

CONTROL OF "DAMPING OFF" DISEASE CAUSED BY *Sclerotium rolfsii* SACC. USING ACTINOMYCETES AND VAM FUNGI ON SOYBEAN IN THE DRY LAND BASED ON MICROORGANISM DIVERSITY OF RHIZOSPHERE ZONE

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ABSTRACT

One of the obstacles in the efforts to increase soybean production in Indonesia is disease such as damping off which is caused by *Sclerotium rolfsii*. In East Java, the intensity of *S. Rolfsii* reached approximately 8.61% that spread all over Indonesia region, even in our neighbor countries such as Malaysia, Thailand and the Philippines. This research was carried out to determine the efficacy of Actinomycetes and VAM (Vesicular Arbuscular Mycorrhizal) against damping-off attack and the diversity of microorganisms in rhizosphere. Research conducted in the laboratory and screen house on Plant Protection Department, Faculty of Agriculture, University of Brawijaya and in Lawang District Malang. Observation variables include level of pathogen attack and infection rate of damping-off pathogen. Plant height, number of pods, pod weight, seed weight and weight of 100 seeds from each treatment, diversity and identification of microorganisms in rhizosphere were also observed. The results showed that Actinomycetes and VAM application could decrease the percentage of plant death due to damping-off. Application of Actinomycetes and VAM gave effect on microorganism diversity of Ratai Rhizosphere but not on Wilis.

Keywords: *Sclerotium rolfsii*, vesicular arbuscular mycorrhizal, actinomycetes, and rhizosphere

up to 2.2 million tons per year. Only 20 to 30% of the demand can be supplied by domestic product, while 70 to 80 % depends on the import. It is important to increase soybean production in Indonesia. However there are several obstacles that could hamper efforts to increase national soybean production, and one of the obstacles is disease outbreak.

One of the diseases of soybean plant is damping off caused by *Sclerotium rolfsii*. In East Java, the intensity of *S. rolfsii* reached to 8.61%. *S. rolfsii* attack the soybean seedling and cause damping off disease on early age. Difficulty on damping off disease control is the presence of primary inoculum on dormancy stage in soil. In the soil, this *Sclerotium* can survive until one year or more and still has the viability to grow and germinate when there is a host plant (Fitchner, 2009).

Some microbes like Actinomycetes, bacteria and fungi can be implemented to control plant pathogens. Actinomycetes can produce secondary metabolites which are biologically active, especially as antibiotics (Loria *et al.*, 1997). VAM can increase the minerals absorption and alter the structure and biochemical aspects of root cells. The effects of these changes are a healthier plant, resistance to stress environment and tolerant (Linderman, 1994; Muhibuddin, 2008). This research aims to observe the influence of application of Actinomycetes and VAM against the attack of *S. rolfsii* and diversity of microbes on soybean plant rhizosphere.

INTRODUCTION

Soybean is a major agricultural commodity in Indonesia. National demand of soybean reached

MATERIALS AND METHODS

The research was conducted at the Laboratory of Mycology, Pest and Disease of Plant

Ika Rochdjatun Sastrahidayat *et al.*,: Control of "Damping OFF" Disease.....

Department, Faculty of Agriculture, University of Brawijaya and at rice field at Bedali Village, Lawang District-Malang in June 2010.

Two treatments were: inoculation of Actinomycetes + VAM and control (without inoculation Actinomycetes + VAM). Two soybean varieties such as Ratai and Wilis were used. Each treatment was repeated five times. The data were analyzed by t- test in 95% confidence level.

Mass Production of Actinomycetes and VAM

Actinomycetes isolates were obtained from collection of Ika Rochdjatun Sastrahidayat, Mycology Laboratory, Faculty of Agriculture, University of Brawijaya, firstly duplicated and regenerated on PDA (Potato Dextrose Agar) medium and than incubated for five days. After the incubation, the fungi were transferred in an oatmeal-sand (OS) medium. Pure culture of Actinomycetes on PDA substrate was taken five pieces using cork borer (Ø 5 cm disk) and then inserted into the OS medium. Those inocula were incubated at room temperature for approximately two weeks before they were ready to use.

