Ilmu Pertanian (Agricultural Science)
Vol. 1 No. 3 December, 2016 : 094-099
Available online at http://journal.ugm.ac.id/jip
DOI: doi.org/10.22146/ipas.12564

Various Shallot Seed Treatments with *Trichoderma* to Increase Growth and Yield on Sandy Coastal

Stefany Darsan[1]*, Endang Sulistyaningsih[1], Arif Wibowo[2]
[1]Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada
Jln. Flora no. 1, Bulaksumur, Sleman, Yogyakarta 5528, Indonesia
[2]Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada
Jln. Flora no. 1, Bulaksumur, Sleman, Yogyakarta 5528, Indonesia
***Corresponding email: stefanydarsan721@yahoo.co.id**

Received: 9th August 2016 ; Revised: 16th November 2016 ; Accepted: 18th August 2017

ABSTRACT
Sandy coastal area is a poor land, with minimal nutrients and low ability to store water and nutrition. Therefore, plant is difficult to grow well, consequently the treatments for improving sandy coastal is required before planting to support their growth. Application of *Trichoderma* as a plant-growth promoting and controlling pathogens had been known. Shallot seed treatment by *Trichoderma* agents was intended to improve and support plant growth and yield in the sandy coastal land. The aim of experiment was to determine the most effective treatment to improve growth and yield of shallot on sandy coastal land. The experiment had been carried out in Yogyakarta during August - November 2015. The factorial treatments of cultivar and *Trichoderma* applications were arranged in Completely Randomized Design with three replications. The shallot cultivars consisted of Tiron, Crok, and Biru, while *Trichoderma* application consisted of control (no treatment), sprayed with *Trichoderma*, soaked in *Trichoderma*, matricontioning media i.e. rice husk charcoal and brick in combination with *Trichoderma*. The collected data related to plant growth and yield. The result showed that seed treatment methods with *Trichoderma* increased the plant height, the leaf area index, extensive root, net assimilation rate (NAR), plant growth rate (PGR), Nitrate Reductase Activities (NRA), total chlorophyll, and fresh bulb weight. The best technical treatment for cultivar Tiron was soaked with *Trichoderma*, and Crok was sprayed with *Trichoderma*, while Biru cultivar was using brick in combination with *Trichoderma*.

Keywords: Sandy Costal, Shallot, *Trichoderma*

INTRODUCTION

Shallot (*Allium Cepa* L. Aggregatum group) was one of the important vegetable crops with high value in the tropical regions. Because of its pungency for food, spice, and seasoning, shallot was often used as a traditional medicine for various ailments and a source of biofar (Sulistyaningsihet *et al*., 2002; Lanzotti, 2006; Merhi *et al*., 2008). Therefore, shallot's demands always increase. Production of shallot bulbs with leaves in D.I. Yogyakarta in 2013 was 10.68 tonnes per hectare, while in 2014 the production was 9.60 tons per hectare decreased. The overall decline in production was due to lower harvested area in each district (Anonymous, 2015) and limited fertile land for shallot cultivation. Cultivation of shallot in sandy coastal land could be an alternative for shallot development (Rajiman, 2014). Sandy coastal was a poor land, it is containing low clay, dust had minimal nutrients so the ability to store water was low (Rajiman *et al*., 2008).

Sowing the high quality of shallot seed was necessary for commercial production. The low seed quality of shallot would result in low seed emergence and the young plants would susceptible to abiotic and biotic pressures, and in turn would result in decreased yield (Ilyas, 2006; Sutariati *et al*., 2014). Treatments to improve quality were very important to produce healthy seeds before planting so that they can support their growth and development in sandy coastal conditions and high produtivity. Seed treatment such as seed soaked, sprayed, and congenital media combined with microorganism to protect seed which was called *biomatriconditioning* (Ilyas, 2006; Sutariyati *et al*., 2013) was usually applied to overcome seed problems.

