Effectiveness of Antagonist Fungi (*Trichoderma* sp., *Gliocladium* sp., and *Verticillium* sp.) against *Sclerotium Rolfsii* Causes Damping-Off Disease on Soybean *Glycine Max* L. Merill.

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ABSTRACT

Efforts to increasing soybean production is inseparable from the various constraints. One of them caused by plant disease like soil. Soil borne diseases is one of the factor that reduce leguminous plants production. The leguminous plants attacked by soil born disease which is known as *S. rolfsii*. One of the alternative control isthe use of biological agents. Bilogical agents that used in this research were *Trichoderma* sp., *Gliolcadium* sp., and *Verticillium* sp. The pusrpose of this research were to know the effectiveness of *Trichoderma* sp., *Gliolcadium* sp., *Verticillium* sp. against *S. rolfsii.* The research was conducted 3 tests, all three tests used completly randomized design and if there is siginificant difference, continuedwith LSD test. The first test was direct oposition test, consist of 4 treatments and 5 replications. The second test was steam culture test, consist of 4 treatments and 5 replications. The third test was antagonist test in the glass house that consist of 4 treatments and 6 replications. The result of this research in direct oposition test on 7 days after inoculation, which is *Trichoderma* sp., can inhibit the pathogen by 61,71%, *Gliocladium* sp. 59,74% and *Verticillium* sp. 34%. On the steam culture method test *Trichoderma* sp., *Gliocladium* sp., and *Verticillium* sp., can inhibit the pathogen with the pathogen colony diameter by 3,70 cm, 3,24 cm dan 6,82 cm. The third test was antagonist test in the glass house, and the results were attack percentage with treatment *Trichoderma* sp., 51,67%, *Gliocladium* sp. 52,83%, *Verticillium* sp. 91%, Control (without antagonist) 98%.

Keywords: Antagonist, *Sclerotium rolfsii*, *Trichoderma* sp., *Gliocladium* sp., *Verticillium* sp., Soybean.

INTRODUCTION

Soybean is number 3 crop plant in the world. The needs of soybean in Indonesian is increasing along with population growth and per capita-Gross Domestic Product (Irwan, 2006). Efforts to increasing soybean production is inseparablefromthe variousconstraints. One of the constraint is caused by plant disease. Soil borne diseases is one of the factor that reduce leguminous plants production (Sumartini., 2011; Muhibudin, 2009). The leguminous plants attacked by soil born disease which is known as *S. rolfsii* (Semangun, 2008).

The damping off disease needs the control that adapted how the disease can survive in natural habitat. Crop rotation is hard to do, because the disease has a large host plant. Chemical fungicide control is not recommended because the use of chemical fungicide must be applied frequently and in accordance with the soil character. The use of chemical fungicide can contaminate the environment, soil water and the natural enemy can be killed by chemical fungicide. The use of botanical pecticide safe for the soil, water, and air. However, the material of botanical pecticide easy to degraded and vaporized. The application of it's have to several times. (Sumartini, 2011).

The antagonist agent isolated from the soil. In natural conditions, interaction betwen microorganism antagonist and plant pathogen widespread in the nature (Bosah, *et al.,* 2010). Soil in the field or in the glass house each gram contains 5-10 million bacteria, 10.000-10 million actinomycetes, 10.000-1 million fungi, algae and other microfauna. The number of that microorganism there is a role as antagonist against plant pathogen (Sastrahidayat, 2011).

Trichoderma sp. is the common soil fungi. This fungi has reported as biological agent that capable to inhibit the growth of several pathogens (Nuraini *et al*. 2013). The othe fungi that has a potention as biological agent is *Gliocladium* sp. This fungi has effective to control of *Fusarium* wilt on chrysanthemum (Hartal *et al.* 2010; Cahyani, NKMD, *et. al*). *Verticillium* sp. is the other antagonist fungi that has reported can reduce *Rhizoctonia solani* attack intensity on potato (Demirchi *et al.*, 2009).

The purpose of this research is to know the difference of antagonist fungi effetiviness *Trichoderma* sp., *Gliocladium* sp., *Verticillium* sp. on inhibiting pathogen fungi growth cause damping off disease *S. rolfsii.*

METHOD AND MATERIALS

Time and Place

The research was conducted in Mikology Laboratory, Plant Pest and Disease Departement and Glass House, Agriculture Faculty, Brawijaya University. The research was conducted from April 2014 to January 2015.

