The Effect of Liquid Bio Fungicides Dosage *Trichoderma* spp. against Fusarium Wilt Diseases, Growth and Yield of Onion

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Abstract. The study aimed to determine the effect of liquid bio fungicides dosage *Trichoderma* spp. against Fusarium wilt diseases, growth and yield of onion. This study used an experimental method with experiments on field conditions carried out in Sembalun Bumbung Village Sembalun District East Lombok Regency from May to August 2018. The experiment used a Randomized Block Design with liquid bio fungicides Dosage *Trichoderma* spp. which consists of 9 treatments, namely, 0 ml/plant, 2.5 ml/plant, 5 ml/plant, 7.5 ml/plant, 10 ml/plant, 12.5 ml/plant, 15 ml/plant, 17.5 ml/plant and 20 ml/plant. Each treatment was repeated three times so that there were 27 experimental units. Experimental parameters included the percentage of disease incidence, plant growth (height and number of plant leaves), and yield of onion (number of tillers and weight of harvested onion). Data were analyzed using Analysis of Variance at 5% level and Honestly Significant Different Test at 5% level. The results showed that *Trichoderma* spp. can increase the induced resistance of onion to Fusarium wilt diseases and increase the growth and yield of onion. The conclusion of this experiment is liquid bio fungicides *Trichoderma* spp. starting with a dose of 5 ml/plant caused the onion plants not infected with Fusarium wilt diseases. Similarly, at the starting dose of 5 ml/plant increase the weight of harvest onion.

Keyword: bio fungicides, Fusarium, onion, *Trichoderma* spp., wilt diseases

1. Introduction

One obstacle in increasing onion production is the high attack rate of Fusarium wilt disease. At the beginning of growth since the age of 0-10 days after planting is often attacked by *Fusarium oxysporum* fungi which cause plants to wither quickly, leaves turn yellow, leaves twisted and the base of the stem rot. Fusarium wilt has caused damage and reduced the yield of the bulb up to 50% [1]. Usually attacks plants if such symptoms are found, the plants are removed and destroyed [2]. Fusarium wilt develops in onion planting centers in NTB, among others in West Lombok, East Lombok, Sumbawa, and Bima, which causes damage and decreases the yield of bulbs to more than 45% [3].

Until now, Fusarium wilt disease is difficult to control, because the fungus *F. oxysporum* has a surviving structure in the form of chlamydospore which can survive in the soil as saprophytes in a relatively long time around three to four years even without a host plant [4].

Therefore, it is necessary to control environmentally friendly by utilizing antagonistic microorganisms. Sudantha [5, 6] have successfully isolated saprophytic fungi *Trichoderma harzianum*
isolates SAPRO-07 isolated from the rhizosphere of vanilla and banana plants and *T. koningii* fungi ENDO-02 isolates isolated from endophytes of vanilla and banana plants can inhibit the development of wilt Fusarium in vanilla and banana plants can even stimulate vegetative and generative growth of plants. Besides, the two antagonistic fungi were also able to inhibit the growth of Fusarium wilt disease in soybean, corn, tobacco. Sudantha [7] reported that the two antagonistic fungi could inhibit Fusarium wilt in vanilla. Furthermore, *T. harzianum* saprophyte isolates SAPRO-07 and *T. koningii* fungi ENDO-02 isolates were formulated in the form of bio fungicides.

Bio fungicides containing *T. harzianum* saprophyte isolates SAPRO-07 and *T. koningii* endophytic fungi ENDO-02 isolates are effective in controlling Fusarium wilt disease in vanilla plants [8], Fusarium wilt disease in soybean plants [9], Fusarium wilt disease in banana plants [10], Fusarium wilt disease in corn plants [11], Fusarium wilt disease on soybean plants and Fusarium wilt disease on vanilla [7]. Sudantha, Suwardji and Fauzi [12] reported that in a greenhouse experiment the use of bio fungicides containing *T. harzianum* fungi Sapro-07 and *T. koningii* isolates Endo-02 as much as 15 g / pot effectively controlled fungi *F. oxysporum* f.sp. *cepa* in onion reached 42.26%. While the use of 10 g / pot bio fungicide can increase plant growth and onion yield. Sudantha et al. [13] reported that in a greenhouse bio fungicide application containing *T. harzianum* fungi Sapo-07 and *T. koningii* isolates Endo-02 could suppress Fusarium wilt disease and increase the growth and yield of onion.

