

BUKTI KORESPONDENSI ARTIKEL

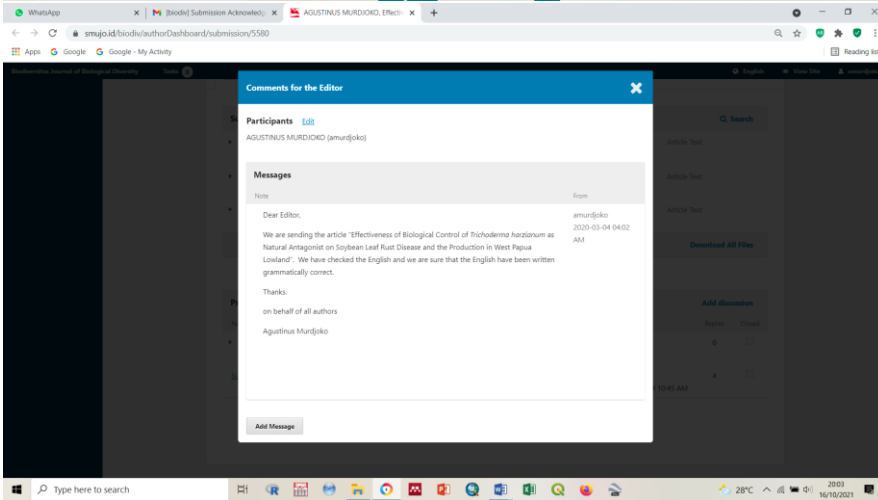
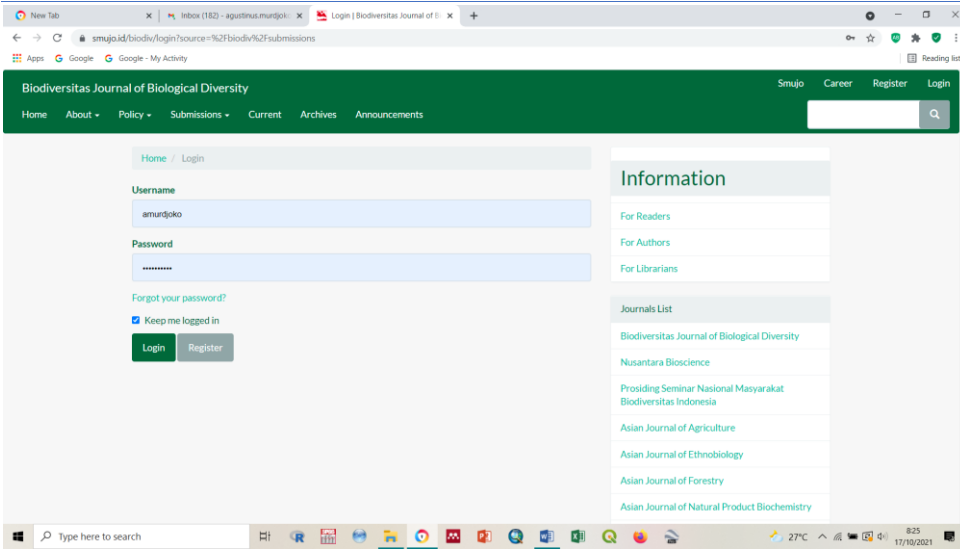
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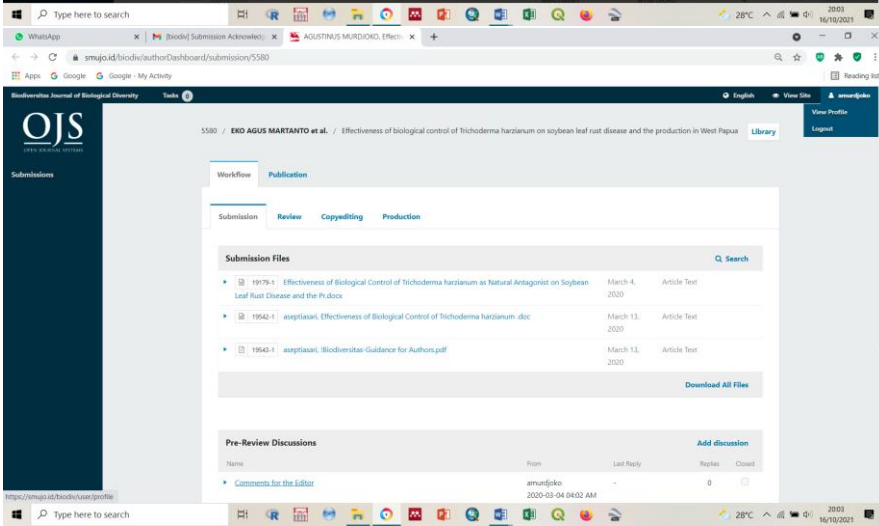