VAM propagation was done by using corn plant as a host on in vivo medium. Mycorrhizal spores will breed as many as 1-2 spores inoculated (2 cm below where the seed has been planted). Upon 45 days after planting, the media will contain many mycorrhizal spores and can be used for the observation. Each inoculum was made in tablet. Each tablet weighs 2 grams mycorrhizal with mycorrhizal spore content of 50-10.

Field Preparation

Field used in this research is an endemic land. Firstly, field was processed by clearing. Every plot size is 4 x 5 m with 30 cm height. The distance between the plot was made as wide as 40 cm of drainage channels.

Seed Preparation

Soybean seed varieties which were used in this research were obtained from Indonesian Legumes and Tuber Crops Research Institute, Kendalpayak-Malang.

Inoculation of VAM and Actinomycetes on Soybean Seed

Soybean seeds were soaked by using machine in a suspension of Actinomycetes for

four hours and planted in the field. VAM inoculation was conducted simultaneously with seed planting.

Cultivation

The planting was conducted two weeks after soil treatment. Seeds were planted with the space of 40 x 15 cm with the treatment namely Actinomycetes combined with VAM inoculation or without inoculation.

Plant Handling

Plant handling was performed by the same standards in all treatments, such as: fertilizing, weeding, watering and pest management. For each treatment, fertilizer recommendations are adjusted with a dose of soybean cultivation. Recommendations of fertilizer used per hectare were 50 kg Urea, 100 kg SP36, and 100 kg KCl. Nitrogen fertilizer (urea) was performed twice, each was given with half of the dose of treatment. Fertilization was carried out during the first planting and the second was to be done four weeks after planting. P and K fertilizer was given along with N (Urea) fertilization, each with a dose of 100 kg/ha SP36 and 100 kg/ha KCl.

Variables Observed

1. Damping-off and infection rate (r) were calculated by counting total plant number which was divided by population of infected plants in each treatment, and this observation was conducted every six days.
2. Growth of the plants includes plant height. The number of plant samples used was 13 plants per plot. The variables observed were the number of pods (filled and empty pods), pod weight, seed weight and weight of 100 seeds from each treatment. Observations of plant yield such as seed weight after going through the drying process were done by drying the beans on the drying tub for 3-7 days, but this depends on the quality and quantity of sunlight.
3. Variety of microbes in rhizosphere was calculated at each soil sampling. According to Waluyo (2005) Microbial population of each soil sample (g) can be calculated using the following formula:

$$P = M \times \frac{1}{10^{-4}}$$

Remarks =

P = Population diversity of microbes per gram of soil

M = Number of colonies per plate dish

4. Isolation of fungi from soil samples uses "soil dilution plate method". 1 ml of soil dilution was poured into PDA media on plate dish. Purification was performed on each microbes colony based on the morphology including color and colony shape differentiation. Each of these microbes was taken with a needle ose, and then grown again in the plate dish containing solid medium PDA. Identification of the genus was based on Illustrated Genera of Imperfect Fungi and Bergey's Manual of Determinative Bacteriology.

RESULTS AND DISCUSSION

Percentage of the Death Plant caused by *Sclerotium rolfsii*

The results indicate that percentage of the death plant due to *S. rolfsii* soybean on both varieties was not significantly different at the 9th, 15th and 21st day after planting (dap), whereas at the age of 27, 33, 39 and 45 dap, it respectively showed significant difference on RAM (Ratai Actinomycetes + VAM) compared to RK (Ratai – Control treatment). On Willis variety at the age of 9, 21, 27, 33, 39 (dap), it did not show significant difference, but it did at 15 dap (Table 1).

Table 1 showed that in the first observation (7 dap) there was no difference on plant death caused by *S. rolfsii* between RAM and RK

treatment. Pathogen attack appears at 15 dap which is showed with the number of dead plants on RK treatment which is more than those on RAM. The percentage of plant deaths on the control plants continues to grow and stops at 45 dap while the addition of percentage mortality on RAM treatment plants stops after 27 dap.

