Trichoderma asperellum inoculation on shallots productivity in coastal sand lands

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Trichoderma asperellum inoculation on shallots productivity in coastal sand lands

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Abstract. This study aims to examine the best responses in growth and yield of shallot cultivars to the inoculation of Trichoderma asperellum cultivated in coastal sand soils. The research was carried out in the coastal sand field in Samas, Bantul, Yogyakarta, from January to March 2017. The experiment was a factorial experiment, consisting two factors arranged in a completely randomized block design (CRBD) with three replications. The two factors are various shallot cultivars, consisting of 20 cultivars, and the inoculation of Trichoderma asperellum isolates, consisting of two levels namely without and with inoculation. Result showed an interaction between Trichoderma asperellum and onion cultivars on total chlorophyll content and total dry weight per plant. Various shallot cultivars show the greenness of the leaves, plant height, number of leaves per plant, and the variety of fresh bulb weight per plant. Inoculation of Trichoderma asperellum produced better leaf greenness, stomata opening width, number of roots, plant height, number of plant leaves, fresh bulb weight per plant than without inoculation. Cultivar that shows best growth and yield was Biru Lancor.

1. Introduction

Trichoderma spp. is a free-living fungus, commonly found in soil ecosystems and roots, belongs to Plant Growth Promoting Fungi (PGPF), which are beneficial fungi that can stimulate plant growth through various mechanisms. Indirect growth stimulation mechanisms, can be in various ways such as antibiotics, competition, parasitism or lysis, inhibition of pathogen production of enzymes or toxins and resistance to plant induction. The direct mechanism, through its ability to produce fitohormones, dissolve phosphates, improve nutrient uptake and decompose organic matter. According to Arora [1], PGPF increases plant growth by producing hormones, mineralization, and suppression of plant pathogens (biocontrol). Likewise stated by Martinez-Medina et al. [2], that some trichoderma isolates can directly influence plant pathogens, but are also known to affect the phytohormonal tissues of their host plants, which will result in improved plant growth and tolerance to stress. PGPF is able to provide nutrients for plants in a form that is easily assimilated. Trichoderma asperellum is reported as a biological controlling agent for various plant pathogenic fungi and is considered a versatile antagonist [3, 4]. T. asperellum strains have a much faster growth rate than T. viride [5]. This fungus produces or releases various compounds that induce local or systemic resistance responses, indicating a lack of pathogenicity to plants. Trichoderma sp. has been widely used in research on various types of plant commodities, both in food crops and horticulture, among them, on wheat crops [6], corn [7], melon [2], mango [5], tomatoes [8], and also shallots [9].
Shallot (*Allium cepa* var. *Aggregatum*) is one of important horticultural commodities for the community both economically and nutritionally [10]. As a horticultural commodity that is widely consumed by the public, the potential for developing shallots is still wide open not only for domestic needs but also abroad [11]. Shallots production of Indonesia from 2013 - 2017 respectively (1,470,155; 1,446,869; 1,229,189; 1,233,989; 1,010,773) tons [12]. Per capita consumption in households a year according to the Susenas (2013 - 2017), respectively (20,649; 24,872; 27,114; 28,261; 25,702) ounces [13]. As the population increases, the need for shallots will also continue to increase. Therefore it is necessary to develop shallot plants through optimal cultivation techniques so that their growth and production can be expected to meet the needs [14]. Increasing the production of shallots directed at meeting domestic needs and increasing competitiveness can be achieved through increasing productivity and expanding new areas [15].

Coastal land is one of the sub-optimal lands in Indonesia that has high potential to be developed as a new area. Coastal sand land has several advantages for agricultural land, such as extensive, flat, rarely flooded, abundant sunlight, and shallow water surface. Planting preparation in coastal sandy land is quite simple just by making beds without making deep trenches, so that there will be cost efficiency from tillage [10]. The disadvantage is having low soil productivity, caused by limiting factors such as low water holding and storing ability, high infiltration and evaporation, very low fertility and organic matter and low water use efficiency [16]. The properties of this soil are thought to cause high levels of nutrient loss due to leaching so that plants get less nutrients [17]. In addition, the soil microorganism population is low and its activity is slow [18]. To improve land productivity, in addition to adding organic matter, one of the things that can be done is by adding beneficial microorganisms to trigger plant growth so that the sand soil can have more potential for crop cultivation [19].