Trichoderma is a genus of fungi that included in

anamorphic fungi isolated from soil or organic matter (Monte, 2001). This fungi group was known effective and safe, as the fungal antagonist reduces multiplication pathogens, provide indirect effect on plant health (such as growth promoters of plants and improve plants fertility), produce enzymes that could degrade fungal pathogens cell wall, produce antibiotics that could kill pathogens, growth promoting and development of plants, as well as plant resistance inducers (Harman, 2000). Therefore, *Trichoderma* was used as seed treatments with various methods. The objective of this study was to obtain the best method for application of *Trichoderma* on shallot seed for production in sand coastal land.

MATERIALS AND METHODS

This study had been carried out during August - November 2015 at Samas Beach, Bantul subdistrict, Yogyakarta and Laboratory of Crop Science, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta. The materials used were three shallot cultivars, *Trichoderma*, rice husk charcoal, and brick.

The factorial treatments of cultivar and *Trichoderma* applications were arranged in Completely Randomized Design with three replications. The shallot cultivars consisted of Tiron, Crok, and Biru, while *Trichoderma* application consisted of control (no treatment), sprayed with *Trichoderma*, soaked in *Trichoderma*, matricontioning media i.e. rice husk charcoal and brick in combination with *Trichoderma*. Thus there were 15 combinations of treatments with three replications with the total of 45 experimental units.

Preparation of *Trichoderma* Application

The application of *Trichoderma* as follow: 1) The sprayed *Trichoderma*: the suspension of *Trichoderma* 25 $g.L^{-1}$ was sprayed to the plants since 0 days old using a handsprayer. The application interval was seven days; 2) Soaked *Trichoderma*: the seeds were soaked in the solution of (suspended) 25 $g.L^{-1}$ *Trichoderma* for 10 hours then exposed in the room temperature for 12 hours; 3) *Biomatriconditioning* method. This method needed matriconditioning (softening the rice husk charcoal or brick, then sift through a sieve of 0.5 mm passes then dried under direct sunlight). *Biomatriconditioning*: 1 ml suspension of *Trichoderma* 25 $g.L^{-1}$ was mixed with powder of matriconditioning 1.5 gr and the seed (2 gr seed mix: 1.5 gr media: 1 ml water). Shallot bulbs treated with *biomatriconditioning* were placed at room temperature for 12 hours then the shallot bulbs were planted directly.

Plant Growth Analysis

Plant height observed by using a measuring ruler at the base of the root to the growing point. Leaf Area Index (LAI), was a leaf area (A) on every unit of soil area (P) that could be expressed in a mathematical formula as follows: LAI = A/P . Root area was measured by using a leaf area meter. Nitrate Reductase Activities (NRA) (mol $NO_2^-.g^{-1}.h^{-1}$) using the method used by Bates *et al*. (1973). The levels of NRA was declared in the amount of NO_2 micromoles.$g^{-1}.h^{-1}$ by the equation:

$$NRA = \frac{AS}{A_0} \text{ x } \frac{1000}{\text{B}} \text{ x } \frac{1}{T} \text{ x } \frac{500}{1000} \quad \text{.....(1)}$$

whereas: AS = the value of absorbance of the solution, A_0 = absorbance value of the standard (0.0106 or 0.0142), B = fresh weight of leaf samples, T = incubation time.

Net Assimilation Rate (NAR) was the rate of dry weight increased at a particular time (T) per unit of soil area (L) that could be expressed in a mathematical formula as follows:

$$\text{NAR} = \frac{W_2 - W_1}{T_2 - T_1} x \frac{\ln La_2 - \ln La_1}{La_2 - La_1} \quad \text{.....(2)}$$

where: W_1 = total plant dry weight at 1st observation, W_2 = total plant dry weight at 2nd observation , T_1 = observation 1st day, T_2 = observation 2nd day, La_1 = leaf area at the 1st observation, La_2 = leaf area at the 2nd observation.