Materials and Tools

The tools that used in this research are petridish (9 cm), test tube, loopful, cork borer (3mm), stick L, erlenmeyer, ruler, scale, plastic wraping, LAFC, alumunium foil, knive, electric stove, baker glass, micropipette, sprayer, plastic 3 kg, media bottles, stationery, camera.

The materials that used in this research are PDA medium, corn meal soil medium, aquades, spirtus, alqohol, soybean seeds, tissue paper, tray, and soil sterile. *Trichoderma* sp., *Gliocladium* sp., *Verticilllium* sp., dan *S. rolfsii* fungi culture

Preparation

S. rolfsii fungi provision

S. rolfsii fungi obtained from collection of Micology Laboratory, Agriculture Faculty, Brawijaya University.

Antagonist fungi isolation

Antagonist fungi isolated from the soil in soybean rhizosfer area by water dilution plate method.

Sterilitation and planting media provision

The soil that used in this research sterilized by autoclave. After sterilitation, the soil already to inoculation by fungus.

Production of Corn meal soil medium

Put corn meal soil into bottle medium with a ratio of 1:2. The materials added by aquades until the corn meal and soil quite humid, stirred, mixture, and sterilitation.

Multiplication of antagonist fungi and pathogen

Aplication fungi on planting medium must be multiplicated on corn meal soil before. Incubation for 7-14 days until the fungi growth fulfill of the medium bottle. *S. rolfsii* applied on planting medium applied 10 gram/kg soil. For aplication antagonist fungi on planting medium each 30 grams/kg soil (Chamzurni *et al.*, 2011).

Provison of soybean seeds

The seeds that used in this research is Burangrang Variety that obtained from BALITKABI Malang.

Research Implementation

Direct oposition test antagonist fungi against *S. rolfsii* fungi

The test was carried out by inoculation antagonist fungi and pathogen fungi on PDA medium that grown within 3 cm in the center of medium. The test used completly randomized design and if there is siginificantly different, continuedwith LSD test. The direct oposition test, consist of 4 treatments and 5 replications. Observation was carried out on *S. rolfsii* fungi colony growth dan calculated by the formula (Sudantha dan Abadi, 2007):

$$I = \frac{(r1 + r2)}{r1} \text{ x } 100\%$$

I= inhibition percentage, r1= radian of pathogen colony that growth opposite to the antagonist fungi, r2= radian of pathogen colony that growth toward to the antagonist fungi.

Steam culture method antagonist fungi against *S. rolfsii* fungi

On this test, put *S. rolfsii* from the culture by cork borer (3mm) then put on the PDA medium in petridish. Made also antagonist fungi cultured on different petridish. Next step is placed the culture petridsih that containing *S. rolfsii* fungi on petridish that containing antagonist fungi. The steam culture method test test, consist of 4 treatments and 5 replications. The observation was carried out by measuring the diameter of fungi colony *S. rolfsii* (Sudantha *et al*., 2008).

Observation of pathogen fungi hyphae and antagonist fungi hyphae interaction

The interaction fungi observed by placing 2 PDA antagonist fungi and pathogen on a glass microcope slide, 5 mm apart from each other. On the first block is antagonist fungi were placed, *S. rolfsii* block was placed in the another side. After that, sterile cover glass was placed on top of 2 blocks fungi (Ortiz dan Orduz, 2000).

Kind of fungi	Inhibition percentage after inoculation (days)		
	5	6	7
Control	0,00 a	0,00 a	0,00 a
Trichoderma sp.	61,14 c	61,71 c	61,71 c
Gliocladium sp.	58,64 c	59,74 c	59,74 c
Verticillium sp.	26,05 b	30,95 b	32,00 b
LSD	7,86	6,05	5,28

Effectivity test of antagonist fungi on tray in the glass house

The glass house test, pathogen fungi and antagonist fungi culture which has multiplicated on semisynthetic medium mixed into the soil. The soil which has inoculated by fungi, inserted into tray as a soybean planting medium. There is 100 planting hole each experimental unit, the test consist of 4 treatments and 6 replications.