The ideal soil for growth and production of shallots is loose soil, fertile and contains a lot of organic matter or humus, because it will encourage the development of bulbs so that the yield of shallots is better. Land with good drainage and aeration conditions is preferred [20]. Coastal sand land with low nutrient content requires soil enhancers to create soil conditions that support the growth of food crops and horticulture crops including shallots [21]. The provision of organic material is expected to overcome various obstacles in coastal sand land considering the important role of organic matter in relation to soil fertility. The sources of organic material that we can use may come from animal waste and waste (manure), crop residues, green manure, municipal waste, industrial waste, and compost. In sandy soil organic matter can be expected to change the soil structure from single grained to lumpy form, thereby increasing the degree of structure and size of the aggregate or increasing the class structure from fine to moderate or rough [22]. The greatest role of organic matter in the physical properties of soil includes structure, consistency, porosity, water binding power, and which is no less important is the increase in resistance to erosion [23]. In addition, organic matter is an energy source for macro and soil micro-fauna. The addition of organic matter in the soil will cause microbiological activities and populations in the soil to increase, especially those related to the decomposition and mineralization activities of organic matter. Some microorganisms that play a role in decomposition of organic matter are fungi, bacteria and actinomycetes [24]. Manure is the main source of organic material for farmers. By applying PGPF, especially *Trichoderma asperellum* on shallot cultivation in coastal sand, coupled with organic material in the form of cow manure, it is expected to meet the needs of shallots in terms of quantity and quality. The purpose of this study was to examine the responses of some onion cultivars to the inoculation of *Trichoderma asperellum* in coastal sand soils to obtain cultivars with the best growth and yield on cultivation in coastal sand fields.

2. Materials and methods

The research was carried out in the coastal sand field in Samas area, Bantul Regency, Yogyakarta, from January to March 2017. The ingredients used are onion seeds, PGPF inoculants (*Trichoderma asperellum*), a collection of Control Technology Laboratory, Dept. HPT Faculty of Agriculture UGM,
organic material (cow manure), NPK fertilizer, milled corn, PDA (*Potato Dextrose Agar*) media, TSM (*Trichoderma-selective medium*) media, chemicals used for biochemical analysis and polybag. The experiment was a factorial experiment, consisting of two factors arranged in a completely randomized block design (CRBD) with three replications. The two factors are various shallot cultivars which are the first factor, consisting of 20 cultivars, and the inoculation of *Trichoderma asperellum* isolates which is the second factor, consisting of two levels, namely without and with inoculation.

Land preparation: this research is a pot experiment using polybag size 35 cm x 35 cm. The planting medium used is a mixture of coastal sand soil and manure, 10 kg per polybag. Measure of manure 20 tons. Ha-1 (70 g per polybag). N-P-K fertilizer with a dose of 200 kg. Ha
\(^{-1}\) (0.7 g per polybag) is added when the plants are 10, 20 and 30 days after planting. Soil (planting media) was inoculated with *Trichoderma asperellum* isolates according to treatment. Preparation of planting material: the seeds used are shallot bulbs that have been stored for 2-6 months, medium size with a diameter of 1.5-2 cm (2.5-7.5 g). Selected seeds that are uniform, intact, normal, and physically healthy. Before planting, the outer skin of the tuber that has dried is cleaned.

Preparation of isolates: *Trichoderma asperellum* isolates which will be inoculated are cultured first. The fungi isolate culture media is made from milled corn that has been washed, then boiled for 25 minutes with a ratio of 1:1 (weight: volume) that is, every one gram of corn needed 1 ml of water. After that, corn is filled in a plastic bag and sterilized. Sterilized corn media was inoculated with pieces of *Trichoderma asperellum* fungus isolates and then wrapped tightly. The culture medium was incubated for 7 days at room temperature.

Planting: before the seeds are planted, the soil is watered first and a planting hole is made. Inoculation of *Trichoderma asperellum* culture was carried out by 100 grams of culture immersed in sand soil media in polybag and then incubated for three days. Then the shallot seedlings are planted in the planting media. Each polybag is filled with one seed, by immersing the bulbs in the planting hole in an upright position and slightly pressed slightly downward until the end of the bulb is flat with the ground surface, then the growth is observed.

