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THE POSSIBILITY OF CONTROLLING *SCLEROTIUM ROLFSII* ON SOYBEAN *(GLYCINE MAX)* USING *TRICHODERMA* AND TEBUCONAZOLE*)

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ABSTRACT

The possibility of controlling S. *rolfsii* on soybean *(Glycine max)* var. Rinjani using *T. aureoviride* and Tebuconazole under field conditions was studied. The experiment was conducted at the experimental plot of SEAMEO BIOTROP.

The pathogen was mixed with the soil (2 kg/plot) 4 days before the inoculation of the antagonist (2.25 kg/plot). The measurement of each plot was 2.5 x 6 m^2. N, P and K (120 kg/ha) were applied at the same day with the inoculation of the pathogen.

Soybean seeds were planted 7 days after the inoculation of the antagonist. The distance between plants and between plots were 20 and 40 cm, respectively.

The fungicide at concentration of 100 g/ha *(in vitro* concentration) and 210 g/ha (field or recommended concentration) were applied using 2 methods, i.e. 1) spraying on the planting hole at the same day as the planting of soybean seeds, and 2) spraying on the soil surrounding the plants 7 days after planting. Soils that were neither inoculated with the antagonist nor the fungicide were used as controls. Three replications (3 plots) were used for each treatment (including the control).

The results showed that the inoculation of the antagonist, the concentrations of the fungicide, and time of application gave very significant differences in the percentages of the plants infected by the pathogen and significant differences in seed production; while the interaction between the inoculation of the antagonist and the concentrations of the fungicide, between the concentrations of the fungicide and the time of application, and between the inoculation of the antagonist, the concentrations of the fungicide and the time of application did not give significant differences either in the percentages of the plants infected by the pathogen or seed production.

The percentage of plants infected by the pathogen was lower on soil inoculated with the antagonist (31.6%) than on soil not inoculated with the antagonist (52.9%).

The percentage of plants infected by the pathogen was lower on soil treated with the fungicide either at *in vitro* concentration (37.5%) or at field concentration (37.4%) than on the soil not treated

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with the fungicide (61.5%). Nevertheless, based on statistical analysis, the fungicide at *in vitro* concentration was not significantly different from that at field concentration.

The percentage of plants infected by the pathogen on the soil sprayed with the fungicide at the same day of seed planting was lower (30.5%) than sprayed 7 days after planting (44.4%).

The seed production on the soil inoculated with the antagonist (1893.3 kg/ha) was higher than on the soil not inoculated with the antagonist (1465.7 kg/ha).

The production on the soil sprayed with the fungicide either at *in vitro* (1758.0 kg/ha) or at field concentration (1817.1 kg/ha) was higher than on the soil not sprayed with the fungicide (1247.2 kg/ha).

The production on the soil sprayed with the fungicide at the same day of seed planting (2010.9 kg/ha) was higher than sprayed 7 days after planting (1564.2 kg/ha).

The combination between the inoculation of the antagonist and the fungicide application at *in vitro* concentration at the same day of seed planting gave higher seed production (2391.2 kg/ha) than the inoculation of the antagonist (1711.7 kg/ha) or the fungicide application either at *in vitro* concentration (1771.9 kg/ha) or at field concentration (1939.1 kg/ha) at the same day of seed planting. However, based on statistical analysis, the interaction among the three treatments (the antagonist, the concentrations of the fungicide, and the time of application) was not significantly different.

Keywords: *Sclerotium rolfsii, Glycine max, Trichoderma,* Tebuconazole, Antagonist

INTRODUCTION

Sclerotium rolfsii is a soil-borne fungal pathogen that can cause root rot and damping-off of crops, among others, soybean *(Glycine max)* (Agrios 1988).

One of the control methods of the pathogen is by using antagonistic fungi (Cook and Baker 1983).

Some soil fungi especially *Trichoderma* have been reported to be potential biocontrol agents of soil-borne fungal pathogens.