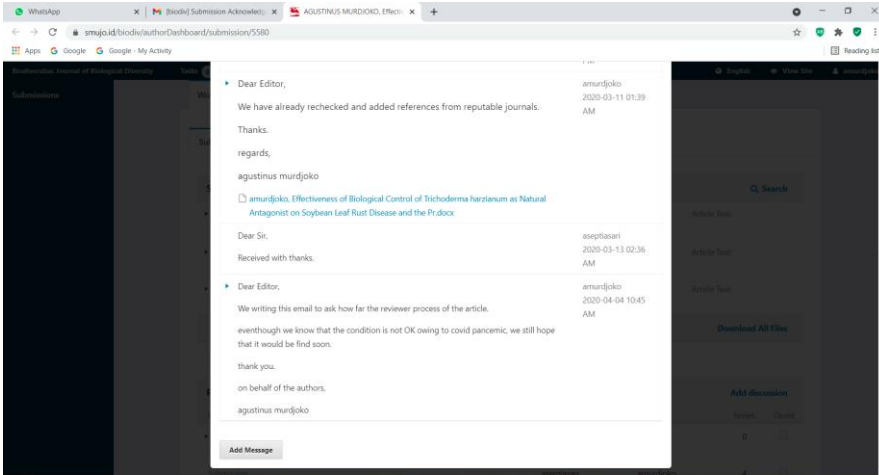
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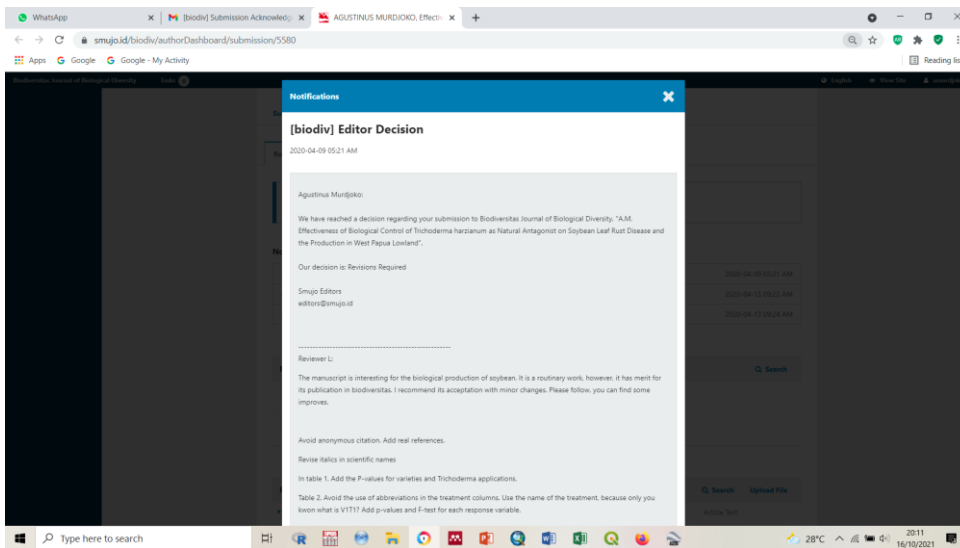
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Pengiriman pertama



