AGRICULTURAL SCIENCE
Journal Of Agricultural Science And Agriculture Engineering
ISSN : 2597-8713 (Online) - 2598-5167 (Print)
Available on :
http://agris cience.scientific -work.org/inde x.php/agris cience

Application of *Trichoderma Harzianum* as Soil Treatment and Additional Treatment for Control of Potato Diseases

Sutarman

Department of Agrotechnology - Faculty of Agriculture Universitas Muhammadiyah Sidoarjo

Jl. Raya Gelam No, 250 Candi – Sidoarjo 61217

Email: sutarman@umsida.ac.id

ABSTRACT

This study aims to determine the effect of biofungicide application with *Trichoderma harzianum* as its active ingredient in the form of soil treatment and additional treatment which includes seed treatment, canopy surface spraying, and combined seed treatment and canopy spraying with Trichoderma biofungicides and active chemical fungicides mancozeb and cymoxanil, against leaf blight disease index caused by *Phytopthora infestans*, stover dry weight, healthy potato tuber weight, rotten tuber weight, and relative quality index of potato plant bulbs. Experiments were carried out in the Split-plot Design using a completely randomized design (CRD). The main plot is soil tretament biofungicide *Trichoderma*, including without soil tretament and with soil treatment. As plot saplings is an additional treatement of *Trichoderma* biofungicide, consisting of: chemical fungicide, seed treatement, canopy spraying, and spraying of canopy and seed treament. Each treatment was repeated 4 times, to obtain 32 experimental units. The variables observed were leaf blight disease index at the end of the vegetative phase, stover dry weight, healthy tuber weight, rotten tuber weight, and relative index of tuber quality. The results showed that the interaction of soil treatment and additional treatment of *Trichoderma* biofungicide had a very significant effect on the disease index of potato leaf blight, rotten tuber weight per plant, and relative index of quality of potato tubers, but did not significantly affect the dry weight of stover and tubers of healthy potatoes per plant. The combination of soil treatment and additional treatment resulted in a decrease in the disease index of 45.37 to 53.96%, a decrease in rotten tubers from 42.39 to 91.50%, and an increase in the percentage of relative index of tuber quality from 7.8 to 65.5% compared to only using fungicides made from mancozeb and cymoxanil.

Keywords: soil treatment, seed treatment, spraying plant canopies, potato bulbs, biopesticide *Trichoderma harzianum*

1. INTRODUCTION

Potatoes are one of the strategic commodities in Indonesia. Various efforts have been made to increase production to suppress imports and even restore the role of exporters as in the 1990s. However, the threat of loss of results due to disruption of disturbing organisms is one challenge that is not easy to overcome.

The attack of potato canopy blight caused by *Phytopthora infestans* and tuber rot caused by *Fusarium oxysporum* and decaying bacteria (*Pseudomonas solanacearum*) often causes harvest failure. Fungicides have been the main method of controlling disease (Ma et. al., 2009; Guan, 2011). The active fungicide ingredient commonly used to control potato diseases, including a mixture of mancozeb and cymoxcanil (Evenhuis, et al., 1996) which is currently widely used in

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potato farming centers. Mixing these two active ingredients is effective in preventing the emergence of pathogenic resistance to cymoxanil (Paramita & Sumardiyono, 2014). However, this application of contact and systemic funicides is now often unable to save damage and losses in cultivating potatoes. On the other hand, various evidence shows the negative impact of harmful fungicidal active ingredients can disrupt the activity of soil organisms that are beneficial to plants.

The use of biological agents for controlling plant diseases has been carried out. Trichoderma is one type of fungus that is used for the application of biological control to soil borne pathogens. Various types of Trichoderma have a high ability to produce chitinase and β-1,3 glucanases enzymes having the ability of mycoparasites (Benítez et al., 2004; Chowdappa et al., 2013) so as to increase plant resistance to disease and protect plants from pathogenic attacks. Trichoderma is capable of synthesizing various compounds such as proteins and antibiotics which can increase its ability to control pathogenic fungi (Al-taweil et al., 2009).