According to Upadhyay and Mukhopadhyay (1986) *T. harzianum* was able to control the disease of sugarbeet seedlings as high as 88% under greenhouse conditions. Under field conditions, integration of PCNB (Pentachloronitro-benzene) and *T. harzianum* significantly reduced the incidence of *Sclerotium* root rot (76% disease control) and increased the root, green foliage and sucrose yield per ha.

Cole and Zvenyika (1988) reported that *T. harzianum* integrated with Triadimenol fungicide enhanced disease control in tobacco caused by other soil-borne fungal pathogens, i.e. *Rhizoctonia solani* and *Fusarium solani.*

Under greenhouse conditions, four strains of *T. harzianum* suppressed damping-off of snapbean caused by S. *rolfsii* (Papavizas and Lewis 1989).

Tebuconazole (Folicur 250 EC) is a fungicide that can control the pathogen (Bayer 1990).

The objective of the study is to determine the effect of *Trichoderma* combined with Tebuconazole to control S. *rolfsii* on soybean.

MATERIALS AND METHODS

This research was conducted at the experimental plot of BIOTROP, Bogor, Indonesia. The soil type is latosol (pH ± 5).

Soybean variety, isolates of *S. rolfsii* and *Trichodernna,* and fungicide

S. rolfsii isolate BIO-1 and soybean variety Rinjani which was the most susceptible variety to the pathogen, were used in this study (Dharmaputra and Retnowati 1992).

T. aureoviride BIO-5 was used as antagonist because it caused the highest percentage of inhibition to the pathogen on Potato Dextrose Agar (PDA) of pH 5 (Dharmaputra and Retnowati 1992).

Tebuconazole was used as a fungicide to control the pathogen (Bayer 1990).

Preparation of pathogen and antagonist inocula

Inoculum of the pathogen was prepared based on Riker and Riker (1936), while the inoculum of the antagonist was based on Dharmaputra and Suwandi (1989).

For the preparation of the inoculum of the pathogen, a mixture of sand : corn : water (2:2:3) was put in plastic bags (2.5 kg/bag), sterilized in an autoclave for 1 h, and then incubated at room temperature for one night.

For the preparation of the inoculum of the antagonist, a mixture of sand : husk : water (2:4:5) was treated in the same manner as the preparation of the pathogen's inoculum.

Five pieces of the pure cultures (5 mm in diameter each) of the pathogen and the antagonist (3 days old on PDA) were grown on each medium. They were then incubated at 28 °C for 7 days.

Inoculation of the pathogen and the antagonist, application of the fungicide, and planting of soybean

The pathogen was inoculated 4 days before the inoculation of the antagonist. It was mixed homogeneously on the surface of the soil in each plot (2.5 x 6 m^2). The inocula of the pathogen and the antagonist were 2 and 2.25 kg/plot, respectively.

N, P and K (120 kg/ha) were given at the same day as the inoculation of the pathogen.

Soybean seeds were planted 7 days after the inoculation of the antagonist (AIA). The distance between plants and between plots were 20 and 40 cm, respectively.

Tebuconazole at concentrations of 100 g/ha *(in vitro* concentration) and 210 g/ha (field concentration) were applied using 2 methods:
a) spraying in the planting hole at the same day as planting of soybean seeds
b) spraying on the soil surrounding the plants (7 days after planting).

Soils that were neither inoculated with the antagonist nor the fungicide were used as controls.

Three replications (3 plots) were used for each treatment (including the control).

Observation of the percentage of infected plants was carried out 14 days after planting (DAP), and of the soybean production at 90 DAP.

Factorial in Randomized Completely Block Design (RCBD) was used in this study consisting of 3 factors: a) the antagonist (not inoculated and inoculated with *T. aureoviride* BIO-5), b) the concentration of Tebuconazole (0, 100 and 210 g/ha), and c) the time of Tebuconazole application.

RESULTS AND DISCUSSION

Percentage of plants infected by the pathogen

Analysis of variance showed that the inoculation of the antagonist and the concentrations of the fungicide gave very significant differences in the percentage of plants infected by the pathogen; the time of fungicide application gave significant difference. The interaction between the inoculation of the antagonist and the concentrations of the fungicide; the concentrations of the fungicide and the time of fungicide application; among the inoculations of the antagonist, the concentrations of the fungicide and the time of fungicide application did not give any significant difference (Table 1).

