gain of dry weight.

The individual application of beneficial microorganisms did not give significantly different on a number of bulb per cluster, bulb diameter, bulb weight, bulb fresh weight per cluster, and harvest index (Table 4). In fact, the increased leaf area as affected by mycorrhizal fungi had not been able to

Table 3. Net assimilation rate (g $(dm^2)^{-1}$ $week^{-1}$) and crop growth rate (kg $(m^2)^{-1}$ $week^{-1}$) of Biru Lancor and Crok Kuning at 3–6 WAP and 6–9 WAP

Treatment	Net Assimilation Rate ($g.(dm^2)^{-1}.week^{-1}$)		Crop Growth Rate ($kg.(m^2)^{-1}.week^{-1}$)	
	3–6 WAP	6–9 WAP	3–6 WAP	6–9 WAP
Cultivar				
Biru Lancor	0.51 p	0.070 p	0.029 q	-0.0012 p
Crok Kuning	0.45 p	0.110 p	0.037 p	0.0080 p
Microorganism				
Control	0.42 a	0.068 a	0.028 a	0.0040 a
Trichoderma sp.	0.57 a	-0.018 a	0.041 a	0.0004 a
Mycorrhiza	0.44 a	0.105 a	0.035 a	0.0043 a
Bacillus thuringiensis	0.47 a	0.128 a	0.036 a	0.0107 a
Mycorrhiza–Trichoderma sp.	0.51 a	0.245 a	0.029 a	0.0136 a
Combination of three microorganisms	0.44 a	0.036 a	0.033 a	-0.0014 a
CV (%)	22.80	28.83*	30.99	30.76
Interaction	(-)	(-)	(-)	(-)

Note: Mean values in the column followed by same letters indicated no significant difference by DMRT at α = 5%; (-) indicated an no interaction between cultivar and microorganism, *: analysis was performed on root transformation.

Table 4. Number of bulb per cluster, bulb diameter (cm), bulb weight (g), bulb fresh weight per cluster (g), and harvest index, of Biru Lancor and Crok Kuning

Treatment	Number of Bulb per Cluster	Bulb Diameter (cm)	Bulb Weight (g)	Bulb Fresh Weight per Cluster (g)	Harvest Index
Cultivar					
Biru Lancor	7.11 q	2.08 p	5.21 p	26.44 q	0.566 p
Crok Kuning	9.35 p	2.05 p	5.53 p	43.78 p	0.582 p
Microorganism					
Control	7.45 a	2.00 a	5.05 a	32.40 a	0.533 a
Trichoderma sp.	8.78 a	1.89 a	4.51 a	33.83 a	0.634 a
Mycorrhiza	8.88 a	2.17 a	6.40 a	41.53 a	0.539 a
Bacillus thuringiensis	8.56 a	2.18 a	5.98 a	41.02 a	0.633 a
Mycorrhiza–Trichoderma sp.	8.11 a	1.91 a	4.88 a	30.43 a	0.634 a
Combination of three microorganisms	7.61 a	2.18 a	5.13 a	31.62 a	0.480 a
CV (%)	8.78*	12.59	21.98	19.09*	18.12
Interaction	(-)	(-)	(-)	(-)	(-)

Note: Mean values in the column followed by same letters indicated no significant difference by DMRT at α = 5%; (-) indicated an no interaction between cultivar and microorganism, *: analysis was performed on root transformation.

accelerate net assimilation rate and crop growth rate significantly, so that bulb yield components were not significantly different compared to those of in control. Several factors might contribute to this effect, one of them was the invasion of plant pest organism (PPO). The invasion of PPO might reduce the plant yield up to 100% yield loss as risky as increase the potency of crop failure (Sakinah, 2013). In the field shallot, was attacked by *Spodoptera exigua*, Stemphylium leaf blight,and Purple Blotch.

CONCLUSIONS

The application of mycorrhizal fungi could improve leaf area of shallot and effectiveness and implication of mycorrhizal fungi would decrease if the application was combined with other microorganisms. However, the application of beneficial microorganisms had not been able to increase plant yield of Biru Lancor and Crok Kuning.

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