However, how big is the role of *Trichoderma* spp. in controlling Fusarium wilt disease and increasing yield on onion in the field, it has not been widely revealed. Therefore, a study was conducted which aimed to determine the effect of bio fungicide doses (containing *Trichoderma* spp. fungi) on Fusarium wilt disease and the results of onion.

2. Method

The bio fungicide used is a liquid bio fungicide. Liquid formulations are based on Sudantha (2009) [10] method. The fungal cultures used were *T. koningii* fungus isolates ENDO-02 and *T. harzianum* SAPRO-07 isolates grown on PDA media and used for coffee leaf powder liquid fermentation.

The onion seeds used are Keta Monca varieties which are purchased from seed growers. Onion seeds that are good to use are healthy and quality seeds with a shelf life of 2 months and there appear to point growing at the roots. The day before planting the seeds are cut around ¼ part.

This study used an experimental method with experiments on field conditions carried out in Sembalun Bumbung Village Sembalun District East Lombok Regency from May to August 2018. The experiment used a Randomized Block Design with liquid bio fungicides dosage *Trichoderma* spp. which consists of 9 treatments, namely, 0 ml / plant, 2.5 ml / plant, 5 ml / plant, 7.5 ml / plant, 10 ml / plant, 12.5 ml / plant, 15 ml / plant, 17.5 ml / plant and 20 ml / plant. Each treatment was repeated three times so that there were 27 experimental units.

Soil processing is done by using hoes to flatten the soil and make plots measuring 2 m × 4 m for each treatment plot. After processing the soil, basic fertilization is carried out using Phonska fertilizer 100 kg/ha (50% of recommendations). The provision of basic fertilizer is done by immersing it next to the planting hole. Fungal inoculation of *F. oxysporum* was carried out because of the fungal population of *F. oxysporum* <40 x 10^4 Colony Form Unit/g soil or disease incidence <50%. Inoculation was carried out by 25 ml of *F. oxysporum* fungi suspension poured around the roots or shallot tuber in the two weeks after planting (WAP). Irrigation is carried out in the morning or evening and is done through the experimental land being dilated through a channel on the experimental plot to wet soil. Weeding is done by removing weeds that grow around the plants. Follow-up fertilization is done when the plant is 3 WAP. Follow-up fertilization using urea fertilizer 165 kg/ha and KCl 50 kg/ha. Fertilization is done by placing fertilizer next to the base of the plant stem with a distance of ± 2cm. Planting is done twice, namely when the plants are 5 WAP and 8 WAP. Embedding is done by raising the soil around the plant to the base of the plant stems. Harvesting of onion is done when the plant is 62 WAP.
Observations of variables were carried out on sample plants, namely 20 plot plants and random sampling. Observation of disease events was carried out by counting the number of plants that withered, observations were made on plant age 7 days after planting (DAP), 14 DAP, 21 DAP, 28 DAP, and 35 DAP. Disease events (%) are calculated using the following formula:
\[ I = \frac{a}{b} \times 100 \% \]

Where:
- \( I \) = Percentage of disease incidence
- \( a \) = Number of plants showing symptoms of the disease
- \( b \) = The total number of plants observed

Observation of yield components is carried out at harvest, i.e., at the age of more than 62 days after the plant. Observation of tuber weight was done using how to weigh tubers at harvest per sample plant. Observations were made by counting the bulbs of each plant after the plants were harvested. Data from observations were analyzed using Analysis of Variance (ANOVA) with a 5% level of significance using Minitab for Windows Rail. If there are variations, further testing is carried out using the Honestly Significant Difference (HSD) at the 5% level.