Plant Growth Rate (PGR) was defined as every increased dry weight per every unit of soil area (W) that can be expressed in a mathematical formula as follows:

$$\text{PGR} = \frac{W_2 - W_1}{T_2 - T_1} \quad \text{.....(3)}$$

where: W_1 = total plant dry weight at the 1st observation, W_2 = total plant dry weight at the 2nd observation, T_1 = observation 1st day, T_2 = observation 2nd day. The total chlorophyll content was calculate by using a formula= 0.0202 x A645 + 0.00802 x A663 ; where:A645 is the absorbance at a wavelength of 645 nm, A663 is the absorbance at a wavelength of 663 nm. Bulb fresh weight.Bulb fresh weight measurements at eight weeks old after the plants were weighted by using an analytical balance.

Data were analyzed by ANOVA with $\alpha = 0.05$ using SAS. If there were significant differences, the analysis was continued with Duncan's Multiple Range Test (DMRT) at $\alpha=0.05$.

RESULT AND DISCUSSION

Seed treatment could continue to increase the quality of yield (Ilyas, 2006; Sutariati *et al*., 2014).

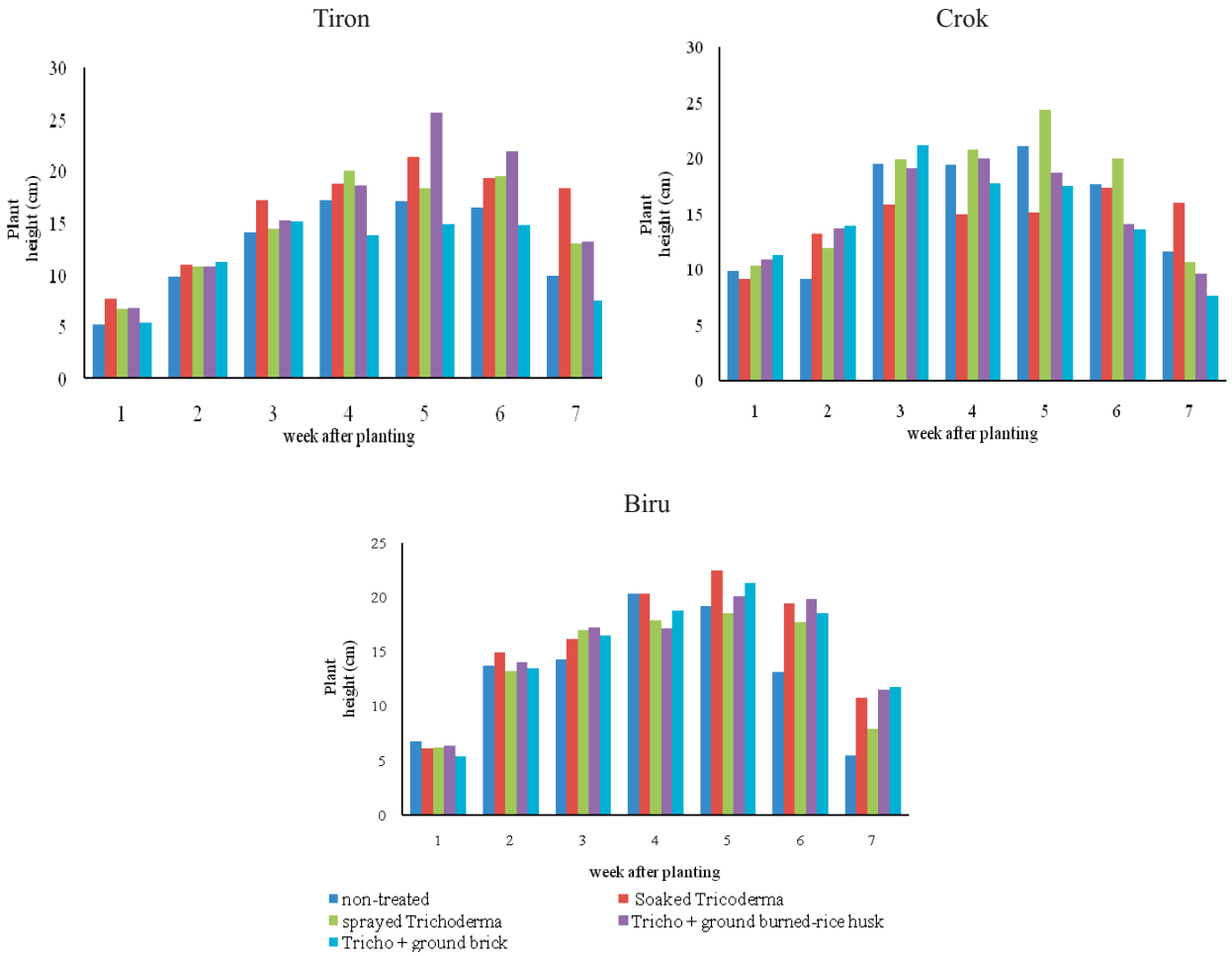

Figure 1. Effect of various seed treatment methods with Trichodermato plantheight onTiron, Crok, and C = Biru

Efforts to develop treatment techniques to protect seeds from disease and promote plant growth to maximize their yield qualities was done. The treatments used were soaking, spraying and matriconditioning with *Trichoderma* in order to protect seeds from pathogenic agents and to increase growth.

Compared with the non-treated, treatments of seed had no significant effect on improving shallot, either *Trichoderma* treatmentor cultivar types (Figure 1). Compared with non-treated, *Trichoderma* treatments increased plant height in early growth to late growth, except in *Trichoderma* integrated with brick treatment at four weeks after planting for Tiron cultivar. Compared with non-treated, *Trichoderma* treatments increased plant height in early growth, at one to three weeks after planting. At four to five weeks, the growth decreased because of the attack of *Spodoptera*, especially in Crok cultivar soaked with *Trichoderma*. Compared with non-treated, *Trichoderma* treatments did not increase plant height at early growth, but at 3 weeks after planting, plant height began to increase. At four weeks, plants were attacked by *Spodoptera* pest. In the weeks subsequent to plants treated with *Trichoderma*, significantly effect was shown on plant height improvement.

