Response of chili (Capsicum annuum L.) to bioslurry fertilization and enrichment of Trichoderma asperellum on planting media

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Abstract. This study aimed to determine the response of chili plants (Capsicum annuum L.) to the treatment of bioslurry fertilizer and enrichment of Trichoderma asperellum on planting media. The study was conducted from October 2017 to January 2018 and set based on randomized block design (RBD). The treatment of Trichoderma asperellum as the first factor consisted of five levels, namely: 0 g plant⁻¹ (control), 5, 10, 15, and 20 g plant⁻¹. The second factor was bioslurry fertilization treatment consisted of five levels, namely: 0 mL L⁻¹ (control), 100, 200, 200, and 400 mL L⁻¹ per application. The results show that the application of Trichoderma asperellum 10 g plant⁻¹ gave the highest fruit length of 12.03 cm. The interaction between Trichoderma asperellum 10 g plant⁻¹ and 100 mL L⁻¹ bioslurry per application showed the best results on the highest number of productive branches (45.17 stems), the highest number of harvested fruit (12.17 fruits), and the heaviest fruit weight (129.82 g).

1. Introduction
Chili is one of the vegetables with substantial development and marketing prospects in the national market in Indonesia. The fruit of this plant is consumed as a cooking spice, a basic ingredient of traditional medicinal herbs, a mixture of ingredients in the food and beverage industry. Large chili is also processed into chili flour for overseas export markets [1]. To maintain the supply for the domestic market, the continuity of the production of this commodity is necessary. The unstable production of chili plants is caused by several factors, including poor land management which results in a decrease in the level of physical, chemical, and biological soil fertility. In addition, plants often experience wilting and death due to attack by bacteria and soil-borne pathogens [2].

One of the poor land management is the use of immature manure. This allows the existence of fungi and bacteria and soil pathogens that have not been decomposed properly in the soil. This resulted in the emergence of sources of disease for plants [3]. Therefore, the provision of good organic fertilizer is important in land management to ensure good growth as well. The application of organic material is one way to improve the physical and biological properties of the soil. Organic matter can improve soil structure, increase water holding capacity, pore aeration, and infiltration rate, and facilitate root penetration so that land productivity and crop yields can increase [4–6].
One of the organic fertilizer that can be used is Bioslurry. Bioslurry fertilizer contains the main nutrients (macro) needed by plants such as NPK (nitrogen, phosphorus, and potassium) and supplementary nutrients (micro) such as magnesium (Mg), calcium (Ca), and sulfur (S). Bioslurry fertilizers also contain "pro-biotic" microbes that are beneficial for improving fertility and health of agricultural land so that they are expected to have an impact on improving the quality and quantity of harvests. Bioslurry as an organic fertilizer is widely used in agricultural areas in Indonesia for leaf and fruit vegetable commodities, tubers, fruit trees, and food crops [7].

Research using Bioslurry as an organic fertilizer on vegetable plants that have been conducted by Musdalifah [8] stated that the application of Bioslurry and ABmix fertilizers with concentrations of 20 mL L\(^{-1}\) and 200 mL L\(^{-1}\) per plant, respectively, resulted in the best products for the growth and production of chili plants. The enrichment of planting media with microorganisms is also a step to improve the quality of the planting media [9]. Growing media on chili plants can be enriched with the addition of *Trichoderma* sp., *Aspergillus* sp., Azotobacter, and other microbes that can perform a symbiotic mutualism with plant roots [10]. Research shows that *Trichoderma* sp. is one of the fungi that can be a biocontrol agent because it is antagonistic to other fungi, especially those that are pathogenic [11]. Antagonistic activity in question can include competition, parasitism, predation, or the formation of toxins such as antibiotics. For biotechnology purposes, this biocontrol agent can be isolated from *Trichoderma* and used to deal with plant damage due to pathogens [12].