The application of some Trichoderma isolates as well as biocontrol agents can also act as biofertilizer active ingredients. Trichoderma acts to degrade soil organic matter (Hu et al., 2015) given its ability to produce important enzymes such as ß-1,3-glucanase, and cellulase (Verma et al., 2007; Saravanakumar et al., 2016), so that and produce essential nutrients for plants (Buysens et al., 2016) that are beneficial for crop production (Ali et al., 2015; Hu et al., 2016).

Conidiospores of Trichoderma fungi can be formulated in at least two kinds to facilitate application in the field, namely the form of bulk solid formulas whose application is as soil tretament and liquid formula or suspension for the need for seed treatment and spraying plant canopies. As a soil treatment, the application of this biofungicide is carried out once before planting, whereas by spraying the canopy it can be carried out several times starting from planting to the end of the vegetative phase.

As a biofungicide, the effectiveness of Trichoderma as an active ingredient needs to be tested, so that it can be measured to what extent it can be an alternative chemical fungicide in the field of potato control measures in the field.

This study aims to determine the effect of Trichoderma biofungicide formulated in bulk solids as soil treatment combined with additional treatment in the form of seed treatment and canopy spraying with biofungicide on the index of potato leaf blight symptoms caused by P. infestans, dry weight of plant stover, rotten tuber weight, total healthy tuber weight, and relative quality index of potato tuber. The additional tretament applied consisted of: canopy spraying with biofungicide, seed treatment with biofungicide, seed treatment and spraying the canopy with biofungicide, and spraying the canopy with chemical fungicides made from mancozeb and cymoxanil.

2. METHODS

2.1. Research design

The research was carried out on land that was usually planted with potatoes, mustard greens, and carrots in Sumberbrantas Village, Bumiaji District, Batu City, East Java Province at an altitude of 1,600 m above sea level. in May-September 2018. Experiments were arranged using a factorial split-plot design using a completely randomized design. by using a completely randomized design (CRD). The main plot is a soil treatment Trichoderma biofungicide consisting of soil treatment and non soil tretament. As a sub-treatment, the additional treatment includes: fungicide application, seed treatment and fungicide application, spraying biofungicides on the canopy, and spraying biofungicides on the canopy and seed treament. In this experiment the seed treat using Trichoderma biofungicide. The treatment in this experiment was repeated 4 times, thus obtained 32 experimental units. Each experimental unit is a bed measuring 1.5 m x 3.0 m containing an average of 24 plants.

2.2. Implementation of research

The isolate of Trichoderma harzianm fungi (code isolate Tc-Jjr-02) as the active ingredient of biofungicide is a collection of the Laboratory of Agricultural Microbiology, Universitas Muhammadiyah Sidoarjo. The potato seeds used are from Granola varieties obtained from local seed growers.

Isolates of T. harzianum are grown on PDA-m media (Vargas Gil et al., 2009). After 10 days of incubation, the culture of the fungi isolates was smoothed and stirred evenly and dilution series up to 10^8. Using a sterile syrup was taken 1 ml of suspension containing conidiospores and sprayed onto the surface of the PDA-m media in a petri dish. The number of greenish spots on the surface of the media in a petri dish shows the number of colonies or population density per ml of suspension, then multiplied by the dilution factor. In this experiment obtained a suspension containing conidiospores as much as 5 x 10^8 cfu/ml which is ready to be used as a biofungicide.

For the purpose of soil treatement, biofungicides in bulk form are made with a carrier material that is sterilized chicken manure (at 121 ^oC 1 atm for 30 minutes). The conidiosporic suspension that has been prepared is mixed evenly with sterile chicken manure as a carrier into a bulk biofungicide with a density of conidiospores 10^8 cfu/gr. This bulk formula biofungicide is given to the soil during final tillage or together with making a planting hole with a dose of 200 grams per planting hole. For treatments that do not use soil treatment, sterile chicken manure is also given at a dose of 200 gr per planting hole but does not contain Trichoderma as an active biofungicide ingredient.