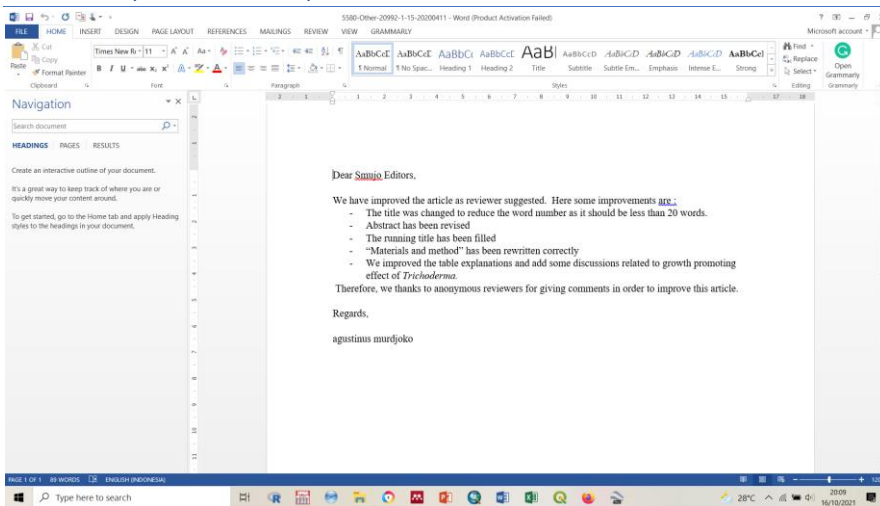


Keputusan editor



Perbaikan artikel

Surat respon terhadap komentar reviewer dan revisi



Effectiveness of Biological Control of *Trichoderma harzianum* as Natural Antagonist on Soybean Leaf Rust Disease and the Production in West Papua Lowland

Abstract. 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 District, Manokwari Regency, West Papua Province. 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*, *Dena-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 eradicate environmentally the leaf rust disease. Seeds weight per plot for *Dena-1* variety was higher compared to other varieties. The combination of *Detam-1* varieties with *Trichoderma* treatment 2 times resulted in the best production.

Keywords: leaf rust, production, Soybean, *Trichoderma harzianum*, West Papua.

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INTRODUCTION

Soybean is one of the important crops in Indonesia in which foods made of soybean contain high carbohydrates and isoflavon 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 (Sudjudi et al. 1989).

Sidey Makmur Village (Residential Unit 11th) is one of the development center for soybean commodities in Manokwari with specific advantages which 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 pachyrizi*. 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 District 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*. *Trichoderma* 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. 2019). *Trichoderma* 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 (Octriana 2011). The chitinase enzyme produced by *Trichoderma* sp. more effective than chitinases produced by other organisms in inhibiting various pathogenic fungi (Umrh 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 *Trichoderma 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 *Trichoderma* on leaf rust control in soybean plant and its production is relevant to conduct. This study aims to evaluate the utilization of *Trichoderma* in controlling the leaf rust disease on soybean and its production.

MATERIALS AND METHODS

Study area

This study was conducted in Laboratory of Plant Pests and Diseases, Faculty of Agriculture, University of Papua (latitude 00°70' 76' S, longitude 134°06' 79' E, latitude 126), and in Residential Unit 11th of Sidey District, Manokwari regency (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 *Trichoderma harzianum*), T1 (application of *Trichoderma harzianum* 1 time) and T2 (application of *Trichoderma harzianum* 2 times). Whereas the variety factor consisted of V1 (*Burangrang* variety), V2 (*Grobogan* variety), V3 (*Dena-1* variety), V4 (*Anjasmoro* variety), and V5 (*Detam-1* variety).

Antagonist and its application. Isolates of *Trichoderma harzianum* 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 *Trichoderma harzianum* spores was 107 spores.ml⁻¹ water. 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 $IP = \frac{\sum n_i \times v_i}{N \times V} \times 100\%$ where, IP = Disease Intensity, ni = Number of plant from attack category, vi = 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

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

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

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 *Trichoderma* was 31.35% and lower compared to treatment with 1-time *Trichoderma* (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).

Table1. Observed disease intensity in soybean varieties and *Trichoderma* treatments. Values followed by a similar letter in the same column are not significantly different according to Duncan Multiple Range Test at 5% level.