3. Result and Discussion

3.1. Effect of Trichoderma spp. Bio fungicide Dosage of Fusarium Wilt Disease in Onion

The results analysis of variance showed that the dosage treatment of bio fungicides was significantly different from the incidence of Fusarium wilt disease in onion at the age of 7 days to 35 days. The results of further testing of the incidence of Fusarium wilt disease of onion can be seen in Table 1.

| Treatment Bio fungicide Dosage | The incidence of Fusarium wilt disease in the onion (%)* | 7 DAP | 14 DAP | 21 DAP | 28 DAP | 35 DAP |
|-------------------------------|--------------------------------------------------------|-------|-------|-------|-------|-------|
| 0.0 ml/plant                  | 18.67 c**)                                              | 19.00 c**) | 23.00 c**) | 23.67 c**) | 23.67 c**) |
| 2.5 ml/plant                  | 11.67 b                                                  | 11.67 b | 11.67 b | 11.67 b | 12.33 b |
| 5.0 ml/plant                  | 7.67 a                                                   | 7.67 a | 8.67 a | 8.67 a | 9.33 a |
| 7.5 ml/plant                  | 7.50 a                                                   | 7.50 a | 7.67 a | 8.67 a | 9.33 a |
| 10.0 ml/plant                 | 7.67 a                                                   | 7.67 a | 8.67 a | 8.67 a | 9.33 a |
| 12.5 ml/plant                 | 7.67 a                                                   | 7.67 a | 8.67 a | 8.67 a | 9.33 a |
| 15.0 ml/plant                 | 7.67 a                                                   | 7.67 a | 8.67 a | 8.67 a | 9.33 a |
| 17.5 ml/plant                 | 7.67 a                                                   | 7.67 a | 8.67 a | 8.67 a | 9.33 a |
| 20.0 ml/plant                 | 7.67 a                                                   | 7.67 a | 8.67 a | 8.67 a | 9.33 a |
| **HSD 5%**                    | 3.21                                                     | 2.70  | 2.21  | 2.86  | 2.31  |

*) Numbers have been transformed \( \sqrt{x + 1/2} \).

**) The numbers followed by the same letters in the same column are not significantly different from the HSD 5% test.

In Table 1, it can be seen that the treatment of bio fungicide doses has a significantly different effect on the incidence of Fusarium wilt disease in onion aged 7 days to 35 DAP. All biofungicidal doses were significantly different from controls (without bio fungicide treatment). This can be seen in the incidence of Fusarium wilt disease on onion gave bio fungicides lower than the treatment without-bio fungicide (control). The effect of the best bio fungicide treatment began to appear at doses of 5 ml/plant up to 20.0 ml/plant.

