Effectiveness of biological control of *Trichoderma harzianum* on soybean leaf rust disease and the production in West Papua Lowland, Indonesia

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Abstract. Martanto EA, Tanati AE, Baan S, Tata HR, Murdjoko A. 2020. Effectiveness of biological control of *Trichoderma harzianum* on soybean leaf rust disease and the production in West Papua Lowland, Indonesia. *Biodiversitas* 21: 1935-1939. This study aims to evaluate the application of *Trichoderma harzianum* in leaf rust disease on soybean and its production. The study was conducted for three months starting from May to July 2017, at Residential Unit 11th Sidey Subdistrict, Manokwari District, West Papua Province, Indonesia. This study was designed using Factorial Complete Randomized Design consisting of two treatment factors and repeated three times. There was no treatment of pathogen inoculation in the field. The tested varieties had a different response to plant height. *Burangrang, Grobogan, Denae-1, Anjasmoro*, and Detam-1 varieties were categorized as moderate resistant varieties. The *Trichoderma harzianum* application should be applied in the whole crops of soybean to control environmentally the leaf rust disease. Seeds weight per plot for Denae-1 variety was higher compared to other varieties. The combination of Detam-1 varieties with *Trichoderma* treatment 2 times resulted in increased production.

Keywords: Leaf rust, production, soybean, *Trichoderma harzianum*, West Papua

INTRODUCTION

Soybean is one of the important crops in Indonesia in which foods made of soybean contain high carbohydrates and isoﬂavones which is an anticancer (Astawan and Fabrinda 2009). Moreover, the high protein content at the level of 30.3-34.6% can be used as an alternative protein source (Yuwono et al. 2012). The development of foods and feeds industry with soybean as their raw materials is followed by the population growth causing the demand for soybean in Indonesia increases signiﬁcantly. The development of foods and feeds industry with soybean as their raw materials is followed by the population growth causing the demand for soybean in Indonesia to increase significantly (Swastika et al. 2009).

Papua has abundant local food stock potency, particularly soybeans as a food source. Statistical data indicates that the soybean production for Manokwari reaches 1.1 ton/ha (Anonymous 2017). The total population of Papua is about 2.2 million with a population growth of 3.14% per year. The increasing population number has caused increased food demand. Therefore, soybean production increases, either by intensification or extensification should be constantly performed to reduce the dependence on imported soybeans (Sudjudo et al. 1989).

Sidey Makmur Village (Residential Unit 11th) is one of the development centers for soybean commodities in Manokwari with speciﬁc advantages that are not found in other regions in Indonesia, particularly the planting period 4 times in a year. This superiority is a potency that can increase yield productivity, but the obtained product is not as expected. One of the obstacles in increasing soybean productivity is pests and diseases attack. The important disease of soybean plants is leaf rust caused by *Phakopsora pachyrhizi*. Leaf rust disease, an important disease in soybean, occurs during summer (Semangun, 2008: Sumartini 2010: Soesanto 2015). Production loss due to leaf rust disease could reach 40-90% in Indonesia (Sudjono et al. 1985). Most of the planted varieties in Sidey Subdistrict are Anjasmoro variety. According to a description from Baliktabi Malang Indonesia, Anjasmoro variety resistance to rust is moderate. The planting of Anjasmoro variety continually will produce a new pathogenic race that can break the resistance of the variety. Therefore, an alternative variety is needed.

Biological control of diseased soybean plants can be done by utilizing microbes that are antagonistic to pathogenic fungi (Agrios 1996). Plant diseases control using biological agents can reduce chemical pesticide use. One of the antagonistic biological agents and can control the disease is *Trichoderma harzianum*. *T. harzianum* has ability as biological control with a wide spectrum in various crop plants. Several studies indicated that the antagonistic agent can reduce the plant diseases development from various pathogen attacks such as Elsinoe batatas in sweet potatoes (Martanto et al. 2015), *Verticillium* sp. in Strawberries (Suryawan et al. 2017), and *Fusarium oxysporum* in tomatoes (Fitrianingsih et al.
The intensity of leaf rust disease. Disease intensity was calculated according to the percentage of occurring disease symptoms. Disease intensity was observed 3 weeks after planting (WAP), repeated 4 times with 2 weeks interval between observation. The intensity of leaf rust was calculated with the following formula:

\[
IP = \frac{\sum n_i \times x_i}{N \times V} \times 100\%
\]

where, \( IP \) = Disease Intensity, \( n_i \) = Number of plant from attack category, \( x_i \) = Scale value of each attack category, \( N \) = Number of observed plants, and \( V \) = Highest scale value. Attack value category referred to Safitri et al. (2015) with the following category: 0 = No attack, 1 = 0-25% of leaf surface area attacked, 2 = 26-50% of leaf surface area attacked, 3 = 51-75% of leaf surface area attacked, and 4 = 76-100% of leaf surface area attacked.