3. Results and discussions

3.1. The physiological response of plants

The results of the observation of the effect of *Trichoderma asperellum* on various shallot cultivars on total chlorophyll content, leaf greenness, and leaf stomata opening width can be seen in Table 1. The observation of the characters and physiological processes in plants, it was found that there was an interaction of the influence of shallots and inoculation of *Trichoderma asperellum* on total chlorophyll. Without inoculation, Mentes cultivars had high total chlorophyll levels but no changes were significantly different from the treatment of *Trichoderma asperellum*. The highest increase in total chlorophyll levels occurred in Bima Brebes cultivars inoculated with *Trichoderma asperellum* compared to without inoculation, which was 31%. The inoculation of *Trichoderma asperellum* also had a positive effect on the greenness of leaves and the width of stomata opening (Table 1).
Table 1. Total chlorophyll content, leaf greenness level, leaf stomata opening width (μm) of various onion cultivars, without and inoculated with *Trichoderma asperellum*

| Cultivars        | Total chlorophyll content (mg / g leaves) | Greenness of leaves | Width of leaf stomata opening (μm) |
|------------------|------------------------------------------|---------------------|-----------------------------------|
|                  | Without inoculation | Inoculated         |                                   |                                   |
| Bima Brebes      | 0.39 b–i            | 0.51 abc           | 47.68 pq                         | 5.76 p                            |
| Bauji            | 0.43 b–h            | 0.40 b–i           | 40.70 rs                         | 4.78 p                            |
| Biru Lancor      | 0.40 b–h            | 0.50 abcd          | 52.60 p                          | 5.93 p                            |
| Bima             | 0.48 a–f            | 0.29 hi            | 39.93 rs                         | 4.91 p                            |
| Bima Nganjuk     | 0.45 b–g            | 0.34 d–i           | 48.65 pq                         | 5.78 p                            |
| Bali Tabanan     | 0.45 b–g            | 0.47 b–f           | 48.53 pq                         | 6.71 p                            |
| Crok Kuning      | 0.37 c–i            | 0.45 b–g           | 41.68 qrs                        | 5.93 p                            |
| Kuning           | 0.35 d–i            | 0.33 fghi          | 33.90 st                         | 5.44 p                            |
| Katumi           | 0.44 b–g            | 0.41 b–h           | 46.10 pq                         | 5.97 p                            |
| Kuning Tablet    | 0.34 c–i            | 0.31 ghi           | 29.40 t                          | 6.07 p                            |
| Manjoung         | 0.40 b–i            | 0.39 b–i           | 52.90 p                          | 7.52 p                            |
| Mentes           | 0.62 a              | 0.54 ab            | 52.33 p                          | 4.78 p                            |
| Pikatan          | 0.40 b–i            | 0.35 d–i           | 46.48 pq                         | 5.24 p                            |
| Super Biru       | 0.39 b–i            | 0.49 a–e           | 42.60 qrs                        | 5.75 p                            |
| Sembrani         | 0.38 c–i            | 0.39 b–i           | 53.88 p                          | 6.03 p                            |
| Trisula Brebes   | 0.37 c–i            | 0.40 b–h           | 41.65 qrs                        | 6.67 p                            |
| Thailand         | 0.38 c–i            | 0.49 a–e           | 47.78 pq                         | 7.31 p                            |
| Tajuk            | 0.40 b–h            | 0.47 b–f           | 40.60 qrs                        | 6.20 p                            |
| Tiron            | 0.44 b–g            | 0.24 i             | 50.10 pq                         | 6.09 p                            |
| Trisula          | 0.41 b–h            | 0.34 d–i           | 48.63 pq                         | 6.59 p                            |
| Average          | 0.41                | 0.41 (+)           | 45.30 (-)                        | 5.97 (-)                           |

Values followed by the same letter show not significantly different in Duncan's Multiple Range test at 5% level (+): interaction; (-): no interaction