Infected and non-infected plants at 14 DAP are presented in Figure 1. The percentage of plants infected by the pathogen was lower (34.1%) and significantly different on the soils inoculated with the antagonist than on the soils not inoculated with the antagonist (56.8%) (Table 2). The percentage of plants infected by the pathogen was lower on the soil sprayed with the fungicide either *in vitro* (37.5%) or field (37.4%) concentration compared to the soil not sprayed with the fungicide (61.5%). Nevertheless, the fungicide application at *in vitro* concentration did not differ significantly from field concentration.

The percentage of plants infected by the pathogen was lower and significantly different when the fungicide was applied at the same day as the planting of soybean

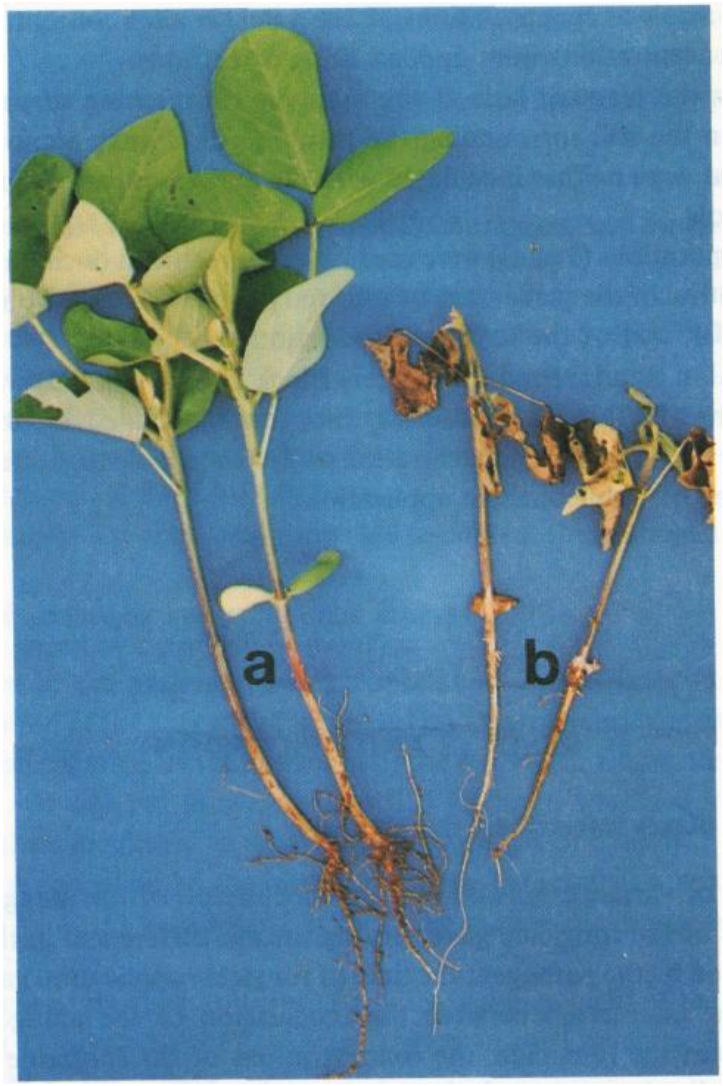

Figure 1. Non-infected (a) and infected (b) plants by *Sclerotium rolfsii* 14 DAP

Table 1. Analysis of variance on the effect of *Trichoderma aureoviride* BIO-5, Tebuconazole and the time of fungicide application on the percentage of plants infected by *Sclerotium rolfsii*

Source of var.	Df	SS	MS	F-value
A	1	4646.688	4646.688	35.840**
C	2	4624.055	2312.027	17.833**
T	1	774.688	774.688	5.975*
A x C	2	334.068	167.031	1.288
C x T	2	438.391	219.195	1.691
A x C x T	2	11.047	5.523	0.043
Block	2	30.383	15.191	0.117