**Antagonist effectiveness.** Antagonist agent effectiveness was calculated according to Abbott (Ditjen PSP 2004). The formula is

\[
EI = \left[ \frac{Ca - Ta}{Ca} \right] \times 100\%
\]

where, \( EI \) = Efficacy of Antagonist Agent (%), \( Ca \) = Disease intensity in control/without treatment, \( Ta \) = Disease intensity in treatment/after application. The effectiveness value of antagonist fungus was categorized as follow: E > 69% very good, E = 50-69% good, E = 30-49% less good, and E < 30% not good.

**Plant height (cm).** Plant height was measured starting from stem base to highest leaf. Plant height was measured at the age of 3 weeks after planting, repeated 4 times with 2 weeks interval between measurement.

**Weight of 100 seeds (g).** 100 seeds were collected randomly from the plant sample and weighted.

**Seed weight per plot (g/plot).** The beans harvest in each trial square was weighted.

**Data analysis.** The data were analyzed using Analysis of Variance when the real effect of treatments then continued with DMRT (Duncan Multiple Range Test) on the level of 95% (Gomez and Gomez 2010).

**RESULTS AND DISCUSSION**

**Disease intensity.** At 5 WAP, leaf rust symptoms had been observed in all studied soybean varieties. Symptoms were observed on leaves, initially manifested as gray-brown small patches and then changed into deep brown or brown patches. At 9 WAP, the intensity of rust was highest in Anjasmoro variety (38.45%), and the lowest in Detam-1 variety (31.04%) (Table 1). The intensity of rust disease with the treatment of 2-time \( T. harzianum \) was 31.35% and lower compared to treatment with 1-time \( T. harzianum \) (33.12%) and control (36.38%). The effectiveness of antagonist fungi with two applications was 13.83%, better than those with one application (8.96%) (Table 1). Disease intensity with combined treatment at 9 WAP was lowest in V2T2 (27.45%) and highest in V1T0 (42.34%) (Table 2).

**MATERIALS AND METHODS**

**Study area.** This study was conducted in Laboratory of Plant Pests and Diseases, Faculty of Agriculture, University of Papua, Manokwari, Indonesia (latitude 00°70′76″S, longitude 134°06′79″E, latitude 126), and in Residential Unit 11th of Sidey Subdistrict, Manokwari District, Indonesia (latitude 00°45′34″S, longitude 133°32′39″E, altitude 39).

**Experimental design and protocol.** This study used factorial Complete Random Design consisting of antagonist factor and variety, repeated three times. The antagonist factor consisted of T0 (without application of \( T. harzianum \)), T1 (application of \( T. harzianum \) 1 time) and T2 (application of \( T. harzianum \) 2 times). Whereas the variety factor consisted of V1 (Buranangr variety), V2 (Grobogan variety), V3 (Dena-1 variety), V4 (Anjasmoro variety), and V5 (Detam-1 variety).

Antagonist and its application. \( T. harzianum \) that isolated from rhizosphere in this area were multiplied in the rice husk medium. Isolates were applied by spraying on to the leaf surfaces at 4 weeks after planting. The density of \( T. harzianum \) spores was 10⁷ spores.mL⁻¹ water.

**Tabel 1.** Plant response to leaf rust disease using the formula by Yuspamella et al. (2013)

| Description                  | Response scale  |
|------------------------------|----------------|
| No rust                      | Very resistant |
| 1-10 % of leaf infected      | Resistant      |
| 11-40 % of leaf infected     | Moderate resistant |
| 41-65% of leaf infected      | Susceptible    |
| 66-100 % of leaf infected    | Very Susceptible |

2019). \( T. harzianum \) also has ability as parasite and antibiotic because it produces enzymes that actively degrading pathogenic cells, causing the lysis of pathogenic fungus cells and release tritoxin that can kill pathogenic fungi (Oc triania 2011). The chitinase enzyme produced by \( T. harzianum \) sp. more effective than chitinases produced by other organisms in inhibiting various pathogenic fungi (Umrah et al. 2009).