There was no interaction between cultivars and various *Trichoderma* treatment on leaf area index (LAI) (Table 1). There was no significantly different LAI between cultivars. Compared with the non-treated treatment, *Trichoderma* treatments were more effective on increasing LAI. LAI of plant treated by *Trichoderma*+brick treatment was higher compared with other *Trichoderma* treatments. The lowest LAI occured in the plant grown from seeds soaked *Trichoderma* treatment.

Based on the root area measurement, there was an interaction between cultivars and various *Trichoderma* treatment in enhancing root area (Table 2). Tiron cultivar soaked in *Trichoderma* produced the highest root area compared with other *Trichoderma* treatments. The lowest root area was found on Crok cultivar soaked in *Trichoderma*.

Based on net assimilation rate (NAR), plant growth rate (PGR), chlorophyll total, and Nitrate Reductase Activity (NRA) measurement, there was no interaction between cultivars and various *Trichoderma* application methods (Table 3). Cultivar treatments had no significant effect in increasing ANR, NAR,

Table 1. Leaf Area Index (LAI) Plant Shallot at 8 weeks after planting (WAP)

Cultivar	*Trichoderma* Treatment					Average
	Non-treated	Soaked Tricho	Sprayed Tricho	Tricho+rice husk charcoal	Tricho+brick	
Tiron	38.67	100.83	52.67	86.67	52.67	66.30 a
Crok	64.00	65.33	81.33	51.33	34.67	59.33 a
Biru	40.83	60.00	40.67	70.67	42.50	50.93 a
Average	47.83 ab	75.39 a	58.22 ab	69.56 ab	43.28 b	(-)
CV	23.64					

Remarks: The numbers in the columns followed by the same letters are not significantly different according to Duncan Multiple with α = 5%; (-): There is no interaction between factors being tested; Data was transformed with a log x.

Table 2. Root area (cm^2) of shallot at 8 weeks after planting (WAP).