A = the antagonist
C = the concentrations of the fungicide
T = the time of fungicide application
A x C = the interaction between the antagonist and the concentrations of the fungicide
C x T = the interaction between the concentrations of the fungicide and the time of fungicide application
A x C x T = the interaction among the antagonist, the concentrations of the fungicide and the time of fungicide application
* = significantly different at 95% confidence level
** = very significantly different at 99% confidence level

Table 2. The effect of the inoculation of *Trichoderma aureoviride* BIO-5, the concentrations of Tebuconazole and the time of fungicide application on the percentage of plants infected by *Sclerotium rolfsii*

Effect	Percentage of infected plants*)
T. aureoviride BIO-5	
Inoculated	34.1 a
Not inoculated	56.8 b
Concentrations of Tebuconazole (g/ha)	
0	61.5 c
100	37.5 d
210	37.4 d
Time of fungicide application	
At the same day with the planting of soybean seeds	40.8 e
7 days after planting	50.1 f

*) Numbers followed by the same letter do not differ significantly according to DMR Test at 95% confidence level

seeds (40.8%) than when the fungicide was applied 7 DAP (50.1%) (Table 2). It was assumed that the plants were already infected by the pathogen before spraying.

It is interesting to note that the percentage of infected plants on soil sprayed with the fungicide at *in vitro* concentration combined with the inoculation of the antagonist (SFT_1A_1 = 20%) was lower than on soil sprayed with the fungicide at field concentration (SF_2T_0A = 35.3%) (Table 3), but based on statistical analysis the interaction among the inoculation of the antagonist, the concentrations of the fungicide and the time of fungicide application did not give any significant difference (Table 1).

Figure 2 shows the plants on soil inoculated with the pathogen 14 DAP; while Figure 3 shows the plants inoculated with the pathogen and sprayed with the fungicide at field concentration (210 g/ha) at the same day of seed planting.

The plants on soil inoculated with the pathogen, sprayed with the fungicide at concentration of 100 g/ha (in *vitro* concentration) at the same day of seed planting, and inoculated with the antagonist is presented in Figure 4.

According to Upadhyay and Mukhodhyay (1986), a combination of PCNB with *T. harzianum* was able to control basal stem rot in sugar beet caused by *S. rolfsii* up to 76%.

Seed production

Analysis of variance showed that the inoculation of the antagonist and the concentrations of the fungicide gave very significant differences in seed production; the time of fungicide application gave significant difference. The interaction between the inoculation of the antagonist and the concentrations of the fungicide; the concentrations of the fungicide and the time of fungicide application, and among the inoculation of the antagonist, the concentrations of the fungicide and the time of fungicide application did not give any significant differences (Table 4).

The seed production of the soil inoculated with the antagonist was higher and significantly different (1863.3 kg/ha) than that of the soil not inoculated with the antagonist (1351.8 kg/ha) (Table 5).

According to Elad *et al.* (1980) *T. harzianum* was able to control the disease on bean, cotton or tomato caused by *S. rolfsii* and *Rhizoctonia solani.* The antagonist was also able to increase the production of bean.

The soybean production of the soil sprayed with the fungicide either at in *vitro* concentration (1758.0 kg/ha) or at field concentration (1817.1 kg/ha) was significantly different from that not sprayed with the fungicide (1247.2 kg/ha) (Table 5). Nevertheless, the soybean production of the soil sprayed with fungicide at field concentration was higher than that of the soil sprayed with the fungicide at *in vitro* concentration.

Table 3. Percentage of soybean plants infected by *Sclerotium rolfsii* with different treatments

Treatment	Percentage of infected plants
SF_0T_0	76.3
SF_0T_1	46.7
SF_1T_0A	44.0
SF_2T_0A	35.3
SF_1T_1A	20.0
SF_2T_1A	22.7
SF_1T_0B	54.7
SF_2T_0B	54.3
SF_1T_1B	31.3
SF_2T_1B	37.3