Treatment	Disease intensity at.... (%)			
	3 WAP	5WAP	7 WAP	9 WAP
V1 : <i>Burangrang</i>	0,00	8,65abc	14,76ab	34,08
V2 : <i>Grobogan</i>	0,00	11,46a	15,83ab	32,28
V3 : <i>Detam-1</i>	0,00	8,25bc	12,44b	32,23
V4 : <i>Anjasmoro</i>	0,00	10,51ab	17,18a	38,45
V5 : <i>Detam 1</i>	0,00	6,34c	12,73b	31,04
T0 (No application)	000	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
<i>Trichoderma</i> effectiveness (%)				
T1 (1 application)	0,00	14,30	6,70	8,96
T2 (2 applications)	0,00	-12,65	-0,02	13,83

Table 2. The effect of the combination of variety and *Trichoderma* on observed parameters. Values followed by a similar letter in the same column are not significantly different according to Duncan Multiple Range Test at 5% level.

Treatment	Disease intensity (%) 9 MST	Plant height 9 MST	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	46,53 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

Observations at 5 WAP began to detect leaf rust symptoms in all tested varieties. The delayed occurring symptoms were caused by the fact that during study no inoculation of leaf rust pathogen was performed. The study location was a region with continuous soybean planting making the inoculum sources are always available. Observation at 9 WAP, *Anjasmoro* variety was the most vulnerable variety with the highest disease intensity, whereas *Detam-1* had the lowest disease intensity. The high disease intensity can contribute to decreased production. This is in accordance with Djauhari (2008), that rust disease attack can reduce production by 60-80%. According to the occurring disease intensity extent, all tested varieties were categorized as moderately resistant to rust disease. The disease intensity difference in each variety was related to different resistance of each variety. The developing leaves in all varieties causing the microclimate around the plant supports the development of the pathogen. Zadoks and Schein (1979) suggested that micro climate 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 *Trichoderma* application was lower compared to the intensity in control. Taufik (2010) suggested that *Trichoderma* sp. has an ability to 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 Trichodermin which is a toxin to pathogens. The toxin reduce the growth and reproduction of the pathogens (Rivera-Méndez et al. 2020; Alamri et al. 2019; Ghorbanpour 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; Mercl 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 *Trichoderma* applications (67.72 cm) was higher than the treatment of 1 *Trichoderma* 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).

Table 3. Plant height in soybean variety and observed *Trichoderma* treatments. Values followed by a similar letter in the same column are not significantly different according to Duncan Multiple Range Test at 5% level.

Treatment	Plant height			
	3 WAP	5 WAP	7 WAP	9 WAP
V1 : <i>Burangrang</i>	23,73b	49,09a	69,36a	69,38b
V2 : <i>Grobogan</i>	25,60a	44,98bc	49,62c	49,09d
V3 : <i>Detam-1</i>	23,36b	48,53ab	71,18a	75,89a
V4 : <i>Anjasmoro</i>	24,04b	46,71abc	59,78b	66,16bc
V5 : <i>Detam 1</i>	21,31c	43,16c	58,76b	63,29c
T0 (No application)	24,33a	46,83ab	63,11a	61,61b
T1 (1 application)	24,48a	48,27a	63,13a	64,95a
T2 (2 applications)	22,01b	44,39b	58,97b	67,72a

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 *Trichoderma* causes the plant height is higher compared to those without antagonists. In addition to its ability in suppressing pathogen growth, *Trichoderma* is also a phosphate dissolving microorganism that can be used by plants, so the growth of plant height occurs in good progress. This is in accordance with Setiadi (1989) that the presence *Trichoderma* in the soil is in synergy with the phosphate dissolving bacteria (Mercl 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 *Dena-1* variety (945.56 g/square) and in the treatment of 2 *Trichoderma* applications (655.33 g) (Table 4). Soybean seeds weight per square was highest in the combination of V3T2 (1086,86 g) (Table 2).

Table 4. Weight per 100 seeds, and seed weight per square of tested soybean variety and *Trichoderma* application. Values followed by a similar letter in the same column are not significantly different according to Duncan Multiple Range Test at 5% level.