There is a fact that the doses of bio fungicides containing *T. koningii* fungi Endo-02 isolates and *T. harzianum* isolates Sapro-07 can inhibit the development of Fusarium wilt disease in onion because it is thought that these fungi act as antagonists that can suppress fungi *F. oxysporum*. This is supported by the results of several studies conducted on various plants, namely Abd-El Moity and Shatla (1981) [14], *Trichoderma* spp. is a mycoparasite that can penetrate the *S. rolfsii* mycelium and fungal
sclerotia resulting in lysis and crystallization. Furthermore, Papavizas [15] states that the mechanism of mycoparasitism begins with softening host cells by enzymes produced by mycoparasites before the damage and death of host cells. According to Hadar et al. [16], *T. harzianum* fungi produce β-(1,3) glucanase and chitinase enzymes which can damage *R. solani* cell walls. Cook and Baker [16] say that certain strains of *Trichoderma* produce viridin antibiotics which can inhibit the growth of other fungi. Sudantha [5] reported that invitro *Trichoderma* spp. effectively inhibits fungal growth of *F. oxysporum* f. sp. *vanillae* physically (space competition and mycoparasites) and excrete antibiotics. Sudantha and Abadi [17] say that *T. koningii* fungi isolate Endo-02 effectively inhibits Fusarium wilt disease in vanilla plants. Sudantha [8] revealed that the use of *Trichoderma* spp. can suppress the development of Fusarium wilt disease in soybean plants. Ernawati and Sudantha [18] say that the application of endophytic fungi *Trichoderma polysporum* isolates endo-04 and *T. harzianum* saprotot fungi isolate Sapro-07 can improve induced resistance to stem rot diseases of several vanilla clones. Latifah *et al.* [19] stated that *T. harzianum* in soil was able to inhibit the development of pathogenic fungi by conducting competition, both in terms of space and nutrition. *T. harzianum* can use many sources of nutrients for growth by destroying cellulose, starch, lignin, and other soluble compounds such as protein and sugar. Besides, *Trichoderma* can also inhibit the growth of spores and pathogenic hyphae with their ability to produce furonan group antibiotics [20]. Sudantha [4] said that *Trichoderma* spp. can be packaged in the form of bio fungicides, biocomposites, and bio activators which can reduce the development of soil-borne diseases. Sudantha, Kusnarta, and Sudana [21] also reported that *T. koningii* endophytic fungi found in banana plant tissue can improve the induced resistance of banana seedlings to Fusarium wilt disease and spur growth in the number of leaves. Sudantha and Ernawati [22] say that *Trichoderma* spp. endophytic fungus use. can increase the induced resistance of vanilla seedlings to Fusarium wilt. Sudantha [7] said that *Trichoderma* spp. can increase the induced resistance of vanilla seeds to stem rot disease. Sudantha and Suwardji [23] revealed that the use of *Trichoderma* spp. can increase the growth and yield of onion. Hasanah, Ernawati, and Sudantha [24] revealed that the use of *Trichoderma* spp. and vegetable fungicide extract can control Fusarium wilt disease in chili plants. Likewise, it was reported by Yudhiarti, Sudantha, and Fauzi [25] that the use of *T. koningii* fungi can spur the growth of soybean plants.

As an illustration of Fusarium wilt disease in onion as shown in Figure 1, it appears that onion plants infected with fungi *F. oxysporum* show symptoms of poor growth, some leaves are curved and twisted, leaf color is pale green to yellow and over time dry out.

![Figure 1. Healthy onion plants with the treatment of *Trichoderma* spp. (A). Onion infected with Fusarium wilt disease (without bio fungicide treatment) (B)](image)

The symptoms of Fusarium wilt disease in this study are following the results of a study by Duriat *et al.* [26] that plants wither quickly, the root of rotten plants, such plants will collapse, and yellowish leaves with a slightly curved shape. Supriyadi *et al.*, [27] in their study also reported that red-bottom
plants infected with Fusarium sp. show symptoms of yellowing leaves from the tip to the base of the leaf, leaf twisted plants, and in further attacks cause plants to collapse and die.

3.2. Effect of *Trichoderma* spp. Bio fungicide Dosage on Onion Plant Height

The results of the analysis of variance showed that the treatment of bio fungicide doses was significantly different from the height of onion plants at the age of 7 to 35 DAP. The results of further testing of shallots can be seen in Table 2.