The implementation of biological control can be applied as a single method or combined with other control techniques simultaneously. The use of \( T. harzianum \) and the use of several resistant soybean varieties is the combined control expected to control the attack of P. pachyrhizi. So far, the effectiveness of spraying and the use of several soybean varieties in controlling leaf rust is not known. Thus, the study on soybean to know the effect of \( T. harzianum \) on leaf rust control in soybean plant and its production is relevant to conduct. This study aims to evaluate the utilization of \( T. harzianum \) in controlling the leaf rust disease on soybean and its production.
Table 1. Observed disease intensity in soybean varieties and *T. harzianum* treatments. Values followed by a similar letter in the same column are not significantly different according to Duncan Multiple Range Test at 5% level while the values not followed by letter are not significantly different. WAP stands for weeks after planting.

| Treatment  | Disease intensity at (%) | 3 WAP | 5 WAP | 7 WAP | 9 WAP |
|------------|--------------------------|-------|-------|-------|-------|
| P1: Burangrang | 0.00 | 8.65 abc | 14.76 ab | 34.08 |
| V2: Grobogan | 0.00 | 11.46 a | 15.83 ab | 32.28 |
| V3: Detam-1 | 0.00 | 8.25b c | 12.44 b | 32.23 |
| V4: Anjasmoro | 0.00 | 10.51 ab | 17.18 a | 38.45 |
| V5: Detam 1 | 0.00 | 6.34 c | 12.73 b | 31.04 |
| T0: No application | 0.00 | 9.09 | 14.92 | 36.38 |
| T1: 1 application | 0.00 | 7.79 | 13.92 | 33.12 |
| T2: 2 applications | 0.00 | 10.24 | 15.21 | 31.35 |

*T. harzianum* effectiveness (%)

| Treatment  | Disease intensity (%) | 9 WAP |
|------------|-----------------------|-------|
| P1: Burangrang | 23.73 b | 49.09 a |
| V2: Grobogan | 25.60 a | 44.98 bc |
| V3: Detam-1 | 23.36 b | 48.53 ab |
| V4: Anjasmoro | 24.04 b | 46.71 abc |
| V5: Detam 1 | 21.31 c | 43.16 c |
| T0: No application | 24.33 a | 46.83 ab |
| T1: 1 application | 24.48 a | 48.27 a |
| T2: 2 applications | 22.01 b | 44.39 b |

Table 2. The effect of the combination of variety and *T. harzianum* on observed parameters.

| Treatment | Disease intensity (%) | Plant height 9 WAP | Weight/100 seeds (g) | Seed weight per square (g) |
|-----------|-----------------------|------------------|---------------------|--------------------------|
| V1T0      | 42.34                 | 67 cd            | 18.96               | 516.66                   |
| V1T1      | 27.81                 | 70.47 bc         | 19.00               | 523.33                   |
| V1T2      | 32.09                 | 70.67 bc         | 18.5                | 543.33                   |
| V2T0      | 36.09                 | 46.93 g          | 15.5                | 443.33                   |
| V2T1      | 33.29                 | 46.3 g           | 18.87               | 416.66                   |
| V2T2      | 27.45                 | 53.8 f           | 19.87               | 506.66                   |
| V3T0      | 35.77                 | 75.27 ab         | 13.1                | 856.66                   |
| V3T1      | 32.49                 | 73.27 abc        | 12.9                | 893.33                   |
| V3T2      | 28.42                 | 79.13 a          | 13.2                | 1086.66                  |
| V4T0      | 39.42                 | 59.33 ef         | 16.7                | 393.33                   |
| V4T1      | 40.23                 | 66.2 cde         | 17.93               | 370                      |
| V4T2      | 35.69                 | 72.93 abc        | 18.56               | 520                      |
| V5T0      | 28.26                 | 59.53 ef         | 16.83               | 483.33                   |
| V5T1      | 31.78                 | 68.27 bcd        | 20                  | 533.33                   |
| V5T2      | 33.08                 | 62.07 de         | 18.93               | 620                      |

Note: Values followed by a similar letter in the same column are not significantly different according to Duncan Multiple Range Test at 5% level while the values not followed by letter are not significantly different. WAP stands for weeks after planting. The symbols of treatments are: V1: Burangrang, V2: Grobogan, V3: Detam-1, V4: Anjasmoro, V5: Detam 1, T0: No application, T1: 1 application and T2: 2 applications.