The conidiosporous suspension obtained from dilution with a density of 5 x 10^8 cfu/ml as much as 1,000 ml was poured into the tray and used as a liquid biofungicide for seed treatment. Potato seeds in the form of tubers that have germinated or are ready for planting are soaked in the liquid biofungicide for 1 minute, then drained until there is no suspension dripping from the potato tubers to be planted. For treatment with seed treatment, the seed that has been treated is placed in the planting hole that has been prepared.

Spraying tretament is used by conidiospora suspension with a density of 5 x 10^8 cfu/ml which is diluted by mixing 200 ml of suspension into the hand sprayer tank and mixed with water until the mixture volume becomes 10 liters. Spraying is done by soaking the entire surface of the canopy and stem of the potato until the mixture of the biofungicide is exposed evenly to the entire surface of the plant above the ground. Spraying starts at 3 weeks after the plant (WAP) and is repeated every week until the age of 10 WAP. During spraying, the surrounding beds (one experimental unit) which are treated are covered with a plastic screen to prevent the droplets from being deposited on the surface of the plant in another experimental unit. The same thing was done when spraying fungicides for additional treatment that did not apply biofungicide spraying and that applied biofungicide spraying but got the seed treament.

All plants were given basic fertilizers in the form of urea, ZA, SP 36, and KCl each with a dose of 12 gr, 8 gr, 15 gr, and 5 gr per planting hole before planting. Insecticides with active ingredients of chlorpyriphos with a concentration of 5 cc/ l are given by spraying the entire surface of the soil during planting in such a way that the soil of the planting media around the tubers of potato seeds contains residues of active ingredients; this was done to prevent the attack of the caterpillar (*Agrotis ipsilon*), and orong-orong (*Cryllotalpa* sp.). For applications that use chemical fungicides with active ingredients a mixture of mancozeb and cymoxanil with a concentration of 5 gr/l, carried out every week from the age of 3 to 10 WAP only in aditional treatment in the form of no biofungicide or not given to treatment using biofungicides for canopy spraying, seed treatment. and spraying the canopy and seed tretament.

2.3. Observation

Observations were made on: (i) disease index carried out at the age of 11 WAP which at that time vegetative growth in the form of canopy had begun to stop using formula (1) obtained from the calculation of symptom scores based on symptom criteria (Table 1), (ii) weights stover dry weight (gr), (iii) total weight of tubers healthy or not rot and/or not defective attacked by pathogens (gr), (iv) total rotten tubers (gr), and (v) relative index of quality of tubers performed at the time harvest or at 17 MST.

Formula for determining disease index:

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$$I_i = \sum_{i=1}^{k=4} (i\,ni)/Nk \quad ....(1)$$

with the provisions: Ip is a disease index that shows the intensity of the attack symptoms, i is the lowest score, k is the highest score, this is the number of individual plants with symptom criteria of -*i* score, N is the number of plants observed.

Table 1. Criteria for symptoms of blight and wilting canopy of potato plants

Value (score)	Criteria for symptoms
0	There are no symptoms
1	> 0-10% canopy with blight symptoms
2	> 10-20% canopy with blight symptoms or the canopy looks rather wilted
3	> 20-40% canopy with blight symptoms or the canopy looks quite wilted
4	> 40-70% canopy with blight symptoms or 20-40% symptomatic blight and wilt or the plant experiences wilting quite heavily
5	> 70% of crowns are blighted until the plant dies or heavy wilted plants until total wilting to death

The relative index of tuber quality was calculated using formula (2) obtained from determining the calculation of the quality score based on the potato tuber quality criteria as shown in Table 2.
The formula for determining the relative index of tuber quality:

$$I_i = \sum_{i=1}^{k=4} (i\,ni)/Nk\left(\frac{x_t}{x}\right) \quad ....(2)$$

with the provisions: *Ir* is the relative index of the quality of normal tubers, healthy without foul defects, *i* is the lowest score, *k* is the highest score of tuber quality, ni is the average tuber weight per plant with the-*i* tuber quality criteria, N is the number of plants observed, xt = the weight of normal tubers per plant (healthy not decayed or symptomatic by pathogens) for each experimental unit, x = average tuber weight per plant of the entire population in the experiment.

