Trichoderma biofungicides formulations on shallot growth, yield and fusarium wilt disease resistance

I M Sudantha¹* and S Suwardji²

¹Agroecotechnology Study Program, Faculty of Agriculture University of Mataram, Indonesia
²Soil Science Study Program, Faculty of Agriculture University of Mataram, Indonesia

*Corresponding Author: sudantha@unram.ac.id

Abstract. The impact of climate change is an increase in extreme climatic events that make plants susceptible to disease. Fusarium wilt disease is one of the endemic diseases in extreme climatic areas in the high and lowlands. To deal with this climate change adaptation, a study was carried out that aims to determine the effect of the biofungicide Trichoderma spp. in controlling Fusarium wilt disease in several shallot varieties. This study used an experimental method carried out in the Highlands of Sembalun Bumbung Village, East Lombok using a Randomized Block Design with factorial experiments. The main factor is the formulation of biofungicides, namely: without biofungicides (control), liquid biofungicides, tablets and powders. The simple factor is shallot varieties, namely: Bali Karet, Ampenan, Keta Monca, Super Philip, and Super Cross. The results showed that liquid, tablet and powder of biofungicide could suppress Fusarium wilt disease up to 0%, while in control the percentage of disease reached 60%. Bali Karet varieties are very adaptive in the highlands, so they are very resistant to Fusarium wilt, Ampenan and Keta Monca adaptive in the highlands, while Super Philip and Super Cross are less adaptive. Bali Karet varieties showed the highest growth and yield.

1. Introduction

The impact of climate change is an increase in extreme climatic events that make plants susceptible to disease. Fusarium wilt disease is one of the important diseases in shallot whose development is highly dependent on climate change. Fusarium disease is more developed at high temperatures around 25 – 30° C because at high temperatures generally shallot are more stressful and more susceptible [1]. The impact of climate change can be direct on plants or indirectly through pathogens [2].

Fusarium wilt disease caused by the fungus Fusarium oxysporum f. sp. cepae is one of the important diseases of shallot. Fusarium wilt disease has caused damage and reduced the yield of coated tubers in NTB by up to 50% [3]. The fungus F. oxysporum f. sp. cepae has a surviving structure in the form of chlamydospores that can survive in the soil as saprophytes for a relatively long time, so it becomes difficult if controlled using chemical fungicides [4].

One of the control techniques that have good prospects in the future is biological technology using biofungicides fermented with the fungus Trichoderma spp. Biofungicide Trichoderma spp. is a fungicide whose active ingredient is in the form of microbes or one of the antagonistic fungi to control pathogenic
fungi in this case the fungus *F. oxysporum f. sp. cepae*. One of the microbes used for biofungicide fermentation is especially the saprophytic *Trichoderma harzianum* and the endophytic *T. koningii* [5].

The results of preliminary in-vitro studies showed that the saprophytic antagonist *T. harzianum* and *T. koningii* endophytic fungi isolated from the rhizosphere and shallot tissue were effective in controlling the fungus *F. oxysporum f. sp. cepae* with 45% percentage inhibition [6]. The mechanism of antagonism by means of space and nutrient competition, mycoparasites and antibiosis [7]. As a comparison, the use of *T. harzianum* and *T. koningii* fungi on other plants such as vanilla has been shown to be effective in controlling Fusarium wilt disease [8, 9]. The use of both antagonistic fungi in soybean is effective in controlling Fusarium wilt disease [10]. Fusarium wilt disease in banana plants can be controlled using antagonistic fungi *T. harzianum* and *T. koningii* [11]. Likewise, Fusarium wilt disease in maize can be controlled using the antagonistic fungi *T. harzianum* and *T. koningii* [12].

Fusarium wilt control using shallot varieties which have induced resistance has good prospects in the future. Induced resistance is the resistance of plants to pathogenic infections because the plant has been previously infected by other microorganisms, either of the same type or from other types [13]. The results of preliminary research indicated that Bali Karet, Ampenan, Keta Monca, Super Philip and Super Cross varieties were induced by the fungus *Trichoderma* spp. induces immunity to Fusarium wilt disease and induced resistance to Fusarium wilt disease [14].

To deal with extreme environmental adaptation, a study was conducted with the aim of knowing the effect of the biofungicide formulation *Trichoderma* spp. against Fusarium wilt disease, growth and yield of five shallot varieties. in field conditions in the highlands.

2. Methodology

2.1. Research materials

The biofungicide used was made from fermented coffee leaf extract with Sapro-07 isolate *T. harzianum* fungus and Endo-02 isolate *T. koningii* fungus for 7 days. The manufacturing procedure is in accordance with the procedure Sudantha [15] and Sudantha et al. [16].