Chamzurni et al. [13] showed that *T. harzianum* and *T. virens* can be used in controlling *R. solani* with average seed growth and plant height of 75% and 9.25 cm, respectively. Other studies have shown that the response of chili growth due to *T. harzianum* can increase the number of lateral roots, chlorophyll content and dry weight of chili plants. The application of *T. harzianum* 15 g kg\(^{-1}\) soil has been shown could control the attack of *Fusarium oxysporum* f.sp capsicin which causes wilting disease in red chili [14]. In addition, based on research conducted by Sepwanti et al. [15] suggested that the best dosage of compost-enriched *Trichoderma harzianum* is at a maximum dose of 20 g plant\(^{-1}\). Similarly, the application of a combination of Trichoderma enrichment and Boron fertilizer can increase fruit length with *Trichoderma asperellum* 4 g plant\(^{-1}\) and Boron 1 mg L\(^{-1}\) doses [16].

Based on the description above, this study was conducted to determine the response of chili plants (*Capsicum annuum* L.) to the application of Bioslurry and enrichment of planting media with *Trichoderma asperellum*.

### 2. Methodology

The study was conducted at the Experimental Farm of the Faculty of Agriculture, Hasanuddin University, Makassar from October 2017 to January 2018. This trial was conducted in the form of an experiment based on randomized block design (RBD). Application of *Trichoderma asperellum* (T) on the planting media set as the first factor consisted of four levels T0 = without *Trichoderma asperellum*; T1 = *Trichoderma asperellum* 5 g plant\(^{-1}\); T2 = *Trichoderma asperellum* 10 g plant\(^{-1}\); T3 = *Trichoderma asperellum* 15 g plant\(^{-1}\); and T4 = *Trichoderma asperellum* 20 g plant\(^{-1}\). As the second factor, application of Bioslurry fertilizer (P) that consisted of four levels, namely P0 = 0 mL L\(^{-1}\) per application; P1 = 100 mL L\(^{-1}\) per application; P2 = 200 mL L\(^{-1}\) per application; P3 = 300 mL L\(^{-1}\) per application; and P4 = 400 mL L\(^{-1}\) per application. Each treatment combination was repeated three times resulted in a total of 75 experimental plots.

Prior to planting, the chili seeds of the Pillar 1 variety were immersed in 1 mL L\(^{-1}\) of Atonic solution for 24 hours to accelerate germination. Seedlings at 25 days after sowing (DAS) were pruned to stimulate the growth of stems and leaves. Simultaneously, the selection was conducted for healthy, strong and uniformly grown seedlings. The 25 days old seedlings were then transferred to the field after being soaked with enough water. The seedlings were carefully removed from the polybag and immediately planted in the holes provided.

Application of the *Trichoderma asperellum* in the planting hole was carried out at the transplanting and placed right about at the rhizosphere of the seedlings. The application of bioslurry was conducted from 9 days after planting (DAP) to 51 DAP (8 times). Supplementary fertilization using AB-Mix as
much as 20 mL L$^{-1}$ [8] was applied once a week. Fertilization is applied by watering the roots of plants with a volume of 200 mL plant$^{-1}$, fertilizing is applied in the morning.

Observation data were analyzed using analysis of variance (ANOVA). The treatment that shows the significant effect was further tested using Tukey’s Honestly Significant Difference (HSD) analysis at the 0.05 level.

3. Results

3.1. Number of productive branches

Analysis of variance results shows that the treatment of *Trichoderma asperellum*, Bioslurry concentration and the interaction of two factors significantly affected the productive branches of the chili plants. Table 1 shows that the highest number of productive branches (45.17 branches per plant) was found in the interaction between application of *Trichoderma asperellum* 10 g plant$^{-1}$ and concentration of 100 mL L$^{-1}$ bioslurry (T2P1). Better responses of the chili plant to application of different concentration of bioslurry were observed in control growing media without the fungi application. Second largest number of productive branches was shown by P1T0 combination treatment (application of 100 ml L$^{-1}$ of bioslurry in 0 g per plant *Trichoderma asperellum*).