| Treatment Bio fungicide Dosage | Onion Plant Height (cm) |    |    |    |    |
|-------------------------------|------------------------|----|----|----|----|
|                               | 7 DAP  | 14 DAP | 21 DAP | 28 DAP | 35 DAP |
| 0.0 ml/plant                  | 6.30 a*)| 15.00 a*)| 20.00 a*)| 25.60 a*)| 33.60 a*)|
| 2.5 ml/plant                  | 10.60 b | 19.60 b | 25.60 b | 33.60 b | 38.30 b |
| 5.0 ml/plant                  | 13.60 c | 23.50 c | 30.10 c | 36.60 c | 40.40 c |
| 7.5 ml/plant                  | 13.70 c | 23.50 c | 30.15 c | 36.70 c | 40.45 c |
| 10.0 ml/plant                 | 13.10 c | 23.60 c | 30.20 c | 37.10 c | 40.50 c |
| 12.5 ml/plant                 | 13.15 c | 23.65 c | 30.30 c | 37.10 c | 40.60 c |
| 15.0 ml/plant                 | 13.42 c | 23.65 c | 30.50 c | 37.20 c | 40.70 c |
| 17.5 ml/plant                 | 13.50 c | 23.68 c | 30.60 c | 37.40 c | 40.80 c |
| 20.0 ml/plant                 | 13.60 c | 23.70 c | 30.70 c | 37.50 c | 41.00 c |
| HSD  5%                      | 2.20   | 2.70   | 2.20   | 2.80   | 2.00   |

*) The numbers followed by the same letters in the same column are not significantly different from the HSD 5% test.

In Table 2, it can be seen that the treatment of bio fungicide doses has a significantly different effect on the height of onion plants aged 7 to 35 DAP. All bio fungicidal doses were significantly different from controls (without bio fungicide treatment). This can be seen in the height of onion plants in the treatment dosage of 5.0 ml/plant up to 20.0 ml/plant higher than in the treatment dose of 2.5 ml/plant and in the control. The fact that liquid *Trichoderma* bio fungicide can increase the height of onion plants is suspected because the role of *T. koningii* fungi Endo-02 isolates and *T. harzianum* isolates Sapro-07 contained in bio fungicides can control Fusarium wilt disease. In addition, the direct role of *T. koningii* fungi Endo-02 isolates in influencing the growth of onion plant height. Previous researchers such as Sudantha [5] reported that the role of *T. koningii* endophytic fungi isolates ENDO-02 in plant tissues can stimulate ethylene thus spurrying vegetative growth in plants. Windham *et al.* [28] reported that *T. harzianum* fungi could increase seed germination and plant growth.

3.3. Effect of *Trichoderma* spp Bio fungicide on Onion Results

Effect of *Trichoderma* spp. bio fungicide treatment on the weight of wet tubers and the weight of dried tubers have a significant effect. The results of further tests on the weight of wet tubers and the weight of dried tubers of onion can be seen in Table 3.

| Treatment Bio fungicide Dosage | Weight of Wet Tubes | Weight of dry bulbs |
|-------------------------------|---------------------|---------------------|
|                               | Weight kg/plot | Weight ton/ha | Weight kg/plot | Weight ton/ha |
| 0.0 ml/plant                  | 4.48 a *) | 5.60 | 3.90 a *) | 4.88 |
| 2.5 ml/plant                  | 7.79 b | 9.74 | 7.29 b | 9.11 |
| 5.0 ml/plant                  | 9.09 c | 11.36 | 8.10 c | 10.13 |
| 7.5 ml/plant                  | 9.32 c | 11.65 | 8.13 c | 10.16 |
| 10.0 ml/plant                 | 9.60 c | 12.00 | 8.27 c | 10.34 |
| 12.5 ml/plant                 | 9.60 c | 12.60 | 8.27 c | 10.34 |
| 15.0 ml/plant                 | 9.60 c | 12.60 | 8.27 c | 10.34 |
| 17.5 ml/plant                 | 9.60 c | 12.60 | 8.27 c | 10.34 |
| Treatment Bio fungicide Dosage | Weight of Wet Tubes | Weight of dry bulbs |
|-------------------------------|---------------------|-------------------|
| HSD 5%                        | Weight kg/plot      | Weight kg/plot    |
|                               | Weight ton/ha       | Weight ton/ha     |
| 20.0 ml/plant                 | 9.60 c              | 12.60             | 8.27 c | 10.34 |
|                               | 1.26                | 0.45              |

*) The numbers followed by the same letters in the same column are not significantly different from the HSD 5% test.