Willis looks at the varieties where the average percentage of plant mortality between WAM (Willis Actinomycetes + VAM) and WK (Willis Control) at the age of 9 dap result is the same, each amounting to 0.15% and at the age of 15 dap average percentage of plant mortality was lower WAM WK than the average value of the percentage of plant death WAM of 0.30%, but not lower than 1.67% WK. At the age of 33 dap, WAM average percentage mortality was 6.89% and amounted to 10.30% WK, and the value is fixed until 45 dap. At the end of observation, the percentage of dead plants in soybean varieties Ratai and Willis showed the number of dead plants more than the control.

The low average percentage of deaths caused by *S. rolfsii* on soybean crop Willis varieties is allegedly due to the interaction antagonism in getting the nutrients which increased plant resistance against *S. rolfsii*. Because of the biological agent, at the beginning of planting Actinomycetes, VAM provides an opportunity for more flexibility in competing in the infected seeds. Actinomycetes as the biological agent of *S. Rolfsii* has mechanisms to inhibit the growth of *S. rolfsii* allegedly due to producing antibiotics and enzymes such as chitinase hydroxamate-siderophores.

Table 1. Plant mortality percentage due to *S. rolfsii*

Variety	Treatment	Percentage of plant deaths (%) in the Age of Plants (dap)						
		9	15	21	27	33	39	45
Ratai	Actinomycetes + VAM	0.00 a	0.76 a	4.85 a	6.67 a	6.67 a	6.67 a	6.67 a
	Control	0.00 a	1.36 a	6.82 a	9.24 b	9.47 b	9.85 b	9.85 b
Willis	Actinomycetes + VAM	0.15 a	0.30 a	4.92 a	6.44 a	6.89 a	6.89 a	6.89 a
	Control	0.15 a	1.67 b	7.42 a	9.32 a	10.30 a	10.38 a	10.38 a

Remarks = Number followed by same letter in same column are not significantly different at 95%t test level

Widyastuti (2003) stated that cell wall of *S. rolfsii* contains 1,3-glucans and chitin. Chitinase is an enzyme that can hydrolyze chitin

and khitodekstrin. Actinomycetes can utilize chitin as a source of carbon and nitrogen. Goodfellow and Williams (1983 *in* Khamna *et al.*,

2008) added that Actinomycetes can protect roots by inhibiting the growth of potentially pathogenic fungi by producing enzymes that degrade fungal cell walls or produce anti-fungal compounds (antibiotics). Oskay *et al.*, (2004) has reported that 10 of agricultural soil samples obtained 50 isolates of Actinomycetes. Approximately 34% (17 isolates) produces broad and narrow spectrum antibiotics. Muller *et al* (1984); Muller and Raymond (1984); Tokala *et al* (2002) in Khamna *et al.* (2008) reported that Streptomyces produces hydroxamate-siderophores that can inhibit the growth of fitopatogen in the competition for iron element in rhizosphere. VAM (*Glomus* sp.) as a biological agent can protect the root zone of plants by infecting the cortex of soybean root zone of plants and helps plants absorb nutrients needed by plants, thereby increasing plant resistance to *S. rolfsii* by releasing compounds that can prevent infection by pathogens such as lignin, phenol, and fitoaleksin.

Caron (1989) stated that mycorrhizae infection could increase the resistance of plants against pathogen attack through increase plant nutritional absorption ability, reduce environmental stress on plants and improve soil microorganisms diversity. VAM can also hold up an availability of nutrients for pathogen (Elsen *et al.*, 2003; Muhibuddin, 2007).