The shallot seeds used are Bali Karet, Ampenan, Keta Monca, Super Philip, and Super Cross varieties purchased from seed breeders. Preparation of shallot seedlings for this study was according to procedures Sudantha et al. [17].

2.2. Research methods

This study used an experimental method carried out in the Highlands of Sembalun Bumbung Village, East Lombok using a Randomized Block Design with factorial experiments. The main factor is the formulation of biofungicides which consists of 4 levels, namely: without biofungicides (control), liquid biofungicides, tablets and powders. The simple factor is shallot varieties which consists of 5 levels, namely: Bali Karet, Ampenan, Keta Monca, Super Philip, and Super Cross. The treatment was a combination of *Trichoderma* spp. biofungicide formulations and shallot varieties with three replications, so that there are 60 experimental units.

2.3. Ways of working

Processing was carried out by using a hoe to level the soil and make experimental plots measuring 2x4 m for each treatment plot. Furthermore, the experimental plots were covered with plastic mulch and the planting holes were made 20x20 cm apart. The basic fertilizer used is Phonska fertilizer as much as 100 kg/ha or 50% of the recommendation. When the plant is 5 weeks after planting, the follow-up fertilization is carried out using urea fertilizer 165 kg/ha and KCl 50 kg/ha.
T. harzianum biofungicide is given according to the treatment, namely: liquid formulation biofungicide is given together with the planting of shallot bulbs by soaking the shallot bulbs, the tablet formulation is immersed next to the planting hole and the formulation is immersed next to the planting hole. Planting is done by placing the onion seed tubers into a hole with a depth of 2 cm and the hole is closed again with soil. Harvesting of shallots is carried out when the plants are 62 days after planting (dap)

2.4 Observation of variables
The incidence of Fusarium wilt disease was carried out by counting the number of wilted plants, up to the maximum vegetative growth of 35 dap. The incidence of Fusarium wilt is calculated using the formula:

\[
I = \frac{a}{b} \times 100 \%
\]

Where:
I = Percentage of disease incidence
a = The number of plants showing symptoms of disease
b = The total number of plants observed

For the dry tuber weight of the harvest was done by weighing all parts of the harvested dry plants when the plants were 62 dap.

2.5 Data analysis
Data were analyzed using Analysis of Variance at 5% level and Honestly Significant Different (HSD) Test at 5% level.

3. Results and discussion

3.1 The effect of Trichoderma spp. on the incidence of fusarium wilt disease
The results of the analysis of variance showed that the treatment of the biofungicide formulation Trichoderma spp. and shallot varieties and their interactions showed significant differences in the incidence of Fusarium wilt in shallot plants at 35 dap. The results of further tests on the interaction of the biofungicide formulation Trichoderma spp. and shallot varieties using HSD 5% against the incidence of Fusarium wilt in shallot plants at 35 dap observations are presented in Table 1.

| No. | Trichoderma spp. Biofungicide Formulation | Bali Karet | Ampenan | Keta Monca | Super Philip | Super Cross |
|-----|------------------------------------------|------------|---------|------------|--------------|-------------|
| 1.  | Without biofungisida                     | 30.00 c    | 50.50 b | 50.50 b    | 60.80 a      | 60.90 a     |
|     | A (2)                                    |            | A       | A          | A            | A           |
| 2.  | Liquid                                   | 0.00 c     | 10.20 b | 10.30 b    | 30.00 a      | 30.20 a     |
|     | B                                        | B          | B       | B          | B            | B           |
| 3.  | Tablet                                   | 0.00 c     | 10.60 b | 10.60 b    | 30.20 a      | 30.30 a     |
|     | B                                        | B          | B       | B          | B            | B           |
| 4.  | Powder                                   | 0.00 c     | 10.70 b | 10.70 b    | 30.40 a      | 30.50 a     |
|     | B                                        | B          | B       | B          | B            | B           |

Information: 1) The numbers in each column followed by the same letter are not significantly different.
2) The numbers on each line followed by the same letter are not significantly different.
In Table 1, it can be seen that the treatment of the biofungicide formulation *Trichoderma* spp. compared with control or without biofungicide treatment, it can reduce the incidence of Fusarium wilt disease in shallot plants. The incidence of Fusarium wilt disease in control or without the biofungicide *Trichoderma* spp. the Super Philip and Super Cross varieties reached more than 60%, the Ampenan and Keta Monca varieties reached 50%, while the Bali Karet varieties only reached 30%. The incidence of disease after biofungicide treatment was seen in Super Philip and Super Cross varieties reaching 30%, Ampenan and Keta Monca varieties reaching 10% and Bali Karet varieties reaching 0.0% or there was induced resistance or became immune after treatment with the Biofungicide *Trichoderma* Petrini [18] states that induced resistance in plants can be done by treating the seeds or plants with a suspension of endophytic fungi derived from these plants. According to Guest [19], resistance to disease induction occurs due to a combination of passive inhibition with local responses due to oxidative events, cell death and accumulation of antibiotics in the form of phytoalexin.

