The Resistance of Potatoes by Application of *Trichoderma viride* Antagonists Fungus

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Abstract. Leaf blight disease caused by pathogenic fungus *Phytophthora infestans* is the major disease in potato that can reduce its production up to 100%. The use of biological agent *Trichoderma viride* as an inducing potato resistance against leaf blight disease has been considered potential method. The purpose of this study was to evaluate the use of biological agent *Trichoderma viride* in inducing potato plant resistance. The parameters observed were the growth of the potato plant and leaf blight intensity. Experimental research with complete randomized design with 6 treatments was applied. The results showed that the application of *Trichoderma viride* could reduce the intensity of leaf blight disease and increase the growth of the potato plant. *Trichoderma viride* application could improve the systemic resistance of potato plants.

Keywords: **Trichoderma viride, leaf blight intensity, Phytophthora infestans.**

1. INTRODUCTION

Potato is an important horticulture commodity that plays an important role in Indonesian economy. In addition to supporting food diversification program, potato is an alternative food substitute for rice because it has high carbohydrate, protein, fat, and vitamin C [1]. Until 2012, Central Java Province of Indonesia is one of the potato production centers with the widest planting area, 16.102 ha, that produce 252,608 tons [2]. One major obstacle in potato cultivation is leaf blight disease/late blight by *Phytophthora infestans* pathogens that can cause loss to 100% [3,4] Leaf blight disease is very destructive and difficult to control, because *P. infestans* is a pathogenic fungus with various degree of pathogenicity, as this fungus is heterothallic [5]. To control this pathogenic fungus, biological control needs to be applied.

Among the biological control, as a potential alternative method, is antagonistic fungus to the pathogen of *P. infestant* called antagonistic fungus of *Trichoderma spp*. [6,7]. *Trichoderma spp*. is not only capable of inducing host resilience against potential pathogens but also has a characteristic of hyperparasitic and micoparasitic. [8-11]. Controlling the leaf blight disease on potato using induced resistance with antagonistic fungus *Trichoderma spp*. is part of biological control because it utilizes non-pathogenic microorganisms to induce plant resistance.

As a result of the resilience induced by inoculation of biological agents, reduction in the symptoms of disease and changes in biochemical factors in host plants take place causing plants to resist disease-causing pathogens. The induced resilience of cultivation by biological agent is caused by the activation of the potential genetic endurance of the host plant.

Given this situation, the aim of this research was to identify the resistance of potato plant to leaf blight disease that had been induced by *Trichoderma viride* antagonist fungus. Among indicators of potato plant resistance against leaf blight disease was the decreased intensity of leaf blight disease during the growth of the plant, the measurement of plant growth hormone levels such as gibberellin hormone, and the increased growth and yield of potato crops.

Trichoderma viride application with seed treatment before planting conducted in greenhouse showed a decrease in germination of *Rhyzoctoni solani sclerotia* by 88%. The ß- (1.3) *glucanase* enzyme produced by the *T. viride* fungus is capable of destroying the moldy fungi *Sclerotinia sclerotiorum*. The *T. viride* produced antibiotics and various enzymes such as *exogluconase, endoglucanase, selobiase* and *chitinase* that is able to destroy cell walls of plant pathogenic fungi (Papavizas, 1985). The plant protection mechanisms by *T. viride* do not only involve in attacking on pathogens, but also involving in the production of some secondary metabolites serving to increase the growth of plants and roots of host plants [12].

2. RESEARCH METHODS

This study was conducted at the potato home garden of the Vegetable Research Institute (BALITSA), located in Cikole Village, Lembang District, Bandung City, West Java Province; altitude ± 1200 meters above sea level, from March to July 2013. The potato seedlings of Granola

(G2), a variety susceptible to leaf blight disease, were obtained from BALITSA. The planting medium of the potato seed was a mixture of soil and compost/manure with a ratio of 3:1 placed in a 50 cm diameter of polybag. The soil mixture was first physically sterilized with soil sterilizer (steamer) before the seed was planted in the polybag medium; one seed per polybag. The potato seeds in the polybag then were maintained for approximately 4 months.