Table 1. Average number of productive branches of chili on different concentration of bioslurry planted in growing media enriched with *Trichoderma asperellum*.

| Concentration of Bioslurry | Dose of *Trichoderma asperellum* (g plant$^{-1}$) |
|----------------------------|-----------------------------------------------|
|                            | T0 (Control) | T1 (5 g) | T2 (10 g) | T3 (15 g) | T4 (20 g) |
| P0 (Control)               | 27.89 ghi    | 24.33 i   | 24.33 i   | 25.39 hi  | 32.89 efg  |
| P1 (100 ml L$^{-1}$)       | 42.19 ab     | 20.06 i   | 45.17 a   | 30.78 efg | 29.22 ghi  |
| P2 (200 ml L$^{-1}$)       | 31.72 efg    | 38.44 bcd | 38.61 bcd | 29.83 fghi| 39.83 abcd |
| P3 (300 ml L$^{-1}$)       | 40.00 abcde  | 39.56 bcd | 34.89 def | 25.22 i   | 24.89 i    |
| P4 (400 ml L$^{-1}$)       | 40.22 abcd   | 29.78 fghi| 41.11 abc | 36.11 cde | 35.67 de   |

Tukey’s HSD $\alpha = 0.05$ 5.40

Numbers followed by same letters are not significantly different based on Tukey’s HSD $\alpha = 0.05$.

3.2. Number of fruit at harvest

Analysis of variance results shows that the treatment *Trichoderma asperellum*, bioslurry concentration and the interaction of the two factors significantly affected the number of chilli fruit per plant at harvest. Table 2 shows that the highest number of harvested fruits (12.17 fruits per plant) was found in the interaction between *Trichoderma asperellum* 10 g plant$^{-1}$ with a concentration of 100 mL L$^{-1}$ bioslurry (T2P1) and significantly different from other bioslurry concentrations.

Table 2. Average number of fruit at harvest (fruits per plant) of chili on different concentration of bioslurry planted in growing media enriched with *Trichoderma asperellum*.

| Concentration of Bioslurry | Dose of *Trichoderma asperellum* (g plant$^{-1}$) |
|----------------------------|-----------------------------------------------|
|                            | T0 (Control) | T1 (5 g) | T2 (10 g) | T3 (15 g) | T4 (20 g) |
| P0 (Control)               | 8.11 d       | 4.67 ijk  | 7.17 de   | 6.17 efgh | 3.22 l    |
| P1 (100 ml L$^{-1}$)       | 10.83 b      | 3.94 kl   | 12.17 a   | 5.44 ghij | 6.61 ef   |
| P2 (200 ml L$^{-1}$)       | 5.58 fghi    | 9.33 c    | 6.11 efgh | 6.61 ef   | 8.22 cd   |
| P3 (300 ml L$^{-1}$)       | 5.94 fghi    | 4.33 jkl  | 5.33 hij  | 5.11 hij  | 4.61 ijk  |
| P4 (400 ml L$^{-1}$)       | 7.11 de      | 6.17 efgh | 8.39 c   | 6.5 ef    | 5.89 fgh  |

Tukey’s HSD $\alpha = 0.05$ 1.12
Numbers followed by same letters are not significantly different based on Tukey’s HSD α = 0.05.

3.3. Fruit length
The results of analysis of variance show that the treatment of *Trichoderma asperellum* had a significant effect while the concentration of bioslurry and the interaction of two factors did not significantly affect the length of chilli fruit. Table 3 shows that *Trichoderma asperellum* 10 g plant\(^{-1}\) (T2) resulted in the longest fruit length (12.03 cm) and was not significantly different from *Trichoderma asperellum* 0 g plant\(^{-1}\), 5 g plant\(^{-1}\), and 20 g plant\(^{-1}\).

| Concentration of Bioslurry | Dose of *Trichoderma asperellum* (g plant\(^{-1}\)) |
|---------------------------|-----------------------------------------------|
|                           | T0 (Control)          | T1 (5 g)       | T2 (10 g)     | T3 (15 g)    | T4 (20 g)    |
| P0 (Control)              | 12.57                | 11.74          | 11.60         | 11.74        | 11.71        |
| P1 (100 ml L\(^{-1}\))   | 11.73                | 11.16          | 12.58         | 11.35        | 11.12        |
| P2 (200 ml L\(^{-1}\))   | 11.33                | 10.96          | 11.60         | 10.42        | 11.69        |
| P3 (300 ml L\(^{-1}\))   | 11.96                | 12.00          | 12.70         | 10.44        | 10.91        |
| P4 (400 ml L\(^{-1}\))   | 11.51                | 12.25          | 11.66         | 10.88        | 11.34        |
| Mean                      | 11.82 ab             | 11.62 ab       | **12.03 a**   | 10.96 b      | 11.35 ab     |

Tukey’s HSD \(_{0.05}\) 1.10

Numbers followed by same letters are not significantly different based on Tukey’s HSD α = 0.05.