Table 2. Criteria for quality of potato tubersvalue

Value (score)	Quality	Criteria for potato tubers
1	Grade D	Weight per one tuber <50 gr
2	Grade C	Weight per one tuber 50-99 gr
3	Grade B	Weight per one tuber 100-199 gr
4	Grade A	Weight per tuber ≥ 200 gr

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2.4. Data analysis

Data from the observations were analyzed using variance at the level of 5% to determine the effect of treatment on disease index and production of potato plants. The test was continued using the Honestly Significant Difference (HSD) test at the level of 5% to know the difference between treatments.

3. RESULTS

3.1. Disease Index

The results of the variance analysis showed that soil treatment and additional treatments and their interactions had a very significant effect ($p < 0.01$) on the disease index of potato leaf blight (11 WAP). The mean effect of soil treatment and additional treatment interactions on the potato leaf blight disease index and the percentage difference in weight of healthy tubers to the control (%) is shown in Table 3.

Table 3. Average disease rate for potato leaf blight at the end of the vegetative period of potatoes

Perlakuan fungisida hayati	Disease index		Percentage of disease index difference to controls (%)
Soil Treatement = Seed Treatement- Canopy spraying	22.88	a	53.96 (-)
Soil Treatement =Seed Treatement	23.12	a	53.48 (-)
Soil Treatement =Canopy spraying	25.03	a	49.65 (-)
NonSoil Treatement =Seed Treatement- Canopy spraying	25.82	a	48.05 (-)
NonSoil Treatement =Canopy spraying	26.02	a	47.65 (-)
NonSoli Treatement =Seed Treatement	26.22	a	47.24 (-)
Soil Treatement =chemical fungicide	27.15	a	45.37 (-)
Non Soil Treatement = chemical fungicide (Control)	49.70	b	-
HSD 5%	4.73		

Remarks: Numbers followed by the same letters in the same column show the same effect on the HSD 5% test.

3.2. Dry weight of stover

Based on the results of the variance analysis, it was found that the application of soil treatment had no significant effect on the dry weight of stover ($p > 0.5$), as well as the interaction with additional treatment had no significant effect ($p > 0.05$) based on the results of variance analysis. Additional treatment had a very significant effect ($p < 0.01$) on the dry weight of potato plant stover. Table 4 shows the mean effect of additional treatment on dry weight of potato stover and percentage of weight difference in healthy tubers to controls (%).

Table 4. Average dry weight of potato plant stover

Additional treatment	Dry weight stoverper plant (gr)		Percentage of stover dry weight difference to control (%)
Seed Treatement - Canopy spraying	60.59	a	53.02
Seed treatement	58.46	ab	47.65
Canopy spraying	58.42	ab	47.53
Chemical fungicide (control)	39.60	b	-
HSD 5%	19.86		

Remarks: Numbers followed by the same letters in the same column show the same effect on the HSD 5% test.

3.3 Healthy tuber weight

Additional treatment had a very significant effect (p <0.01) on total tuber weight based on the results of variance analysis; while soil treament and its interaction with additional treatment did not significantly affect the total weight of healthy tuber plants (p>0.05). The mean total tuber weight per plant and the percentage difference in weight of healthy tubers to the control (%) can be seen in Table 5.

Table 5. Average weight of healthy potato tubers

Additional treatment	Healthy tuber weight per plant (gr)		Percentage of weight difference between healthy tubers and controls (%)
Seed Treatement - Canopy spraying	1,523.63	a	38.81
Seed treatement	1,486.88	ab	35.46
Canopy spraying	1,456.69	ab	32.71
Chemical fungicide (control)	1,097.67	b	-
HSD 5%	438.62		

Remarks: Numbers followed by the same letters in the same column show the same effect on the HSD 5% test.

