Systemic inducing resistance against late blight by applying antagonist Trichoderma Viride

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Abstract. Biological control by Phytophthora infestans (Mont.) de Bary, a pathogen causing late blight disease on potatoes, has attracted scholars as an environmentally alternative preference to chemical pesticide. Some species of Trichoderma spp. has proven to be capable of triggering resistance mechanism of plant against fungal pathogens. The aim of this research was to analyze the effect of Trichoderma viride SP1 suspension to induced systemic resistance on potato plant as showing glucanase activity and total phenol content. The method used was Completely Randomized Design with six treatments and seven times repeated. Variables observed were total phenol content and total crop. The result showed that glucanase activity and total phenol content in plants treated with Trichoderma viride and fungicide (1587 µg/g and 1934 µg/g) significantly increased. In addition, T. viride enhanced induced systemic resistance on potato plant and increased potato yield.

Keywords: Induced systemic resistance, late blight, Trichoderma viride, Phytophthora infestans, glucanase activity, total phenol content.

1. Introduction

As a staple food substituting to rice and maize, potato (Solanum tuberosum L.) is in danger in term of its productivity and production [1]. The pathogen Phytophthora infestans causing late blight disease has become the single most important threat to potatoes worldwide. Since then more than $5 billion annually lost worldwide, therefore this disease has been considered a serious threat to global food security [11].

Attempt to control this disease using chemical substances has been conducted but they are not only costly but also harmful to the environment [19]. Meanwhile, non-chemical methods, or natural based practices has proven not to be conclusive yet, nor are there any reliable fungicides available to reduce the infestation [8]. However, lately, scientists have started to investigate Trichoderma spp., a well-known antagonistic fungus against various plant pathogens [9].

Observations upon the function of Trichoderma spp. in enhancing the growth of plants have been conducted for years [8]. Trichoderma spp. employs indirect mechanism in promoting plant growth and
health by doing biocontrol of various plant pathogens [10]. As plant is treated with *Trichoderma*, the deleterious microorganism activity in the rhizosphere of plants reduces and the nutrient status improves. Moreover, *Trichoderma viride* grows faster than its pathogen, as this species is very competitive in using the space and nutrition [9].

*Trichoderma* is also capable of constraining the incubation period intensity of the disease by colonizing plant roots leading to induce growth, stimulate nutritional adsorption, and control plant disease [6]. Therefore, it has been proposed that the mechanism of plant growth promotion takes place as hormone-like secretion metabolites and release nutrients from soil or organic matter [23]. Furthermore, *Trichoderma* spp. as active mycoparasites could control plant pathogens either aerial or soil-borne since they were proven to be successful as a biocide in greenhouse and field applications [5].

Hence, Trichoderma antagonistic mechanism as a complex process correlates with secretion of extracellular enzymes such as chitinases, β-glucanases and proteinases [12]. These compounds affect phytopathogenic fungi by degrading the cell walls to form holes. The systematic activation of plant resistance mechanism against fungal pathogens triggered by *Trichoderma* spp. has been demonstrated in at least ten dicots and monocots including Graminaceae, Solanaceae and Cucubitaceae against major plant pathogens like *Rhizoctonia solani*, *Botrytis cinerea*, *Alternaria* spp., *Colletotrichum* spp., *Magnaporthe grisea* and *Phytophthora* spp [22].

However, as research findings on biological control of late blight of potato are inconclusive, evaluating the efficacy of local isolates *Trichoderma viride* to induction potato plant resistance and potato yield crop needs to be furthered [16]. Therefore, the objective of this study was to investigate the total content of phenol and glucanase activity in potato plant after applying *Trichoderma viride* suspension.