The observation was carried out by observing attack percentage of *S. rolfsii* on soybean. To calculate the attack percentage used the following formula (Sastrahidayat *et al*., 2013):

$$PS = \frac{a}{b} \text{ x } 100\%$$

PS= attack percentage, a= number of plant attacked, b= total number of plant

RESULT AND DISCUSSION

Inhibtion Percentage of Antagonist Fungi against Damping off disease *S. rolfsii* on Direct Oposition Test.

The result showed that the kind of fungi there is significantly different to inhibit *S. rolfsii* fungi at 5-7 days after inoculation. The highest percentage of inhibition obtained by *Trichoderma* sp, dan *Gliocladium* sp. treatments, then *Verticillium* sp. and Control tratments (Table 1). *Gliocladium* sp. fungi has an ability to growth quickly. The fungi can fulfill the petridish on 3 days, this is why the *Gliocladium* sp. can compete with the pathogen

fungi in getting the nutrients (Octriana, 2011; Howel, 2003 *in* Ramadhina, 2013).

Trichoderma sp. is the fungi that can growth quickly, and has an ability to competition with pathogen fungi in getting the growing space, nutrition, and colonizes the pathogen (Chamzurni *et al.*, 2011). The fungi can fulfill the petridish on 3 days, then in suppresing the pathogen fungi *Trichoderma* sp., is the aggresive competitors. (Sudantha dan Abadi, 2007; Sudantha, 2009).

Table 1. Percentage inhibition of antagonist fungi against *S. rolfsii* on direct oposition test

Description: Numbers in a column that followed by the same letter showed there is not significantly different based on LSD test on 5% level.

Colony Diameters of Pathogen Fungi that Placed on Antagonist Fungi Petridsih in Steam Culture Method Test

The result showed that the kind of fungi there is significantly different to pathogen colony diameter (Table 2). Pathogen fungi placed on antagonist fungi petridish, the colony diameter has a slower growth than the pathogen fungi that placed on PDA medium (without antagonist). *Trichoderma* sp. and *Gliocladium* sp. treatments provide the highest suppression based on the pathogen colony diamater. Then following by *Verticillium* sp., and control treatments on 7 days after inoculation.

The pathogen colony growth can be stoped, if the colony placed on *Trichoderma* sp. and *Gliocladium* sp. petridih. The fungus has an antibiotic compounds that can evaporate to the air, then can inhibit and stop the colony pathogen growth. The antagonis fungus produce an antibiotic compunds or alkaloid that is volatile, and can inhibit colony patogen growth (Sudantham 2009). *Trichoderma* sp. dan *Gliocladium* sp. produce a various of secunder metabolism, and some of the secunder metabolism can inhibit the other microorganism without phsically direct contact, the substances are known as antibiotic and the commonly known of that antibiotics are gliotoxin, viridin dan gliovirin (Harman dan Kubicek, 1998).

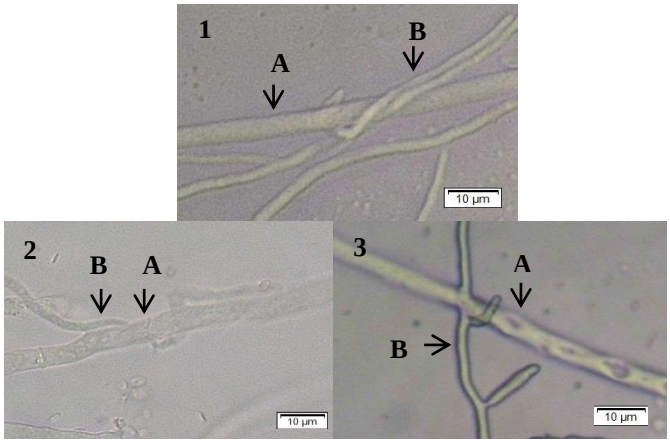
Table 2. Colony diameter averages of pathogen fungi that placed on antagonist fungi petridish 7 days after inoculation

Kind of fungi	Pathogen colony diameter (cm)
Control	9,00 c
Trichoderma sp.	3,70 a
Gliocladium sp.	3,24 a
Verticillium sp.	6,82 b
LSD	0,86

Explanation: Numbers in a column that followed by the same letter showed there is not significantly different based on LSD test on 5% level.