Cultivar	*Trichoderma* Treatment					Average
	Non-treated	Soaked Tricho	Sprayed Tricho	Tricho+rice husk charcoal	Tricho+brick	
Tiron	11.41 cde	31.09 a	17.03 abcd	18.04 abc	13.05 cde	18.12
Crok	13.41 cde	6.67 e	14.64 bcde	13.44 cde	7.88 de	11.21
Biru	15.76 bcde	16.98 abcd	19.70 abc	17.30 abcd	27.01 ab	19.35
Average	13.53	18.25	17.12	16.26	15.98	(+)
CV	17.17					

Remarks: The numbers in the columns and / or rows followed by the same letter were not significantly different according to Duncan Multiple with α = 5%; (+): there was interaction between factors tested; Data was transformed with a log x.

and PGR at three to five and five to eight weeks after planting. Cultivar treatments were significant on improving total chlorophyll content. An increase in total chlorophyll content was observed in Biru cultivar.

Compared with non-treated, *Trichoderma* treatments did not significantly improve NAR at three to five and five to eight weeks after planting. *Trichoderma* treatments were effective in improving PGR at three to five weeks after planting. The effect of treated seed was increase significantly different from the non-treated. An increase of PGR was observed on plants treated with sprayed *Trichoderma*. But at five to eight weeks after planting, the increasing was not significantly different compared with non treated. As an increase of total chlorophyll and ANR were observed at *Trichoderma* soaked treatment.

Based on the bulb fresh weight, there was interaction found between cultivars and various *Trichoderma* treatment in increasing fresh bulb weight (Table 4). Tiron cultivar soaked in *Trichoderma* showed an increase in root area compared with other *Trichoderma* treatment. The lowest increasing of root area was found in Crok cultivar soaked in *Trichoderma*.

Discussion

The results of experiment showed that various method using *Trichoderma* were significantly able to improve growth and yield in shallot through height plant, LAI, root area, PGR, total chlorophyll, and NRA compared with control. The results were in accordance with previous studies. As reported by Shoresh *et al.*(2010), the used of *Trichoderma* could protect plant, increase growth, help nutrient absorption, fertilizer use efficiency, and stimulation of plant defenses against biotic and abiotic damage (Shoresh *et al.*, 2010).

Observation on growth and yield showed that there were differences in response of each cultivar with *Trichoderma* treatment methods. Soaking in *Trichoderma* was more responsive from Tiron cultivar. Tiron cultivar had a small size and lower water content of 81.25 %, and Tiron seeds were more resistant to high humidity. *Trichoderma* treatment which was applied through sprayed was more suitable for Crok cultivar. Crok had a fairly large seed size and higher water content of 86.23 %. The high water content was not suitable. Meanwhile, Biru cultivar was more suitable with *Trichoderma* brick-integrated treatment. Brick could maintain longer presence of *Trichoderma* at the seed. The use of solid materials caused a higher water holding capacity and better ability in maintaining microorganisms (Sutariati *et al.*, 2013). The solid medium humidified with *Trichoderma* could improve the viability and vigor. Moisturization

Table 3. Net assimilation rate (NAR), Plant Growth Rate (PGR) (kg / m2 / week) at 3-5 and 5-8 Weeks of age After planting, Nitrate Reductation activity (NRA) and Chlorophyll Total 5 Weeks After Planting.

Cultivar	NAR		PGR		Chlorophyll Total	ANR
	3-5	5-8	3-5	5-8		
Tiron	0.052 a	-0.012 a	0.4438 a	0.0063 a	0.18 b	1.41 a
Crok	0.004 a	0.008 a	0.2532 a	0.0063 a	0.16 b	1.34 a
Biru	0.005 a	0.013 a	0.2451 a	0.0446 a	0.29 a	1.54 a
Trichoderma						
Tricho Soaked	0.023 a	-0.003 a	0.3103 ab	0.01348 a	0.26 a	1.68 a
Sprayed Tricho	0.035 a	0.004 a	0.5592 a	0.00642 a	0.21 b	1.37 b
Tricho+Rice husk charcoal	0.031 a	0.012 a	0.3062 ab	0.04422 a	0.24 a	1.52 ab
Tricho+brick	0.021 a	0.005 a	0.1419 ab	0.03038 a	0.21 b	1.35 b
Non treated	0.006 a	-0.004 a	0.2527 b	0.00105 a	0.15 b	1.25 b
CV	7.33	3.53	16.61	7.51	29.04	18.49
Interaction	(-)	(-)	(-)	(-)	(-)	(-)

Remarks: The numbers in the columns followed by the same letter werenot significantly different according to Duncan Multiple with α = 5%; (-): No interaction was found between factors tested; Data was transformed with log x.