### 2. Materials and Methods

The study, conducted from June to November 2013, took place in the potato field at Cikole Village, Lembang Sub district, Bandung Regency and at the Laboratory of Biochemistry of Faculty of Science and Mathematics Diponegoro University. The site of the research has an altitude of 1500 m above sea level. The media for bioassay of the strains and culture cultivation was Potato Dextrose Agar (PDA, Oxoid) for *Trichoderma viride*. Vegetable juice (V8) agar (100 mL V8 juice, 1.5 g L\(^{-1}\) CaCO\(_3\), 0.05 g L\(^{-1}\) β-sitosterol and 15 g L\(^{-1}\) agar) was used to isolate *P. infestans*.

Antagonist method application was conducted to evaluate the efficiency in controlling the pathogen. Completely Randomized Design using six treatments and five replicates was applied. The treatments were (T-a) antagonist fungal application (2 weeks before planting), (T-b) antagonist fungal application (1 week after planting), (T-c) antagonist fungal application (2 weeks before planting and 1 week before planting), (T-d) Mancozeb (a chemical fungicide included as a standard check). Negative and positive controls were included, (T-e) negative control (with pathogen fungal application and without antagonist fungal application), and (T-f) positive control (without pathogen fungal and antagonists fungus application).

Six replicates were used for each treatment and the pots were arranged in a completely randomized design method. Three kilograms of clay (pH=5.7), after being autoclaved at 121°C for 30 min, was added to the surface of every sterilized plastic pots of 5 L volume (diameter and depth of 20 cm each). Then, every pot was given appropriate dosage of fertilizers (i.e., 0.6 g di-ammonium phosphate and 0.5 g urea).

Potato Granola cultivar was used in the experiment. A 100 mL *T. viride* spores suspension of 9 days old culture plates grown on PDA at 25°C was prepared. Potato tubers were planted in pots containing sterilized soil, and then antagonist agents were applied to the sterilized soil before or after planting (depending on the kinds of treatments). Meanwhile, for the negative controls, the antagonist agent was not applied.

The other treatment was that after potato tubers were planted in pots containing sterilized soil, *Trichoderma viride* -SP1 suspension was applied via drip and dip adding to the soil at 14 days (two
weeks) before planting and after planting. For the negative and the positive controls, the plants were sprayed with sterile distilled water.

Observation was conducted by visually rating individual plants at weekly interval basis to identify leaf disease with late blight symptom calculated in percentage. Data gathered were statistically analyzed using general linear model procedures with SPSS (version 12). The treatments were analyzed using analysis of variance and least significant test at P<0.05. The glucanase activity in the plants treated with preparation was assayed colorimetrically as described by [13].

In the first experiment, the effect of *T. viride* application on inducing systemic resistance, leaves taken from 30-day-old seedlings were sampled. To assay total phenol content, leaf samples were obtained using sodium acetate (pH 5.0) buffer. The glucose equivalent was calculated using glucose standard graph obtained with pure glucose solution at incremental concentrations. Next, the phenol content extracted using 80% ethanol was estimated using Folin-Ciocalteau reagent. Using gallic acid as standard, the phenol content in the extracted leaves was calculated [14,7].

3. Result and Discussion

3.1. Induction of glucanase activity in potato plants treated with *T. viride* – SP1 suspension

The glucanase activity in untreated potato plants was 92 μg glucose equivalents per min per g compared to treat ones with *T. viride* – SP1, which was 133, 115, and 110 μg glucose equivalents per min per g. The maximum induction of glucanase activity was found in plants treated with application of *T. viride* after planting (T-a) followed by before and after planting (T-c) and before planting only (T-a) (115 and 105 μg glucose released per min per g). The glucanase induction in plants treated with *T viride* SP-1 application was significant compared to control. (Table1 and Fig1).