3.4. Weight of Rotten Tubers

Additional treatment and its interaction with soil treatment had a very significant effect (p<0.01) on the total weight of rotten tubers per potato plant. whereas soil treatment has no significant effect (p> 0.05). The mean effect of interaction between soil treatment and additional treatment on the total weight of rotten tubers and the percentage difference in weight of damaged tubers to controls (%) is shown in Table 6.

Table 6. Average weight of rotten tubers of potato plants

Tretament	Weight rotten tubers per plant (gr)		Percentage of rotten tuber weight difference to control (%)
Soil Treatement = Seed Treatement - Canopy spraying	72.88	bc	42.39 (-)
Soil Treatement =Seed Treatement	72.88	bc	42.39 (-)
Soil Treatement = Canopy spraying	80.50	bc	36.36 (-)
Non Soil Treatement = Seed Treatement - Canopy spraying	9.50	a	92.49 (-)
Non Soil Treatement = Canopy spraying	27.50	ab	78.26 (-)
Non Soli Treatement =Seed Treatement	10.75	a	91.50 (-)
Soil Treatement = chemical fungicide	58.38	ab	53.85 (-)
Non Soil Treatement = chemical fungicide (Control)	126.50	c	-
HSD 5%	61.52		

Remarks: Numbers followed by the same letters in the same column show the same effect on the HSD 5% test.

3.5. Relative Quality of tuber index

The interaction between soil treatement and additional treatments based on the results of the variance analysis showed a significant effect ($p < 0.05$) on the relative index of the quality of potato tubers. as well as additional treatment. Conversely, soil treatment did not have a significant effect ($p > 0.05$) on the relative index of potato plant tuber quality. The average relative index of potato tuber quality and percentage difference in relative index of tuber quality to controls (%) is presented in Table 7.

Table 7. Relative index of quality of potato tubers

Treatment	Relative quality of tuber index		Percentage of difference in relative index of tuber quality to control (%)
Soil Treatement = Seed Treatement- Canopy spraying	0.34	b	17.0
Soil Treatement =Seed Treatement	0.33	b	14.2
Soil Treatement =Canopy spraying	0.33	b	11.4
Non Soil Treatement =Seed Treatement- Canopy spraying	0.48	a	65.5
Non Soil Treatement =Canopy spraying	0.35	b	20.5
Non Soli Treatement =Seed Treatement	0.34	b	17.1
Soil Treatement = chemical fungicide	0.32	b	7.8
Non Soil Treatement = chemical fungicide (Control)	0.29	b	-
HSD 5%	0.10		

Remarks: Numbers followed by the same letters in the same column show the same effect on the HSD 5% test.

4. DISCUSSION

The disease index in the treatment application of mancozeb and cymoxanil fungicides showed the highest value of 49.70. while in other treatments the disease index value was lower (Table 3). The lowest value reached 22.88 in the soil treatment treatment combined with seed treatment and spraying the canopy using Trichoderma biofungicide. The highest disease index value is 49.70 in the treatment of a combination of non-soil treatment and spraying of chemical fungicides. This shows that Trichoderma has inhibited P. infestans activity from the disposition phase. infection. and penetration. given its ability to produce toxins such as diterpenoid harziane and to harmonize pathogens (Harman, 2006; Zhang et al., 2016). produce chitinase and glucanase (Vinale et al., 2008) which can damage the walls of pathogenic fungi. and produce antibiotics (Al-taweil et al., 2009) which can inhibit the growth of pathogenic fungi. In line with that. the application of Trichoderma in the canopy of cacao seedling plants has shown its ability to suppress wound indices between 76.3-89.5% caused by P. palmivora (Sutarman, 2017).