Table 4. Bulbs Fresh weight at 8 Weeks After Planting.

Cultivar	*Trichoderma* Treatment					Average
	Non-treated	Soaked Tricho	Sprayed Tricho	Tricho+rice husk charcoal	Tricho+brick	
Tiron	4.36 def	13.83 a	5.92 bcde	12.18 abc	6.26 abcd	8.51
Crok	5.30 cdef	1.27 f	7.42 abcd	7.06 abcd	1.79 ef	4.57
Biru	5.54 bcd	9.29 abcd	10.07 abcd	9.90 abcd	12.63 ab	9.48
Average	5.07	8.13	7.80	9.71	6.89	(+)
CV	23.21					

Remarks: The numbers in the columns and / or rows followed by the same letter were not significantly different according to Duncan Multiple with α = 5%; (+): there was interaction between factors tested; Data was transformed with a log x.

was done to facilitate the formation of seeds (Elias, 2006).

Response of plant growth caused by *Trichoderma* depends on their ability to survive and thrive in root zone (Naseby *et al*., 2000). It could give a great influence on roots formation and yield (Harris *et al*., 2000). Factors causing differences in plant response were varied, such as application techniques and seeds used. Each cultivar had different morphological characteristics. It was cause of differences in the response three cultivars in associated with *Trichoderma* incorporated to seed.

Trichoderma was able to associated with plant roots to improve early growth of seeds and support the further development. The results were in accordance with previous studies. As reported by Mastouri *et al.* (2010), tomato seed treated with *Trichoderma harzianum* accelerate germination and increases seed vigor. Landa *et al*. (2004) reported that the use of biological agents significantly improved the viability and vigor of Chickpea, compared with the untreated seed.

Trichoderma mechanism to promote plant growth was through an increase in the transfer of nutrients from soil to the roots, which were supported by the ability of *Trichoderma* to colonize the roots. *Trichoderma* was capable of infecting plant roots, and as the result would be able to promote plant growth and protect plants. Another benefit that could be given to plants, such as IAA hormone produced by *Trichoderma* to spur the development of plant roots (Harman, 2000). This was in line with research done by Naseby *et al.* (2000), which showed that *Trichoderma* could increase root weight and dry weight of bean plants, compared with no *Trichodema*.

Healthy plants with properly developed root systems associated with a high yield were reported by Harris *et al*. (2000). It was stated that a healthy plant with root system is well developed to survive in adverse conditions at the beginning of the growth and association

with a high yield. The use of *Trichoderma* acted as biocontrol could reduce multiplication of pathogens and could be a driver of growth and plants development, improve soil fertility, produce enzymes that could degrade cell wall of fungal pathogens, produce antibiotics that could kill pathogens, and as inducers of plant resistance (Harman, 2000).

CONCLUSION

Based on these results, it could be concluded that the method of seed treatment with Trichoderma on the sandy coastal land was able to improve growth and yield of shallot. Soaked, sprayed, or integrated with matriconditioning plus rice husk charcoal or brick in combination with *Trichoderma* agents were effectively improve growth and yield of shallot, compared with non treated plant. The best technical treatment for Tiron cultivar was soaked in *Trichoderma*, and Crok was sprayed with *Trichoderma*, while Biru was applied with brick in combination with *Trichoderma*.

ACKNOWLEDGEMENT

Acknowledgement are due Australian Center for International Agricultural Research (ACIAR) for financial provided to author, appreciate to all employees in the Department of Agronomy Faculty of Agriculture Gadjah Mada University.

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