This experiment was conducted using Completely Randomized Design (CRD) consisting of 6 treatments each of which was repeated 8 times and 6 replications. The six treatments were P1: Application of antagonistic fungus *T.viride* and pathogenic fungal infections *P. infestans* 2 weeks before planting (wbp) the potato seed; (P2): Application of antagonistic fungus *T.viride* and pathogenic fungal infections *P. infestans* 1 week after planting (wap) the potato seed; (P3): Application of antagonistic fungus *T.viride* 2 wbp and 1 wbp as well as infection of *P. infestans* pathogen; (P4): Application of synthetic fungicide with active ingredient of mankozeb (Dithane M-45) and pathogenic infection of *P. infestans*; (P5): Application without *T.viride* inoculation of potato plant but infected with *P. infestans* (positive control); (P6): Potato plants that were not applied *T.viride* and not infected by pathogens *P. infestans* (negative control).

Isolate fungus antagonist *T. viride* was rejuvenated on PDA medium for 8 days in room temperature (25ºC), then the density of its conidia was calculated up to 10^8 conidia/mL. *P. infestans* pathogen isolates were also grown on V8 gelatin juice medium for about 9 days in the incubator at 18° C. Furthermore, the grown sporangia was harvested by scraping the surface of the medium V8 gelatin juice using glass rod. The sporangia is then calculated in density up to 10^3 sporangia/mL. The calculation of conidia and sporangia density was done using haemocytometer. A 250 mL of a conidia *T. viride* suspension solution with that density was poured into the soil in which the seeds of potato seedlings were grown. Furthermore, at 30 days of potato plant life, 300 mL of *P. infestans* zoospore suspension with a zoospore density of 10^6 were also inoculated on 30-day potato leaf except for positive control treatment. The relative humidity of the air was kept at a minimum of 90% and the air temperature was relatively set at 20° C.

3. RESULTS AND DISCUSSION

3.1. Intensity of leaf blight disease

Symptoms of potato leaf blight disease began to appear when the plants were older than 5 weeks after planting (wap), and the older the age of the plant was, the higher the disease intensity in all treatments would be (P1, P2, P3, P4, and P5) except in the control treatment negative (P6) as shown in Table 5. The highest intensity of leaf blight disease was found in positive control treatment (P5), which was 90% at 10 wap and the lowest intensity was identified in the treatment of *T. viride* 2 wap (P1), which was 59% at 10 wap. The symptoms of the leaf blight disease were not appeared in the negative control treatment (P6) because the plant was not infected by *P. infestans* pathogens.

T. viride application with various time variation of application significantly affected the intensity of leaf blight disease (Figure 1). The treatment without application of *T. viride* (P5) showed higher intensity of leaf blight disease compared to the treatment with application of *T. viride* (P1, P2 and P3). The intensity of leaf blight disease in P5 continued to increase along with the increase of potato plant life up to 90% at 10 wap. The increasing intensity of leaf blight disease in P5 and P1, P2 and P3 was probably caused by the absence of plant resistance induced against the *P. infestans* pathogen attack. Therefore, *P. infestans* pathogen was able to freely attack without any defense from the potato plants that eventually triggered the incidence of leaf blight disease (Ortiz et all. 1999 *in* Baihaqi et all. 2013) [13].

In addition, environmental conditions supporting the development of the leaf blight disease such as low temperature (20ºC), high humidity (> 90%), and high rainfall at the time of this study doubled the number of pathogen inoculum in the field. The pathogens spores were easily disseminated from one plants to others to infect the plants. This was consistent with the finding of Goth (1981) that high rainfall (2000 mm/year) and high humidity (90%) are positively correlated with the severity of potato leaf blight disease. The persistence of the pathogenic *P. infestans* in potato leaf tissue was also the cause of leaf blight intensity kept increasing and tended to remain high (Ortiz et al. 1999 *in* Baihaqi et al. 2013) [13].

The intensity of leaf blight disease that tended to be low in the treatment of P1, P2, and P3 was probably caused by the existence of the plant resistance mechanism resulted from the induction of antagonistic fungus *T. virides* before the occurrence of the infection of pathogen *P. infestans* (Driesche and Bellows, 1996). This proved that the application of antagonistic fungus *T.viride* prior to the infection of pathogen *P. infestans* were very effective in suppressing the progress of the leaf blight disease. This state might happen as early antagonistic fungal inoculum administration allows early antagonists to multiply and adapt to the plant environment (Rachmawaty et al., 1995). The longer the distance between *Trichoderma* infestation and the inoculation of pathogenic fungi is, the lower the intensity and the percentage of seeds attacked by the pathogenic fungus *Pythium spp* will be (Sulistiyowati, et al., 1997).

