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The Biocontrol Ability Test of *Trichoderma harzianum* Toward Damping Off Disease On Soybean Seedlings

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Abstract. This study aims to determine the controlling power of *Trichoderma harzianum* against the activity of damping off pathogen (*Rhizoctonia solani* isolate Rs-Clkt-01) on soybean during the period of attack. The first phase of research is descriptive research that explains the potential of damping off pathogen attack isolated from pine seeds in forest land on soybean plant which is part of pine-based agroforestry system. The experimental research are arranged in complete randomized design using 5 kinds of seedlings inoculation treatment ie: without inoculation, inoculated by pathogens, inoculated by *Trichoderma* and 6 hours later inoculated by pathogens, inoculated by pathogens and 6 hours later inoculated by *Trichoderma*, inoculated by pathogens and *Trichoderma* simultaneously. Each treatment was repeated 4 times. For this research observed the damping off disease index. The observed data were analyzed using a variance test followed by a 5% HSD test to determine the differences between treatments. The results showed that *T. harzianum* is able to control the *damping off* pathogen both at 6 hours before and after and simultaneously inoculate with pathogens so as to suppress the disease index up to 5-10 (100) during the period of soybean cultivation. *T. harzianum* Tc-JJr-02 isolate can be used to control the damping off disease caused by *R. solani* in soybean cultivation.

1. Introduction

The national production of soybeans is only 998,000 tons, while the soybean requirement reaches 2.54 million tons [1]. The development of soybean cultivation that utilizes dry land of plantation forest through agroforestry system is one of soybean production alternative. However, indigenous pathogenic disorders of damping off disease (*Rhizoctonia solani*) often cause crop failure. In various area in Indonesia relatively have not found the spread of attack cases on soybean sprouts by *R. Solani* yet. However, preliminary tests conducted by the researchers showed high pathogenicity to the intensity of attack about 70-100%. These pathogens are already known to severely damage major agricultural crops and cause considerable yield loss [2].

Meanwhile, forest land is rich in various sources of germplasm both beneficial and adverse. *Trichoderma harzianum* and several *Trichoderma* isolates have been tested capable of controlling *damping off* disease and various diseases caused by *R. Solani* [3, 4]. *Trichodema* has the ability as a micoparasite [5], producing extracellular compounds that can be toxic to pathogens [6], and produce enzymes that can degrade pathogen cell walls [7].



This study aims to test the ability of *T.harzianum* Tc-Jjir-02 in controlling damping off disease on soybean seeds caused by *R. solani* isolates Rs-CLkt-01 inoculated in various ways.

2. Material and methods

This research held in Laboratory and Green House of Agriculture Faculty, Universitas Muhammadiyah Sidoarjo, Candi, Sidoarjo on January-April 2016.

In this study, we used Rs-CLkt-01 *R.solani* isolate pathogen that caused damping off and *T. harzianum* Tc-Jjr-02 as biocontrol agent which are the collection of Microbiology Laboratory of Faculty of Agriculture Universitas Muhammadiyah Sidoarjo. Both isolates were obtained from Celaket (Pacet) and Jatijejer (Trawas) of Mojokerto Regency, East Java Province, which is an area of agroforestry land of *Pinus merkusii* forest area that has potential to be used for the development of soybean cultivation.

The pathogen and *Trichoderma* isolates were grown for 7 days on PDA media containing chloramphenicol [8]. From those culture, we taken all the propagules and diluted them to get suspensions which containing 10^7 /ml spore for each isolates. Meanwhile, soybean seeds that have been washed with disinfectant (50% alcohol for 2 seconds) were drained. The seeds of 10 grains were placed on the surface of growing medium that containing a sterile soil and sand mixture (1:1) in a plastic container with 20 cm on diameter and 8 cm on thickness for each experimental unit. Furthermore, we inoculated pathogens and *Trichoderma* according to the type of seeds treatment such 4: not inoculated with anything (control), inoculated with pathogen (T0P1), inoculated with *Trichoderma*, and 6 hours later inoculated with pathogen (T1P6), inoculated with pathogen and 6 hours later inoculated with *Trichoderma* (T6P1) inoculated pathogens and *Trichoderma* simultaneously (T1P1). The five treatments were repeated four times to obtain 20 units of experiments that prepared in Completely Randomized Design (CRD). For every day during the damping off period on soybeans or 1-11 days after inoculation (HSI), we observed the disease symptoms to calculate disease index (Formula 1) which determined according to the criteria of symptoms 4 listed in Table 1. The observed disease index data were analysed using variance continued with a 5% HSD test to determine differences between treatments.