Caron (1989) observed that VAM may improve response of plant root system against pathogens. Tomato plants inoculated with VAM were able to boost tolerance to *Fusarium oxysporum* f.sp. *lycopersici* by increasing the synthesis of lignin during metabolism process (Caron, 1989). Accumulation of compounds

such as phenolic and hormones fitoaleksin is as defense response system of healthy plant (Elsen *et al.*, 2003). Caron (1989) has been testing that the compound inoculated by VAM could inhibit *S. rolfsii* growth under in vitro treatment.

Effect of Actinomycetes and VAM Application on the Diversity of Rhizosphere Microorganisms

The results of t-test showed that the diversity of microorganisms in rhizosphere Ratai variety did not differ significantly at samples 1 and 2, while among three samples it was shown that there was significant difference between RAM and RK. Observation on Wilis variety did not differ significantly at samples 1, 2 and 3 on treatment WAM and WK.

Table 2 showed that there was no differences between both microbial populations at sample 1 and 2, while the 3rd sample shows that there was a significantly difference. The mean diversity in Ratai and Wilis which is applied by VAM and Actinomycetes is higher than control treatment.

Linderman (1994) stated that VAM which is associated to plant can change the region (mycorrhizosphere) i.e. rhizosphere areas. This is affected by the presence of VAM on rhizosphere. Actinomycetes has an ability to decompose the organic materials which is very important for other microorganisms, while the VAM can be a symbiotic microorganism during N fixation process by bacteria. Thus, it can increase the availability of N and P in the soil. It is also known that VAM hyphae can produce exudates which stimulate the availability of some required nutrients.

Table 2. Diversity of microorganisms in both varieties

Variety	Treatment	Diversity of Microorganisms Rhizosfer (coloni/g)		
		Sample 1	Sample 2	Sample 3
Ratai	Actinomycetes + VAM	2.0 x 10 ⁴ a	3.3 x 10 ⁴ a	3.3 x 10 ⁴ a
	Control	1.6 x 10 ⁴ a	2.7 x 10 ⁴ a	1.0 x 10 ⁴ b
Wilis	Actinomycetes + VAM	2.0 x 10 ⁴ a	3.3 x 10 ⁴ a	2.7 x 10 ⁴ a
	Control	1.6 x 10 ⁴ a	2.7 x 10 ⁴ a	2.0 x 10 ⁴ a

Remarks= Number followed by same letter in same column are not significantly different at 95%t test level

Kanti (2005) stated that Actinomycetes has a role as a saprophyte decomposing organic material microorganism which is essential to increase soil fertility. Suciati (2006) observed that the soil condition could stimulate the

increasing of soil fungal populations, because the fungus requires humus particles. Rillig (2004) also proved that either directly or indirectly the presence of VAM could change the composition of microbes that was directly

involved in ecosystem processes. The microorganisms found in the table are shown below.

Table 3. Microorganisms found on Ratai

Spesies	Ratai Actinomycetes +VAM	Ratai Control
<i>Penicillium</i> sp	+	+
Not identified fungi no. 1	+	+
Not identified fungi no. 2	+	+
<i>Penicillium</i> sp isolat 2	+	+
<i>Streptomyces</i> sp	+	-
<i>Sclerotium rolfsii</i>	-	+
<i>Aspergillus</i> sp	+	+
<i>Trichoderma</i> sp	+	+

Remarks= (+) is found, (-) not found

Effect of Actinomycetes and VAM Application on the Growth and Yield of Soybean

The results showed that plant height in Ratai in RAM treatment was significantly different to RK on 24, 36, 48 and 60 dap, but it was not significantly different on 12 dap. Meanwhile, plant height in Wilis by WAM treatment was significantly different to WK at all time observation. The mean plant height is presented in Table 5 and the average production is in Table 6.