3.4. Weight of harvested fruits
The results of analysis of variance show that the treatment of *Trichoderma asperellum*, bioslurry concentration and the interaction of the two factors significantly affected the weight harvested fruit of the chili plants. Table 4 shows that the heaviest fruit weight (129 g per plant) was found in the interaction between *Trichoderma asperellum* 10 g plant\(^{-1}\) with a concentration of 100 mL L\(^{-1}\) bioslurry (T2P1) and significantly different from other bioslurry concentrations. The same bioslurry concentration of 100 mL L\(^{-1}\) showed that *Trichoderma asperellum* 10 g plant\(^{-1}\) (T2P1) gave the heaviest fruit weight and was significantly different from other *Trichoderma asperellum* treatment except for the control *Trichoderma asperellum* treatment (TOP1).

| Concentration of Bioslurry | Dose of *Trichoderma asperellum* (g plant\(^{-1}\)) |
|---------------------------|-----------------------------------------------|
|                           | T0 (Control)          | T1 (5 g)       | T2 (10 g)     | T3 (15 g)    | T4 (20 g)    |
| P0 (Control)              | 89.3 d                | 53.63 j        | 80.58 ef      | 68.81 fgh    | 33.79 k      |
| P1 (100 ml L\(^{-1}\))   | 119.48 ab             | 33.33 k        | **129.82 a**  | 59.93 hij    | 69.81 fgh    |
| P2 (200 ml L\(^{-1}\))   | 60.05 hij             | 105.91 bc      | 62.27 hij     | 70.29 fgh    | 99.84 cd     |
| P3 (300 ml L\(^{-1}\))   | 69.62 fgh             | 51.44 j        | 56.61 ij      | 50.98 j      | 70.97 fgh    |
| P4 (400 ml L\(^{-1}\))   | 78.2 efg              | 68.73 fgh      | 95.68 cd      | 57.28 hij    | 65.81 ghi    |

Tukey’s HSD \(_{0.05}\) 13.90

Numbers followed by same letters are not significantly different based on Tukey’s HSD α = 0.05.

4. Discussion
Soil as a plant growing medium is a component of the overall ecosystem and cannot be separated from the health of that ecosystem. In plant cultivation, healthy soils have optimal physical, chemical and biological conditions for crop production and have the ability to maintain plant health and the quality of ecosystems that include water and soil [17]. The soil zone in the root system area, known as the
"Rizosphere", is an area of biological and chemical activity of the soil, influenced by compounds released by roots intensively and is food for soil microorganisms [18].

Utilization of soil bio-technology (utilization of soil microbial services) to increase general crop production and maintain land productivity is an alternative that needs to be made as the primary choice in improving land productivity [19]. The addition of bioslurry and *Trichoderma asperellum* is an alternative to increase soil fertility and help plants produce high production. The relatively higher number of productive branches in the 10 g plant⁻¹ *Trichoderma asperellum* treatment is due to the soil's microbiological ability to absorb nutrients and water for plant needs and help plants to protect from pests and diseases. This is consistent with the opinion of Nasaruddin [20] which stated that the ability of Trichoderma singly or in a double manner can improve plant growth and production. In addition, the addition of bioslurry to plantations adds nutrients so that the plants do not experience nutrient deficits and the needs of plants to produce high production are met. This is according to the opinion of Hartanto and Putri [7] bioslurry is an organic fertilizer that contains essential nutrients for plant growth. Macro nutrients such as Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), and Sulfur (S), as well as micro nutrients such as Iron (Fe), Manganese (Mn), Copper (Cu), and Zinc (Zn).