Treatment	Weight per 100 seeds (g)	Seed weight per plot (g)
V1 : <i>Burangrang</i>	18,82 a	527,78 b
V2 : <i>Grobogan</i>	18,08 a	455,55 b
V3 : <i>Detam-1</i>	13,07 b	945,56 a
V4 : <i>Anjasmoro</i>	17,73 a	427,78 b

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V5 : Detam 1	18,59 b	545,56 b
T0 (No application)	16,22 b	538,67
T1 (1 application)	17,74 a	547,33
T2 (2 applications)	17,82 a	655,33

Two applications of *Trichoderma* caused the pathogens were difficult in attacking soybean plants, so its production is higher. *Trichoderma* 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 *Trichoderma* 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 due to the fact that 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*, *Anjasmoro*, and *Detam-1* varieties were categorized as moderate resistant. The *Trichoderma harzianum* application should be applied regularly in the whole crop of soybean to eradicate environmentally the leaf rust disease. Seeds weight per plot of *Dena-1* variety was higher compared to other varieties. The combination of *Detam-1* varieties with *Trichoderma* treatment 2 times resulted in the best production. Therefore, the use of *Trichoderma 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 *Trichoderma harzianum* to prevent the disease would also provide the organic nutrients in the soil since the biological controls are able to 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. Furthermore, this method could provide the benefit economically for the owner and ecologically for promoting the biodiversity along with its environment.

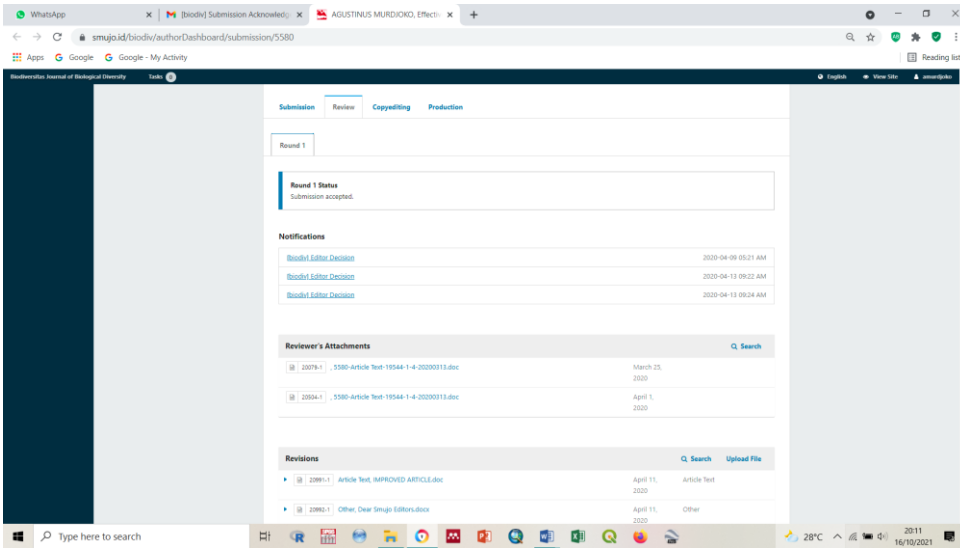
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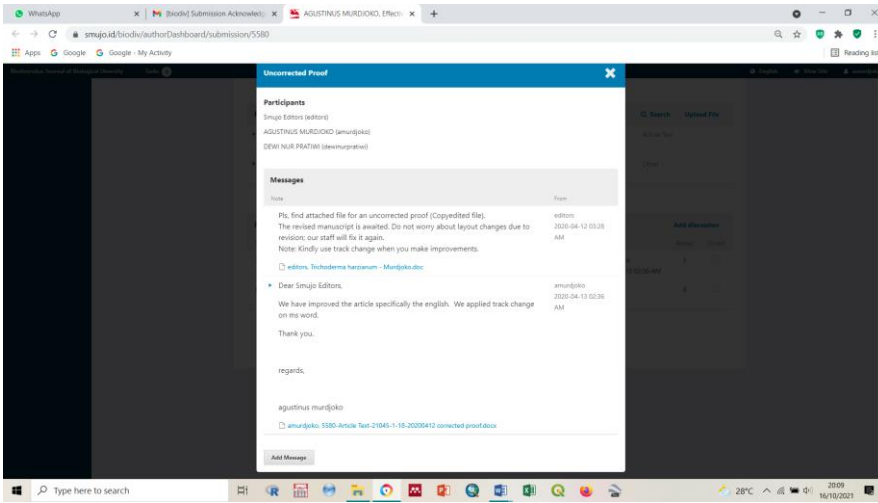
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Keputusan akhir journal



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*, *Dena-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 *Dena-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 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 (Sudjudi 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 (Fitrianiingsih et al. 2019). *Trichoderma* 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 (Octriana 2011). The chitinase enzyme produced by *Trichoderma* 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 *Trichoderma 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.