Table1. Glucanase activity and phenol contents in plants treated with T viride SP-1 suspension application

| Application of T viride SP-1 suspension | Glucanase activity (μg glucose min-1 g^-1) | Total Phenol (μg phenol g^-1) |
|----------------------------------------|-------------------------------------------|-------------------------------|
| T-a                                    | 105, 231                                  | 1487,905                      |
| T-b                                    | 131, 471                                  | 1587,232                      |
| T-c                                    | 115, 636                                  | 1157,46                       |
| T-d                                    | 110,285                                   | 1934,71                       |
| T-e                                    | 92, 152                                   | 460,68                        |
| T-f                                    | 97, 205                                   | 1177,577                      |

Note:

T-a = application of *T. viride* 2 weeks before planting, infection of *P. infestans*
T-b = application of *T. viride* 1 week after planting, infection of *P. infestans*
T-c = application of *T. viride* 2 before planting and 1 week after planting, infection of *P. infestans*
T-d = chemical fungicide, infection of *P. infestans*
T-e = without application of *T. viride*, infection of *P. infestans*
T-f = without application of *T. viride* and without infection of *P. infestans*

3.2. Changes in phenol content in plants treated with application of *T. viride* SP-1 suspension

The phenol content in plants treated with application of *T. viride* SP-1 suspension significantly increased. In untreated plants, the phenol content was 460.68μg g-1, while in treated plants it varied from 1,157.46 to 1,934.71μg g-L. The maximum phenol content observed was found in plants treated with T-d (1,934.71μg), followed by T-b (1,587.232μg), T-a (1,487.905μg), and T-c (1,157.46μg) (Fig 2).
Fig. 1. Glucanase activity (µg of glucose released per min per g of leaf tissue) in potato plants treated with *T. viride* suspension SP-1

Fig. 2. Total Phenol Content in Potatoes in six treatments with antagonist fungal application

**Note:**
- T-a = application of *T. viride* 2 weeks before planting, infection of *P. infestant*
- T-b = application of *T. viride* 1 week after planting, infection of *P. infestant*
- T-c = application of *T. viride* 2 weeks before planting and 1 week after planting, infection of *P. infestant*
- T-d = chemical fungicide, infection of *P. infestant*
- T-e = without application of *T. viride*, infection of *P. infestant*
- T-f = without application of *T. viride* and without infection of *P. infestant*

The application of *Trichoderma viride* was potent inducers of potato defense responses. These responses were systemic called induced systemic resistance (ISR) [17,18]. Unlike systemic acquired resistance (SAR) elicited by inducers of pathogen origin, ISR induced by biocontrol agent does not result in hypersensitive reaction, plant cell necrosis, or phytotoxicity [15]. Meanwhile, the examination of upon the application of *T. harzianum* T39 to soil instead of spraying resulted in a 75-90% reduction in *Sphaerotheca fusca* coverage on the leaves of greenhouse cucumbers showed that the mode of action of *T. harzianum* T39 in powdery mildew control is induced resistance, not mycoparasitism or antibiotic action [6]. Furthermore, [4] reported that *T. koningii* treatment increases photosynthesis and chlorophyll content in cotton seedlings. In addition, the observation of [21] showed that challenging
tomato or tobacco varieties with ethylene-inducing xylanase (EIX) from *T. viride* cause rapid induction of plant defense responses leading to programmed cell death.

In the present study, BALITSSA Cikole Bandung evaluated the capacity of *Trichoderma viride* isolate to induce systemic resistance in potato plants. The result showed that the application of *T. viride* before and after planting could induce more glucanase production in potato plants when used for treating the plants by seedling dip method, although, there was no direct correlation between increased glucanase activity and increased phenol content. However, a significant reduction in stem necrosis in treated plants occurs; even though, *T. viride* applied as seed and soil treatment does not have any physical contact with spores of *P. infestans* or the infected portions of the plant [3].

Meanwhile, the induction of capsidiol in the stems of treated plants was high. That inoculation of cucumber roots with *T. harzianum* T-203 induces chitinase and peroxidase activity was proven by [23]. Cucumber roots treated with *T. harzianum* exhibits higher activities of chitinase, beta-1.3-glucanase, cellulose, and peroxidase more than 72 h post-inoculation compared to untreated control.

The role of induced systemic resistance in the control of the foliar pathogen *Botrytis cinerea* in cucumber using Trichoderma was proven by [5]. Therefore, identification and integration of the formulations of ISR eliciting Trichoderma strains in disease management program were important and helpful in a long way.

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