Table 5. Plant height in soybean plant

Variety	Treatment	Plant height (cm) at the age of the plant (dap)				
		12	24	36	48	60
Ratai	Actinomycetes+VAM	8.62 a	16.25 a	25.52 a	47.27 a	77.86 a
	Control	8.51 a	15.18 b	25.45 b	43.83 b	72.26 b
Wilis	Actinomycetes+VAM	9.40 a	16.78 a	27.94 a	47.75 a	70.76 a
	Control	8.62 b	15.32 b	23.74 b	42.86 b	65.77 b

Remarks= Number followed by same letter in same column are not significantly different at 95% test level

Table 4. Microorganisms found on Wilis

Spesies	Wilis Actinomycetes +VAM	Wilis Control
<i>Aspergillus</i> sp	+	+
Actinomycetes isolat 1	+	-
Actinomycetes isolat 2	+	-
<i>Penicillium</i> sp isolat 1	+	+
<i>Penicillium</i> sp isolat 2	+	+
<i>Penicillium</i> sp isolat 3	+	+
<i>Trichoderma</i> sp isolat 2	+	+
<i>Trichoderma</i> sp isolat 2	+	+
Not identified fungi no. 1	+	+

Remarks: (+) is found, (-) not found

Table 5 showed that the average of plant height was not significantly different on RAM than RK at the age of the plant 12 dap, but significantly different at the others. On the other hand, it was significantly different on WAM and WK at all time observation. It was also known that treatment of Actinomycetes + VAM grows better than the untreated.

Table 6. Production of soybean plant

Variety	Treatments	Growth and crop production					
		Pod Number	Total empty pod	Pod weight (g)	Shoot weight (g)	Seed dry weight (g)	Weight of 100 seeds (g)
Ratai	Actinomycetes + VAM	121.49 a	33.36 a	38.65 a	58.62 a	24.60 a	7.85 a
	Control	77.5 b	47.26 b	30.70 b	48.91 b	18.50 b	7.66 b
Wilis	Actinomycetes + VAM	69.13 a	36.14 a	37.48 a	50.71 a	22.71 a	10.81 a
	Control	47.63 b	29.92 b	24.54 b	34.69 b	16.55 b	10.22 b

Remarks= Number followed by same letter in same column are not significantly different at 95% test level

Based on the results, it is found that yield in treatment applied by Actinomycetes + VAM was significantly different from the untreated on the number of pod, empty pod, pod weight, shoot weight, seed dry weight, and 100 seeds weight.

Mujoko (2005) stated that inoculation of *Streptomyces* on early growth would increase plant growth. Kumalawati (2006) explained that mycorrhizal fungi interact with some kind of soil organisms. This interaction can be inhibited or spurred, some others are competitive and mutualistic. Sometimes VAM infection would give a negative influence on plant growth because of the competition on product of assimilation at the early plant growth.

According to Kanti (2005), Actinomycetes produces some active cellulase enzymes which degrade cellulose. Actinomycetes can enhance plant growth by producing a promoter such as indole 3-acetic acid (IAA) and produce siderophores to help root growth and improve nutritional intake respectively (Khamna *et al.*, 2008). Siderophores is also important for plant as an iron source. In this research, Mujoko (2005) also proved that application of Actinomycetes by suspension soaking can increase the fruit's weight and number of tomatoes. Actinomycetes can stimulate plant growth enzyme, such as auxin and gibberellin.

VAM has an important role in providing and improving nutrition for plant physiological processes that can perform well. According to Turk *et al.* (2006), VAM symbiosis increases plant growth by increasing nutrients absorption, substances production, drought and salt tolerance, and may be synergistic with the beneficial microbes in the binding of N and P. Turk *et al.*, (2006) also proved that VAM hyphae can extend the plant root zone, thus it increases

the root area and the micro-nutrients absorption. The results of Sheng *et al.*, (2008) explained that the VAM can improve the process of photosynthesis in leaves. Sastrahidayat research (1997) on several crops such as corn, onion, watermelon, soy, chili pepper and tomato showed that plants inoculated with mycorrhizal fungi provide growth and better yields than uninoculated plants.

CONCLUSIONS

Inoculation of Actinomycetes and VAM on endemic soil can reduce the plant death percentage, damping-off disease rate on both Ratai and Wilis, and increase. Inoculation of Actinomycetes and VAM on Wilis did not significantly affect the diversity of micro-organisms but it did on Ratai.

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