3.2. Plant growth and yield

The growth response and yield of various shallots cultivars on *Trichoderma asperellum* inoculation can be seen in Table 2. Morphological and growth responses of shallots to *Trichoderma asperellum* inoculation showed that the inoculation of *Trichoderma asperellum* had a positive effect by increasing the number of roots, plant height, and number of plant leaves. The response of yield components and crop yield quality also showed a positive response to the weight of fresh shallot bulbs per clump. The interaction of the influence of shallot cultivar and inoculation of *Trichoderma asperellum* occurred in the observation variables of total dry weight per plant. The Bima Brebes cultivar showed a negative response to *Trichoderma asperellum* inoculation with a decrease in the value of total dry weight (Table 2).
Table 2. Total dry weight per plant (g), Number of roots, plant height (cm), number of plant leaves (strands), fresh tuber weight per clump (g) on various onion cultivars, which were without and inoculated with Trichoderma asperellum

| Cultivar          | Total dry weight per plant (g) | Number of roots | Plant height (cm) | Number of plant leaves (strands) | Fresh tuber weight per clump (g) |
|-------------------|--------------------------------|-----------------|------------------|----------------------------------|---------------------------------|
|                   | Without inoculation | Inoculated      |                  |                                  |                                 |
| Bima Brebes       | 7.95 g - k          | 6.44 jk         | 14.06 p          | 32.59 rs                        | 20.36 uv                        | 36.14 uv                       |
| Bauji             | 17.50 ab            | 24.37 a         | 12.78 p          | 36.96 pqrs                      | 39.11 qrs                       | 86.44 pq                       |
| Biru Lancer       | 16.75 bc            | 13.72 b - g     | 12.67 p          | 37.36 pqrs                      | 51.11 p                         | 96.56 p                        |
| Bima              | 13.72 b - g         | 14.28 bcede     | 15.44 p          | 38.57 pq                        | 40.83 qr                        | 89.17 pq                       |
| Bima Nganjuk      | 16.50 bcd           | 10.53 e - k     | 11.39 p          | 34.64 pqrs                      | 30.75 rstuv                      | 61.22 rstu                      |
| Bali Tabanan      | 10.17 e - k         | 14.08 b - f     | 13.61 p          | 34.52 pqrs                      | 37.50 qrs                       | 54.89 stu                       |
| Crok Kuning       | 10.06 e - k         | 10.72 d - k     | 11.72 p          | 38.76 p                         | 29.11 stuv                      | 67.61 qrst                      |
| Kuning            | 5.53 k              | 7.72 hijk       | 10.94 p          | 32.06 s                         | 24.39 tuv                       | 23.67 v                        |
| Katumi            | 13.06 b - h         | 12.94 b - h     | 13.61 p          | 35.36 pqrs                      | 38.22 qrs                       | 75.67 q - t                     |
| Kuning Tablet     | 7.47 hijk           | 8.78 e - k      | 11.56 p          | 37.30 pq                        | 32.94 rst                        | 61.33 rstu                      |
| Manjoung          | 12.28 b - j         | 8.33 e - k      | 13.56 p          | 33.88 pq                        | 36.94 rs                        | 61.94 p - u                     |
| Mentes            | 8.25 f - k          | 7.22 hijk       | 12.78 p          | 33.79 pq                        | 36.72 rs                        | 67.39 pqq                      |
| Pikatan           | 8.42 e - k          | 10.33 e - k     | 13.11 p          | 33.15 qrs                       | 36.83 rs                        | 63.72 pq - u                    |
| Super Biru        | 11.92 b - j         | 11.06 e - k     | 14.83 p          | 36.76 pq                        | 41.17 q                         | 67.44 pqq                      |
| Sembrani          | 9.89 e - k          | 16.89 bc        | 10.78 p          | 36.24 pq                        | 19.22 v                         | 59.61 rstu                      |
| Trisula Brebes    | 6.72 jk             | 6.95 ijk        | 12.83 p          | 32.30 s                         | 31.17 rstu                      | 54.78 stu                       |
| Thailand          | 6.78 ijk            | 9.08 e - k      | 14.56 p          | 37.95 pq                        | 48.33 pq                         | 80.83 pqr                       |
| Tajuk             | 6.45 jk             | 12.72 b - i     | 12.56 p          | 34.06 pq                        | 38.17 q                         | 62.89 pq - u                    |
| Tiron             | 8.69 e - k          | 9.67 e - k      | 12.89 p          | 33.92 pq                        | 30.83 rstuv                      | 47.83 tuv                       |
| Trisula           | 9.08 e - k          | 13.11 b - h     | 12.39 p          | 35.33 pq                        | 33.67 rst                        | 61.11 pq - u                    |
| Average           | 10.36               | 11.45 (+)       | 12.90 (-)        | 35.27 (-)                       | 34.87 (-)                       | 64.16 (-)                      |