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 *Trichoderma harzianum*), T1 (application of *Trichoderma harzianum* 1 time) and T2 (application of *Trichoderma harzianum* 2 times). Whereas the variety factor consisted of V1 (*Burangrang* variety), V2 (*Grobogan* variety), V3 (*Dena-1* variety), V4 (*Anjasmoro* variety), and V5 (*Detam-1* variety).

Antagonist and its application. *Trichoderma harzianum* that isolated from rhizosphere in this are 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 *Trichoderma 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

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 $IP = \frac{\sum n_i \times v_i}{N \times V} \times 100\%$ where, IP

= Disease Intensity, ni = Number of plant from attack category, vi = 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 $El = \left[\frac{Ca-Ta}{Ca} \right] \times 100\%$ where, El = 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.

Table1. 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	5WAP	7 WAP	9 WAP
V1: <i>Burangrang</i>	0.00	8.65 abc	14.76 ab	34.08
V2: <i>Grobogan</i>	0.00	11.46 a	15.83 ab	32.28
V3: <i>Detam-1</i>	0.00	8.25b c	12.44 b	32.23
V4: <i>Anjasmoro</i>	0.00	10.51 ab	17.18 a	38.45
V5: <i>Detam 1</i>	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

<i>T. harzianum</i> effectiveness (%)				
T1: 1 application	0.00	14.30	6.70	8.96
T2: 2 application	0.00	-12.65	-0.02	13.83

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).

Table 3. Plant height in soybean variety and observed *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	Plant height			
	3 WAP	5 WAP	7 WAP	9 WAP
V1: <i>Burangrang</i>	23.73 b	49.09 a	69.36 a	69.38 b
V2: <i>Grobogan</i>	25.60 a	44.98 bc	49.62 c	49.09 d
V3: <i>Detam-1</i>	23.36 b	48.53 ab	71.18 a	75.89 a
V4: <i>Anjasmoro</i>	24.04 b	46.71 abc	59.78 b	66.16b c
V5: <i>Detam 1</i>	21.31 c	43.16 c	58.76 b	63.29 c
T0: No application	24.33 a	46.83 ab	63.11 a	61.61 b
T1: 1 application	24.48 a	48.27 a	63.13 a	64.95 a
T2: 2 applications	22.01 b	44.39 b	58.97 b	67.72 a

Observations at 5 WAP began to detect leaf rust symptoms in all tested varieties. The delayed occurring symptoms were caused by the fact that during study no inoculation of leaf rust pathogen was performed. The study location was a region

with continuous soybean planting making the inoculum sources are always available. Observation at 9 WAP, *Anjasmoro* variety was the most vulnerable variety with the highest disease intensity, whereas *Detam-1* had the lowest disease intensity. The high disease intensity can contribute to decreased production. This is in accordance with Djauhari (2008), that rust disease attack can reduce production by 60-80%. According to the occurring disease intensity extent, all tested varieties were categorized as moderately resistant to rust disease. The disease intensity difference in each variety was related to different resistance of each variety. The developing leaves in all varieties causing the microclimate around the plant supports the development of the pathogen.

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

Treatment	Disease intensity (%) 9 WAP	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	46.53 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; Ghorbanpour 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; Mercl 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).

Table 4. Weight per 100 seeds, and seed weight per square of tested soybean variety and *T. harzianum* application. 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.

Treatment	Weight per 100 seeds (g)	Seed weight per plot (g)
V1: Burangrang	18.82 a	527.78 b
V2: Grobogan	18.08 a	455.55 b
V3: Detam-1	13.07 b	945.56 a
V4: Anjasmoro	17.73 a	427.78 b
V5: Detam 1	18.59 b	545.56 b
T0 (No application)	16.22 b	538.67
T1 (1 application)	17.74 a	547.33
T2 (2 applications)	17.82 a	655.33

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 (Mercl 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 *Dena-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*, *Anjasmoro*, 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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