Nutrients from bioslurry application can be absorbed by plants for metabolic activities both vegetative formation and generative appearance of plants so that the high amount of bioslurry given indicates the availability of nutrients in plant roots is also high. The provision of adequate nutrition during vegetative periods of the plant would be very helpful in plant development and growth, besides that fertilization would stimulate an increase in growth hormones present in these nutrients which could regulate the course of plant physiology [21].

Fruit formation is influenced by the amount of nutrients available in the soil and the temperature and evaporation through the soil. Application of Trichoderma in this study improved the size of the fruit formed. According to Karmila [22], the absorption of nutrients and water is more maximal because Trichoderma colonizes the roots of chilli plants allowed a plant growth and increase plant weight, especially roots. Applying 100 mL L⁻¹ bioslurry concentration in addition to the application of *Trichoderma asperellum* 10 g plant⁻¹ simultaneously was an optimum concentration of plant needs that plants can produce high production. Provision of bioslurry will increase the number of microbes in the soil that function as decomposers of organic matter in the soil to be available to plants, coupled with the presence of Trichoderma can also break down organic matter into nutrients for the roots. The length of the fruit is associated with the optimal fruit length will prove that there was adequate amount of nutrition for chili plants. This is in agreement with Suwandi et al. [23] that the application of liquid organic fertilizer will increase the weight of crop yields. This shows that the nutrients contained in bioslurry given to the soil can be used directly by plants both in growth and production. Then the microbes contained in the liquid fertilizer will decompose the organic material into nutrients used by large chili plants.

Fruit length is one of the benchmarks of high yield components produced by plants. The treatment that showed the highest fruit length was the treatment of giving *Trichoderma asperellum* 10 g plant⁻¹ with an average value of 12.03 cm. *Trichoderma asperellum* application has a symbiotic mutualism with the roots of the chili plant with the fungus entering the xylem tissue at the root of the chili plant, the presence of *Trichoderma asperellum* in the rhizosphere can function as a water provider for the chili plants because of the hyphae can expand the water extraction area by extending hyphae to the roots of the chili plant in order to maintain the humidity around the fungus. This is in line with Herlina and Pramesti [14] that in addition to the ability as a biological controller, Trichoderma has a positive influence on plant roots, plant growth, and crop production. This trait indicates that Trichoderma also acts as a Plant Growth Enhancer. Therefore, the interaction of treatment between the concentration of bioslurry with the application of *Trichoderma asperellum* can influence the production of chili plants. Bioslurry proves the mechanism that occurs in highly complex soils such as the decomposition of the organic matter carried out by microbes that will be absorbed by the fungus hyphae which are translocated to the roots. The presence of the fungus will keep disruption from evil microbes that will
damage the root system of chili plants. Exploration of bacteria associated with chili peppers' rhizosphere results present that bacteria inoculated from roots and roots area of chili pepper are potential as Plant Growth Promoting Bacteria [24]. A total of 70 bacteria were isolated from the rhizosphere of potato cv. Hartapel grew at an altitude of 700 m above sea level on the island of Buru-Maluku. Of these isolates, 36 isolates were capable of producing IAA, GA, Siderophore and phosphate solubilization [25].

Fruit weight is the character of the yield component used as a measure of the yields. The treatment that produced the highest fruit weight was bioslurry concentration of 100 mL L⁻¹ with the amount of Trichoderma asperellum 10 g plant⁻¹ and the application of Trichoderma asperellum 10 g plant⁻¹ gave a fruit weight that tended to be higher than the other treatments. The treatment of Trichoderma asperellum helps plant roots to absorb phosphates and other macro and microelements that are needed during the growth hence support for higher production of fruit [26]. To obtain high fruit production, sufficient amount of photosynthate must be available through the process of photosynthesis and transplanted into recipient organs (flowers and fruit) [27]. To get large fruits, cell division must occur with cell enlargement [28].

5. Conclusions
Based on the results of the conducted research, it can be concluded as follows:

a) Interaction in the application of Trichoderma asperellum 10 g plant⁻¹ with a concentration of 100 mL L⁻¹ Bioslurry resulted in the best results on the number of productive branches, the number of harvested fruits, and the fruit weights parameters.

b) The application of Trichoderma asperellum 10 g plant⁻¹ showed the highest yield in fruit length parameter.

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