*Trichoderma* spp. biofungicide liquid, tablet and powder formulations in inhibiting the development of the fungus *F. oxysporum* f. sp. *cepae* through competition mechanisms, both in terms of space and nutrition. *T. harzianum* can use a wide variety of nutrient sources for growth by destroying cellulose, starch, lignin, and other soluble compounds such as protein and sugar [20]. In addition, *Trichoderma* can also inhibit the growth of pathogenic spores and hyphae by entrapment and penetration into pathogenic fungal cells [21]. Ghorbanpoura *et al.* [22] said that the antagonism mechanism of the fungus antagonist *Trichoderma* sp. There are five classes, namely: competing with pathogens for space and nutrition, mycoparasitism, antibiotics, mediated by mycovirus cross-protection, and induced systemic resistance.

The difference in the percentage of Fusarium wilt disease in the five shallot varieties is thought to be because genetically these five varieties have different resistance, in addition to their different adaptability to the environment. Sudantha [14] reported that the Bali Karet variety, Ampenan variety, Keta Monca variety, Super Philip and Super Cross on environmental adaptation tests at different altitude locations showed different resistance reactions to Fusarium wilt disease.

**Figure 1.** Healthy shallot with the treatment of Biofungicides *Trichoderma* spp. (A). Shallot infected with Fusarium wilt disease without biofungicides treatment (B).
Figure 1a shows that the plants treated with the biofungicide *Trichoderma* spp. grow healthly with a higher plant height and number of leaves. Figure 1b shows that the shallot plants are infected with the fungus *F. oxysporum* f. sp. *cepa* shows symptoms of poor growth, namely some of the leaves are curved and twisted, the color of the leaves is pale green to yellow and over time they become dry.

3.2. The Effect of *Trichoderma* spp. Biofungicide Formulation on the Yield

The results of the analysis of variance showed that the treatment of the biofungicide formulation *Trichoderma* spp. and shallot varieties and their interactions showed significant differences in the weight of harvested dry tubers. The results of further tests on the interaction of the biofungicide formulation *Trichoderma* spp. and shallot varieties using HSD 5% for the weight of harvested dry tubers are presented in Tables 2.

In Tables 2, it can be seen that the treatment of the biofungicide formulation *Trichoderma* spp. can increase the yield of shallots. All shallot varieties were treated with the biofungicide formulation *Trichoderma* spp. showed the weight of harvested dry tubers were significantly different from the control. Bali Karet shallot varieties show the best results after biofungicide treatment both liquid, tablet and powder.

According to Marin-Guirao et al. [23], the biostimulant effect of the fungus *Trichoderma* sp. as a biofungicide depending on the formulation, the sub-extract for the formulation which contains lots of organic material is very good for the development of the fungus *Trichoderma* sp. Ningsih et al. [24] said that *T. harzianum* fungus has the potential to be developed as a biopesticide and biological fertilizer for the management to increase crop yields.

| No. | *Trichoderma* spp. Biofungicide Formulation | Bali Karet | Ampenan | Keta Monca | Super Philip | Super Cross |
|-----|--------------------------------------------|------------|---------|------------|--------------|-------------|
| 1.  | Without biofungisida                       | 6.10 c<sup>1</sup> | 5.90 b  | 5.80 b     | 4.30 a       | 4.10 a      |
|     |                                            | A<sup>2</sup> | A       | A          | A            | A           |
| 2.  | Liquid                                     | 16.60 c    | 14.20 b | 14.30 b    | 10.10 a      | 10.10 a     |
|     |                                            | B          | B       | B          | B            | B           |
| 3.  | Tablet                                     | 16.70 c    | 14.00 b | 14.30 b    | 10.20 a      | 10.10 a     |
|     |                                            | B          | B       | B          | B            | B           |
| 4.  | Powder                                     | 16.60 c    | 14.10 b | 14.20 b    | 10.10 a      | 10.10 a     |
|     |                                            | B          | B       | B          | B            | B           |

Information:  
<sup>1</sup>) The numbers in each column followed by the same letter are not significantly different.  
<sup>2</sup>) The numbers on each line followed by the same letter are not significantly different.

4. Conclusion

The results showed that liquid, tablet and powder of biofungicide could suppress Fusarium wilt disease up to 0%, while in control the percentage of disease reached 60%. Bali Karet varieties are very adaptive in the highlands, so they are very resistant to Fusarium wilt, Ampenan and Keta Monca adaptive in the highlands, while Super Philip and Super Cross are less adaptive. Bali Karet varieties showed the highest growth and yield.

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