The weight of rotten tubers in the treatment of chemical fungicides (control) (Table 6) reaches 126.50 gr per plant. while the seed treatment and spraying of biofungsida without soil treament reached 9.5 gr of rotten tubers or had a difference of 92.49% lowering rotten tubers compared to controls. Overall the treatment using Trichoderma biofungicide reduced the total weight of rotten tubers between 42.39% to 92.49%. The relative index of tuber quality (Table 7) in the treatment using only chemical fungicides (control) reached the lowest value of 0.29. while all other treatments reached 0.32 to 0.48 or increased the relative index of tuber quality 7.8% to 65% against controls. The treatment of soil treatment and seed treatment of biofungicides creates protection for plants in the rhizosphere against pathogens and fungi and bacteria. This fungi also produces various enzymes that can degrade the propagules of pathogenic fungi (Hu et al., 2015) and are myoparasitic (Harman et al., 2004; Verma et al., 2007). also produces aminolipopeptide compounds. which can act as anti-dormant against bacteria (Pruksakorn et al., 2010).

The average dry stover weight with an additional treatment using Trichoderma biofungicide (Table 4) reached 58.42 gr to 60.59 gr. When compared with the dry weight of potato stover in the treatment of mancozeb and cymoxanil fungicides of 39.60, it was shown that in the treatment using biofungicide the percentage of dry weight increase was between 47.53-53.02% for the treatment of chemical fungicides. The same pattern is shown by the weight of healthy potato tubers (Table 5). The percentage difference in potato tuber weight ranged from 32,715 to 38.81% in biofungicide treatment compared to chemical fungicide treatment. Growth stimulant compounds produced and contained in solutions sprayed onto the canopy surface or through roots in the treatment of Trichoderma biofungicides will be used by plants for their growth. Thus the role of

Trichoderma in this case. including stimulating indole acetic acid activity for plants (Gravel et al., 2007) in addition to the contribution of nutrients from degradation of organic matter from organic fertilizers and soil organic matter (Howell, 2003). thus increasing vegetative growth and production of plant biomass (Buysens et al., 2016; Youssef et al., 2016).

In the application treatment, mancozeb and cymoxanil fungicides gave the lowest plant response in terms of stover dry weight (Table 4) and healthy tuber weight (Table 5) and the relative index of tuber quality (Table 7). but highest in terms of disease index (Table 3) and rotten tuber weight (Table 6). This shows that the two active ingredients are relatively unable to inhibit the pathogen activity of P. infestans in the canopy and F. oxysporum in the tuber. The second mixing of the active ingredients initially aims to prevent the resistance of pathogenic fungi to cymoxanil (Evenhuis et al., 1996). but now these two mixtures of active ingredients are not effective in controlling the pathogens of hahwar leaves of canopies and tuber rot. In this case it is likely that potentially large Trichoderma is used to control dangerous diseases in this potato plant. Trichoderma slows down the development of pathogenic resistance to fungicides (Glare et al., 2012) and is an important consideration in the strategy for controlling disease in a sustainable manner based on natural resources (Hu et al., 2016).

5. CONCLUSION

Soil treatment has a very significant effect on the potato leaf blight disease index, while the additional treatment has a very significant effect on the potato leaf blight disease index. stover dry weight. healthy tuber weight. rotten tuber weight. and the relative index of the quality of potato tubers. Additional treatment using Trichoderma biofungicide increased stover dry weight 47.53-53.02% and increased healthy tuber weight 32.71-38.81% compared to those using mancozeb and cymoxanil fungicides.

The interaction of soil treatment and additional treatment of Trichoderma biofungicide had a very significant effect on the potato leaf blight disease index. total rotten tuber weight per potato plant. and the relative index of the quality of potato tubers. The combination of treatments can reduce the disease index 45.37-53.96%. reduce rotten tubers 42.39-91.50%. increasing the percentage of the relative index of tuber quality from 7.8 to 65,5% compared to using only mancozeb and cymoxanil fungicides.

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ISSN : 2597-8713 (Online) - 2598-5167 (Print)
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