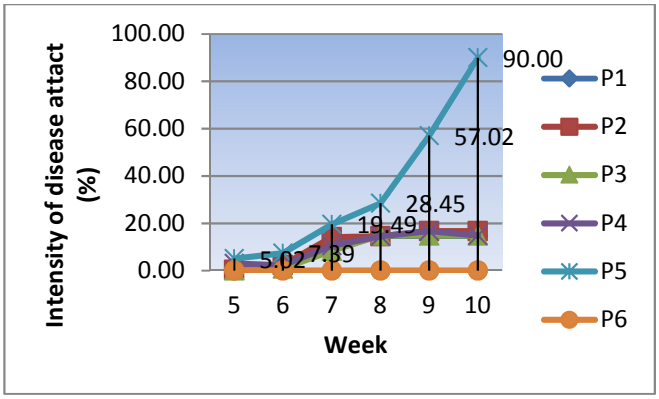

Figure1. The average percentage of leaf blight intensity at 5 to 10 wap

The application of *T. harzianum* in the screen house is able to suppress the development of *Fusarium oxysporum* f.sp. *lycopersicis* up to 92% (Ramezani, 2010). Meanwhile, some isolates of *T. harzianum* are also able to suppress bacterial wilt disease in potato plants caused by *Ralstonia solanacearum* with inhibition percentage up to 63.57% (Hersanti, et al., 2009).

T. viride is a soil fungus that is naturally antagonistic to other fungi; while, the antagonistic fungus *Trichoderma* spp. is also able to induce plant defense against pathogenic fungal attack by the mechanism of plant resistance induction (Driesche and Bellows, 1996). Furthermore, the inoculation of *T. lignorum* on soil media for potato can suppress *P. infestans* attack up to 70% (Purwantisari et al., 2004). The ability of other types of *Trichoderma* is *T. asperellum*, which suppresses leaf blight disease by P. *palmivora* in cocoa seedlings by application of soaking immersion with conidia *Trichoderma* (Azis et al., 2013).

Watering the *T. viristic* suspension before planting the potato seeds would induced resistance the plants to be more effective. This finding was in line with that of [14] that *T. virens* suppression against pathogens can be caused by the ability of these antagonists to spur the formation of compounds in anti-pathogenic plants such as *pathogenesis related* (PR) proteins. PR-proteins will be formed in the intercellular space after penetration of the inducing agent until the plant will synthesize the PR-proteins such as chitinases and β-1,3-glucanase when the plant are infected by pathogens. These two enzyme are able to catalyze the hydrolysis of the polysaccharides, which are the main components of the *P. infestans* pathogen of cell wall causing the plants to resist the pathogen attack.

Harman et al. (2004) [12] argued that *Trichoderma spp*. is a fungus that produces a wide variety of compounds capable of inducing plant resistance locally and systemically against crop disease as well as crop resistance to unfavorable environmental conditions. Further, Djonovic (2005) reported that grain, cotton, and maize treated with *Trichoderma virens* in hydroponic system can increase the resistance of both local and systemic resistance crops to diseases caused by *Pythium ultimum* and *R. solani* through small protein of SM 1 (small protein 1) produced by *Trichoderma virens*.

Singh et al. (2011) also reported that sunflower treated with *T. harzianum* could increase plant resistance to *R. solani* with a mechanism associated with accumulation of ROS gene network: the catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), *ascorbate peroxidase* (APx), and maximum activity of CAT. Salas-Marina et al. (2011) added that inoculation of *T. atroviride* at the root of *Arabidopsis thaliana* is able to induce plant resistance against the attack of *Botrytis cinerea* and *Pseudomonas syringae pv*. tomato on leaves marked by gene expression for the synthesis of *salicylic acid*, *jasmonic acid/ethylene, phytoalexin*, and *camalexin*.

3.2. Yield of Potato Crops (*Solanum tuberosum L.*)

T.viride application on all treatments (P1, P2, and P3) significantly affected potato crop yield. The average number of tubers in treatment P1, P2 and P3 was higher than that of in plant without *T.viride* treatment (P4 and P5) with an increase range of 4 tubers/plant. The highest average number of potato tuber was in P3, which was 16.25 tubers, while the lowest average number of tubers was in P5 that was 2.5 tubers (Figure 3). This suggested that the *T.viride* application was able to increase the number of potato tubers significantly.