Based on Table 3 shows that the treatment of bio fungicide *Trichoderma* spp. gave a significant effect on the weight of wet tubers and dried tubers of onion. This can be seen in the weight of wet tubers and dried tubers of onion planted with *Trichoderma* spp. bio fungicide significantly different from treatment without-bio fungicide (control). Treatment of bio fungicide doses of 5.0 ml/plant showed harvest dry weight of 11.36 tons/ha and stored dry weight of 10.13 tons/ha, and at a dose of 20.0 ml/plant showed harvest dry weight of 12.60 tons/ha and save dry weight 10.34 tons/ha, while in the control for harvest dry weight only 5.60 tons/ha and save dry weight 4.88 tons/ha. In this study, the onion varieties used were the Keta Monca variety. Based on Minister of Agriculture Decree No. 529 / Kpts / PD.210 / 10/2003 [29] that the Keta Monca variety is considered to have 3 - 6 tubers, the weight of the wet bulbs (harvest) ranges from 8 - 25 grams per knoll and the yield potential is 10.70 tons of dried tuber/ha.

The fact that liquid *Trichoderma* bio fungicide can increase the yield of shallots is suspected because the role of *T. koningii* fungi Endo-02 isolates and *T. harzianum* isolates Sapro-07 contained in bio fungicides can control Fusarium wilt disease. Besides the direct role of *T. harzianum* fungi Sapro-07 isolates in influencing the yield of onion. Previous researchers such as Sudantha [5] reported that the role of *T. harzianum* saprophytic fungi isolates SAPRO-07 in spurring generative plant growth. This fungus in the rhizosphere or plant root areas excretes ethylene which is diffused into the plant's body through silem which acts to spur generative growth. Windham et al. [28] reported that *T. harzianum* fungi could increase seed germination and plant growth. Tronsmo and Dennis in Cook and Baker [18] reported that spraying conidia of *T. viride* and *T. koningii* fungi to protect strawberry plants from rotten disease turned out to stimulate early flowering. Salisbury and Ross (1995) [30] say that of the four types of auxin namely gibberellin, cytokinin, abscisic acid, and ethylene, it is suspected that ethylene is a hormone produced by the *Trichoderma* spp. which can stimulate flowering in plants. Latifah, Kustantinah, and Soesanto [31] that the application of *T.harzianum* in onion acts as a biological agent and plant growth stimulator so that it can increase the yield component compared to the treatment without the treatment of *Trichoderma*. Arianci [32] also mentioned that *Trichoderma* spp. can produce certain hormones to increase the weight and number of pods in soybean plants on peatland. Triyatno [33] reported that *Trichoderma* spp. able to stimulate plants in producing gibberellic acid hormones (GA3), Indolacetic Acid (IAA), and benzylaminopurin (BAP) so that plant growth is more optimum, fertile, healthy, sturdy, and ultimately affects plant resistance. Gibberellins and auxin hormones play a role in the extension of roots and stems, and the growth of fruit (tuber) and increase plant growth. As Roco and Perez [34], stated that *Trichoderma harzianum* fungus can stimulate plants to produce certain hormones such as gibberellic acid (GA3), indoleacetic acid (IAA), and benzylaminopurin (BAP) in large quantities. Gibberellins and auxin hormones in plants play a role in lengthening the roots and stems, stimulating flowering and fruit growth and increasing plant growth.

**4. Conclusion**

Liquid bio fungicides Trichoderma spp. starting with a dose of 5.0 ml/plant caused the onion plants not infected with Fusarium wilt diseases. By not getting infected with onion, the plant height growth will be better. The starting dose of 5.0 ml/plant increases the weight of harvest onion. Treatment of bio fungicide doses of 5.0 ml/plant showed harvest dry weight of 11.36 tons/ha and stored dry weight of 10.13 tons/ha, and at a dose of 20.0 ml/plant showed harvest dry weight of 12.60 tons/ha and save dry weight 10.34 tons/ha, while in the control for harvest dry weight only 5.60 tons/ha and save dry weight 4.88 tons/ha.
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