Interaction of Antagonist Fungi Hyphae and S. *rolfsii* Hyphae

The result showed that interaction of antagonist fungi hyphae and *S. rolfsii* hyphae under the microcope, each of fungal hyphae interacting by interact directly on pathogen fungi hyphae *S. rolfsii*. *Trichoderma* sp. hyphae looks wrapped or twisted on pathogen hyphae *S. rolfsii*. The result also showed that *Gliocladium* sp. hyphae that smaller than pathogen hyphae looks coil around pathogen hyphae *S. rolfsii*. Whereas betwen *Verticillium* sp. and *S. rolfsii* there is only looks the contact between antagonist hyphae and pathogen hyphae (Picture 1).



Picture 1. Microscopic appearance of pathogen fungi *S. rolfsii hyphae* and antagonist fungus hyphae interaction.

Description:
1. *S. rolfsii* hyphae twisted by *Trichoderma* sp. hyphae. (A) *S. rolfsii* hyphae, (B) *Trichoderma* sp. hyphae
2. *Gliocladium* sp., hyphae that smaller than *S. rolfsii* hyphae coil around the *S. rolfsii*hyphae.(A) *S. rolfsii* hyphae, (B) *Gliocladium* sp. hyphae

3. There is looks contact between *Vertcillium* sp., hyphae and *S. rolfsii* hyphae. (A) *S. rolfsii* hyphae, (B) *Verticillium* sp. hyphae

Sudantha (2009) reported that commonly the mechanism of *Trichoderma* sp. in inhibiting the pathogen growth by microparasites and competition. That claim is supported by Weindling, 1932 (*in* chamzurni *et al.*, 2011) *Trichoderma* sp. tested against *Rhizoctonia solani*, the mechanism that occur is antagonis fungi twisted, hitch, and then growing within the hyphae of *R. Solani*, then unload the hyphae of *R. Solani*, and the hyphae be empty. Coles *et al.* (2014) reported that *Gliocladium* sp. fungi proven as parasitical against pathogen of *Alternaria radicana*, initially the antagonist fungi spore were clustered on the surface of conidia germ tubes and hyphae of *A. Radicana*, then germ tubes of antagonist fungi had penetrated the hyphae of *A. Radicana*, by 10 days *Gliocladium* sp. fungi was growing within the hyphae of *A. Radicana* and the hyphae walls of *A. Radicana* appeared to be damaged.

Percentage of *S. rolfsii* Attack on Antagonist Test in The Glass House

The result showed that the kind of fungi there is significantly different to attack percentage of *S. rolfsii* fungi on 2-8 days afterr planting (Table 3). That table showed that control and *Verticillium* sp. treatments have the highest attack percentage on 2-8 days after planting. Percentage of the lowest attack were *Trichoderma* sp. and *Gliocladium* sp. treatments on 2-8 days after planting.

Trichoderma sp. and *Gliocladium* sp. fungus have an ability to reduce the attack percentage of damping-off disease caused by *S. roflsii* on soybean in the glass house. That claim is supported by Soenartiningsih (2010); Agustina *et al.* (2012); Ramadhina *et al* 2013); Chamzurni *et al.*,(2011); Hartal *et al.*, (2007) and Pratiwi *et al.*, (2013) that *Trichoderma* sp. and *Gliocladium* sp. can reduce the attack percentage of some of palnts pathogen. The mechanisms which has described above suspected to be one of the factors that can reduces the attack percentage of damping-off disease on soybean in the glass house. The mechanism of antagonist fungus were classified to some mechanism, there were competition, microparasites, and antibiosis. According to Griffin (1972) on in-vivo condition there is not possible these component of mechanism antagonist can work independently.

Table 3.Attack percentage of *S. rolfsii* fungi

Kind of fungi	Attack percentage (days)			
	2	4	6	8
Kontrol	88,83 b	94,33 b	96,50 b	98,00 b
Trichoderma sp.	68,33 a	54,50 a	52,67 a	51,67 a
Gliocladium sp.	75,67 a	54,33 a	53,50 a	52,83 a
Verticillium sp.	91,67 b	91,00 b	91,00 b	91,00 b
LSD	11,65	12,77	13,33	13,41

Explanation: Numbers in a column that followed by the same letter showed there is not significantly different based on LSD test on 5% level.

CONCLUSSION

Based on the result and discussion, it can be concluded that Trichoderma sp. and Gliocladium sp. can significantly inhibit the growth of damping-off disease on soybean caused by *S. rolfsii*. Whereas, *Verticillium* sp. can not inhibit the attack percentage of *S. rolfsii* on soybean.

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