The immense intensity of leaf blight disease caused by *P. infestans* (Figure 1) accumulatively decreased leaf performance as a photosynthesis-producing organ and potato growth process. The intensity of leaf blight disease on potato directly affected the yield of the crop. The higher the intensity of the leaf blight disease of potato plants is, the lower the average yield of the crops will be; in this case the number of the tubers. According to Suhardi (1982), the attack of *P. infestans* on Granola variety appears on the plant at the age of 35-45 days. At that age, susceptibility of plants occurs as the tubers begin to form and the metabolism of the carbohydrate in the leaves changes from unusable state into the form used. Furthermore, the number of leaves increases so the possibility of spores falling to the leaves also increases. The greater number of leaves result in the moisture surrounding the plant is high, due to less evaporation as the intensity of the light is less; therefore, P. *infestans* pathogens can thrive well.

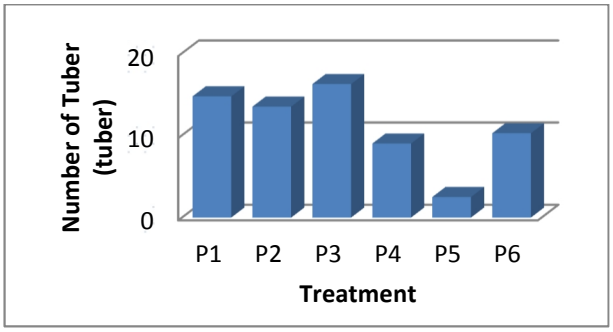

Figure 2: Mean of the number of Tubers at 6 treatments (P1, P2, P3, P4, P5, and P6)

The application of *T.viride* 2 weeks before and 1 week after planting increased the yield of the number of potato tuber. This was probably caused by the time of inoculum *T. virides* given before and after planting provided a great opportunity for antagonistic fungus to secrete secondary toxic metabolite compounds and hydrolytic enzymes to inhibit *P. infestans* pathogens; as a result, small infections took place and eventually tuber production generated maximum.

Unlike P5, treated without *T.viride* application, *P. infestans* pathogenic fungi easily infected the plants without having antagonist fungus application; therefore, plants could not perform their physiological activities smoothly as the photosynthesis activities were disturbed. The interruption of the photosynthesis activities in potato plants resulted in the decrease in the results of photosynthetic that should be stored in potato tuber, which in turn lad to the decrease in potato yields [15]. The finding of this research was in line with the one of Baihaqi et al. (2013) that the high intensity of *P. infestans* attack has a great effect on the decrease of potato yield; the

higher the intensity of the disease is, the lower the yield will be.

Furthermore, Abadi (2003) stated that intensity level of *P. infestans* attack on potato plants correlates with the number of and the weights of potato tubers, due to the intensity of *P attacks*, as *P. infestans* significantly affected the observation variables of the average number of tubers per plant. In addition to number of tubers per plant, the yield is also influenced the intensity of the intensity of the leaf blight disease; the higher the attack is, the lower the harvest will be. Thus, there was a positive correlation between photosynthesis activities and potato crop yields. The decrease of the yield was caused by leaf production, as photosynthetic organ, did not normally active during the attack of the leaf blight disease [15]. The photosynthesis rate (Photosynth accumulated in leaves) indicated as the ability of potato plants to translocate the photosynthesis results from sources into sink (Salisbury and Ross. 1995). The number of Photosynth partition into the sink determined the translocation, while the Photosynth partition was influenced by environmental factors, nutrient status, and age of the plant [15].

The lowest yield of the crop was in P5 treatment (positive control) and the highest one was in the treatment with *Trichoderma viride* applied 2 wap and 1 wbp. The P5 control, treated without protection from antagonistic fungus of *Trichoderma viride*, was more susceptible to *P. infestans attack.* As a result, the plant was difficult to produce because the leaf blight disease by *P. infestans* disturbed of the process of photosynthesis in plants, the infected leaves lose green leaf substances and could not capture sunlight optimally causing the production of the plants decreased (Salisbury and Ross. 1995). Plants attacked by *P. infestants* pathogens causing severe infections will result in all infected leaves becoming rotten, and then eventually die [5].

4. CONCLUSION

Application of antagonistic fungus of *Trichoderma viride* potato plant is able to reduce the intensity of leaf blight disease of potato plants and increase yield of potato crop. By being able to increase the productivity, it is expected that the increased demand for potato could fulfilled as the source of carbohydrate substituting rice.

5. ACKNOWLEDGMENT

We would like to extend our appreciation and gratitude to all staff of Bacteriology and Fungi Laboratory of Vegetable Research Institute (BALITSA) in Cikole Village, Lembang Subdistrict, Bandung, of West Java Province. Without your help, this research might not be accomplished.

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