Determination of disease index:

$$k=4 \\ IP = \frac{\sum(n_i)}{N.k} \times 100 \dots\dots\dots(1) \\ i=1$$

where, IP = damping off disease index, *i* = numerical value (score) of sprouts with concern attack symptom criterion, *n_i*= number of sprouts with concern attack symptom criterion, N = number of observed sprouts, and *k* = highest numerical value (score) with severe attack symptom criterion.

Table 1. Symptom criteria of *damping off*

Score	Symptom criteria
0	No symptom of <i>damping off</i>
1	There are brown/rotten spots that circle up to 1/3 of the sprouts base
2	There are brown/rotten spots that circle up to 2/3 of the sprouts base
3	Brown/rotten spots almost circled around the base of the seedlings stem and/or seeds began to wither
4	Seeds/seedlings withered because stems base circled by spotting rotten until the seeds/seedlings death

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3. Results and discussion

Variance analysis (F test 5% real level) of the *Trichoderma* inoculation effect on disease index shows that *Trichoderma* application had an effect on damping off disease index of soybean 1-11 DAI. The mean disease indexes at 1, 4, 7, and 11 DAI are shown in Table 2.

Table 2. Mean influence of *Trichoderma* and pathogen inoculation to soybean damping off disease index^{a)}

Treatment (Inoculation way)	The ... th observation day after inoculation (DAI)			
	1 DAI	4DAI	7 DAI	11 DAI
Control without pathogen inoculation (K)	0.0 a	0.0 a	0.0 a	0.0 a
Inoculation with pathogen (T0P1)	92.5 b	93.1 b	98.8 b	100.0 b
<i>Trichoderma</i> followed by pathogen (T1P6)	0.0 a	0.0 a	0.0 a	8.1 a
Pathogen inoculation followed by <i>Trichoderma</i> (T6P1)	95.0 b	96.3 b	99.4 b	100.0 b
<i>Trichoderma</i> and pathogen inoculation simultaneously T1P1)	0.0 a	0.0 a	1,3 a	12,5 a
HSD 5%	5.18	4.45	1.83	6.72

^{a)}DAI = hour after inoculation; T = *T. harzianum*, P = *R. solani*, 1 = initial inoculation time (0 DAI), 6 = 6 hours inoculation from initial time; the numbers followed by same letter in same column show no significant difference in the 5% HSD test.

The inoculated pathogens (T6P1 and T0P1), infected rapidly and caused death for soybean at age 5 HSI where it reaches very severe conditions (index of disease 90-95) since 1 HSI and germinated seeds experienced death on the fourth day (disease index 100). This shows that the level of pathogenicity of *R. solani* on soybean seed is very high. Meanwhile, *Trichoderma* application at 6

hours before and after and along with pathogens application can inhibit pathogen infection so that disease intensity can be neglected or very light attack (6-9%).

Although the disease index reached 8.1 (T1P6) and 12.5 (T1P1), but in both treatments the seeds continued to live normally. The results of in vivo experiments were supported by the results of in vitro experiments on liquid cultures showing that *T. harzianum* capable of suppressing and controlling *R solani* [9, 4]. *Trichoderma* has various mechanisms in controlling pathogenic pathogens: competition for niche and nutrients, mikoparasit, antibiotic secretion and/or the production of fungi cell wall degrading enzymes [10, 3]. *T. harzianum* proved could increasing the ability of seed germination to preventing lumber [11], capable of inducing enzymes that play a role in enhancing plant growth [2] as well as plant protection and resistance [12, 13], and also can shorten the germination process [14]. Soybean sprouts that were inoculated by pathogens and also inoculated by these biocontrol agents showed a good growth response up to 11 DAI as a result of *T. harzianum* performance effect.

4. Conclusions

Tc-JJr-02 *Trichoderma harzianum* isolate was able to control the damping pathogen off both at 6 hours before and after and simultaneously inoculated with *R. solani* Rs-Clt-01 during the germination period of soybeans. The Implications of research is *T. harzianum* Tc-JJr-02 isolate can be used to control the damping off disease caused by *R. solani* in soybean cultivation.

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