Zadoks and Schein (1979) suggested that microclimate is an important requirement for the infection cycle, starting from the attachment of spores to the spreading of spores. Disease intensity in the 2-time *T. harzianum* application was lower compared to the intensity in control. Taufik (2010) suggested that *Trichoderma* sp. can compete for space and food and capable of stressing pathogen development in soil and plant tissues and induce resistance and inactivation of pathogenic enzymes. Papavizas (1985) suggested that *Trichoderma* sp. produces Trichoderin which is a toxin to pathogens. The toxin reduces the growth and reproduction of the pathogens (Rivera-Méndez et al. 2020; Alamri et al. 2019; Ghobanpour et al. 2018). Ditjen PSP (2004) added that *Trichoderma* sp. can suppress the growth of pathogens by entwining the pathogenic hyphae, releasing enzymes β-(1-3) (Sumida et al. 2018) and chitinase that can penetrate the host cell wall. According to Purwantisari and Hastuti (2009), *Trichoderma* sp. can colonize the rhizosphere quickly and protecting roots from pathogens attack (Ojaghian et al. 2019; Mercil et al. 2020; Moya et al. 2020).

Disease intensity

At 9 WAP, the plant height of *Dena*-1 variety (75.89 cm) was higher compared other four varieties. Plant height in the treatment of 2 *T. harzianum* applications (67.72 cm) was higher than the treatment of 1 *T. harzianum* application and control (Table 3). The best combination was obtained with *Dena*-1 variety treated with 2 *T. harzianum* applications (V3T2) with a height of 79.13 cm (Table 2).
Plant height is an important variable in observing plant growth because it relates to the number of leaves developed. Study findings indicated that Detam-1 had the highest plant height with the highest production. Malik et al. (2013) suggested that plant height is an important character because it positively correlates to beans yield. Plant height difference occurs due to genetics carried by each of the varieties. Sopialena (2015) suggested that plant variety reaches the highest growth limit according to its genetic traits. The application of antagonists influences plant height. The administration of T. harzianum causes the plant height is higher compared to those without antagonists. In addition to its ability in suppressing pathogen growth, T. harzianum is also a phosphate dissolving microorganism that can be used by plants, so the growth of plant height occurs in good progress as it has growth-promoting effect. This is in accordance with Setiadi (1989) that the presence of T. harzianum in the soil is in synergy with the phosphate dissolving bacteria (Mercel et al. 2020; Ojaghian et al. 2019).

### Weight per 100 seeds, and seed weight per square

There was a significant difference in weight per 100 seeds, and seed weight per square among the tested soybean varieties. The highest seed weight per square was found in Detam-1 variety (945.56 g/square) and the treatment of 2 T. harzianum applications (655.33 g) (Table 4). Soybean seeds weight per square was highest in the combination of V3T2 (1086.86 g) (Table 2).

Two applications of T. harzianum caused the pathogens were difficult in attacking soybean plants, so its production is higher. T. harzianum is capable of decomposing organic materials from rice husk, and in the decomposition process, the antagonist alters elements in a dissolved form so they can be absorbed by plants (Coelho et al. 2020). According to Suryawan et al. (2017) Potassium element in rice husk that can increase plant resistance to disease and antagonist able to inhibit disease progression. Broto et al. (1994) suggested that rice husks used as treatment medium provide abundant inorganic and organic components. The available carbohydrate and cellulose are utilized by T. harzianum sp. as an energy source and carbon source in the decomposition process (Jin and Custis 2011). Pod weight per 100 seeds of Detam-1 variety was the lowest, but its seeds weight per plot was the highest. This was because the seeds produced by Dena-1 variety were higher than other varieties.

### The application of Trichoderma harzianum

The tested varieties had different responses to parameters of plant height. Burangrang, Grobogan, Dena-1, Anjasmorro, and Detam-1 varieties were categorized as moderate resistant. The T. harzianum application should be applied regularly in the whole crop of soybean to regulate environmentally leaf rust disease. Seeds weight per plot of Dena-1 variety was higher compared to other varieties. The combination of Detam-1 varieties with T. harzianum treatment 2 times resulted in the best production. Therefore, the use of T. harzianum indicated that this method is environmentally friendly (Sumida et al. 2018; Khalili et al. 2016) since the method is harmless to biodiversity in soybean crops as well as the environmental condition surrounding the crop. The use of biological control such as T. harzianum to prevent the disease would also provide the organic nutrients in the soil since the biological controls can improve healthy soil biology by supporting nutrient uptake and root growth (Sumida et al. 2018) as the roots are imperative for supporting the metabolism (Hanudin et al. 2014). Therefore, this method supports biodiversity since it allows the organisms to live such as Phakopsora pachyrhizi itself. Even though P. pachyrhizi is pathogen but still part of biodiversity. Furthermore, this method could provide the benefit economically for the owner and ecologically for promoting the biodiversity along with its environment.

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