Trichoderma sp. inoculation

| Cultivar          | Total dry weight per plant (g) | Number of roots | Plant height (cm) | Number of plant leaves (strands) | Fresh tuber weight per clump (g) |
|-------------------|--------------------------------|-----------------|------------------|----------------------------------|---------------------------------|
|                   | Without inoculation | Inoculated      |                  |                                  |                                 |
| Trichoderma       | 11.58 b             | 33.81 b         | 31.99 b          | 58.06 b                          | 50.27 b                         |

Values followed by the same letter show not significantly different in Duncan's Multiple Range test at 5% level

(+) : interaction; (-) : no interaction

The data showed that the inoculation of Trichoderma asperellum was able to improve physiological responses, as well as the growth and yield of shallots, which can be attributed to Trichoderma's ability to act as PGPF as Arora [1] stated that some fungi reported as PGPF are Trichoderma, Penicillium, Phoma, Fusarium, and Gliocladium. Worosuryani et al. [25] also stated that Trichoderma spp. can stimulate plant growth by increasing plant height and dry weight so that it can be referred to as PGPF. Trichoderma colonizes plant roots and causes significant changes in plant metabolism, alters hormone content, dissolved sugars, phenolic and amino acid compounds, photosynthesis, transpiration, and water content ([26]; [27]; [28]). According to Contreras-Cornejo [29], some Trichoderma strains induce root branching and increase shoot biomass as a consequence of cell division, expansion and differentiation due to the presence of auxin-like compounds from fungi. Furthermore, Trichoderma, along with plant roots, can trigger systemic resistance and increase absorption of plant nutrients. Recent research shows that in the early stages of interaction, metabolites such as auxin and protein compounds released by Trichoderma are received by the roots, altering various hormonal mechanisms that control plant growth and development under 'normal' or stressful conditions [30]. Consequently, when the root system is colonized, the association is strengthened, providing protection to the zone against pathogenic microorganisms, and also a strong root system developed to increase the absorption of nutrients and water [31].

Fresh bulb weight per clump is influenced by Trichoderma inoculation, and various shallot cultivars have a variety of fresh bulb weights. Based on the weight of fresh bulbs per clump produced, cultivars can be grouped into three. The group that produced a relatively heavy weight of fresh bulb per clump was Bauji, Biru Lancer, Bima, Katumi, Manjoung, Mentes, Pikatan, Super Biru, Thailand,
Tajuk, and Trisula cultivars. The group of cultivars which have the weight of fresh bulbs per clump that is relatively medium are Bima Nganjuk, Bali Tabanan, Crok Kuning, Kuning Tablet, Sembrani and Trisula Brebes, and the group of cultivars which produced the weights of fresh bulbs per clump relatively light were Bima Brebes, Kuning and Tiron. Plants inoculated with Trichoderma produced fresh tuber weights per clump heavier than without inoculation (Table 2).

In each plant variety there is always a difference in genotypic response to various environmental conditions where it grows, this condition causes differences in the growth of each shallot cultivar [32]. Among the 20 cultivars tested, Biru Lancor gave the heaviest fresh bulb yield per clump. Bulbs on shallots are swollen leaves on the base where the outer petals are always circular, closing the inner leaf petals and over time appear bulging to form a bulb [33]. This is supported by the fact that the Biru Lancor cultivar has the highest number of leaves. The number of leaves also affects plant height. Biru Lancor cultivar, which has the highest number of leaves, apparently did not produce the highest plants, although not significantly different from the highest plants. This is estimated to occur because of competition in obtaining nutrients, water and light, according to a statement from Efendi, (1985) that competition for solar radiation, and growing space affect morphology such as plant height. The large number of leaves will enhance photosynthesis and will produce many photosynthates which will be translocated to bulbs [34]. Increased number of leaves per clump and accompanied by the appearance of green leaves indicate an increase in chlorophyll content that produces photosynthates for plant growth and development [35].

4. Conclusion

There was an interaction between the effect of Trichoderma asperellum and onion cultivars on total chlorophyll content and total dry weight per plant. Various shallot cultivars show the greenness of the leaves, plant height, number of leaves per plant, and the variety of fresh bulb weight per plant. Inoculation of Trichoderma asperellum produced leaf greenness, leaf stomata opening width, number of roots, plant height, number of plant leaves, fresh bulb weight per plant which was better than without inoculation. Cultivar that show the best growth and yield was Biru Lancor.

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