S = soil inoculated with *S. rolfsii*
F_0 = soil not sprayed with Tebuconazole
F_1 = soil sprayed with Tebuconazole at *in vitro* concentration (100 g/ha)
F_2 = soil sprayed with Tebuconazole at field concentration (210 g/ha)
T_0 = soil not inoculated with *T. aureoviride* BIO-5
T_1 = soil inoculated with *T. aureoviride* BIO-5
A = soil sprayed with the fungicide at the time of planting
B = soil sprayed with the fungicide 7 days after planting

Table 4. Analysis of variance on the effect of *Trichoderma aureoviride* BIO-5, Tebuconazole and the time of fungicide application on the production of soybean seeds

Source of var.	Df	SS	MS	F-value
A	1	2351724.484	2351724.484	15.09**
C	2	2356997.985	1178498.992	7.56**
T	1	798401.818	798401.818	5.12*
A x C	2	963998.257	481999.129	3.09
C x T	2	640121.791	320060.895	2.05
A x C x T	2	111343.352	55671.676	0.36
Block	2	264768.500	132384.250	0.85

A = the antagonist
C = the concentrations of the fungicide
T = the time of fungicide application
A x C = the interaction between the antagonist and the concentrations of the fungicide
C x T = the interaction between the concentrations of the fungicide and the time of fungicide application
A x C x T = the interaction among the antagonist, the concentrations of the fungicide and the time of fungicide application
* = significantly different at 95% confidence level
** = very significantly different at 99% confidence level

Figure 2. Soybean plants on soil inoculated with *Sclerotium rolfsii,* 14 DAP

Figure 3. Soybean plants (14 days after planting) on soil inoculated with *Sclerotium rolfsii* and sprayed with Tebuconazole at concentration of 210 g/ha at the same day of seed planting

Figure 4. Soybean plants (14 DAP) on soil inoculated with *Sclerotium rolfsii* and *TricHoderma aure-oviride* BIO-5, and sprayed with Tebuconazole at concentration of 100 g/ha at the same day of seed planting

The soybean production of the soil sprayed with the fungicide at the same day with the planting of seeds was higher and significantly different (1756.3 kg/ha) than that of the soil sprayed with the fungicide 7 DAP (1458.5 kg/ha) (Table 5).

The combination of inoculation of the antagonist, and fungicide application at *in vitro* concentration at the same day as the planting of soybean seeds ($S_1F_1T_1A$ = 2391.2 kg/ha) gave higher soybean production than inoculation of the antagonist ($S_1F_0T_1$ = 1711.7 kg/ha) or the fungicide application either at *in vitro* concentration ($S_1F_1T_0A$ = 1771.9 kg/ha) or at field concentration ($S_1F_2T_0A$ = 1939.1 kg/ha) (Table 6), but based on statistical analysis the interaction among the inoculation of the antagonist, the concentrations of the fungicide and the time of fungicide application did not give any significant difference (Table 4).

Table 5. The effect of the inoculation of *Trichoderma aureoviride* BIO-5, the concentrations of Tebuconazole and the time of fungicide application on the production of soybean seeds

Effect	Seed production (kg/ha)*)
T. aureoviride BIO-5	
Not inoculated	1351.8 a
Inoculated	1863.0 b
Concentrations of Tebuconazole (g/ha)	
0	1247.2 c
100	1758.0 d
210	1817.1 d
Time of fungicide application	
7 days after planting	1458.5 e
At the same day with the planting of soybean seeds	1756.3 f

*) Numbers followed by the same letter do not differ significantly according to DMR Test at 95% confidence level

Table 6. The production of soybean seeds with different treatments

Treatment*)	Seed production (kg/ha)
$S_1F_0T_0$	782.6
$S_1F_0T_1$	1711.7
$S_1F_1T_0A$	1771.9
$S_1F_2T_0A$	1939.1
$S_1F_1T_1A$	2391.2
$S_1F_2T_1A$	1941.5
$S_1F_1T_0B$	1269.5
$S_1F_2T_0B$	1565.2
$S_1F_1T_1B$	1599.3
$S_1F_2T_1B$	1822.6

*) S_1 = soil inoculated with *S. rolfsii*
F_0 = soil not sprayed with Tebuconazole
F_1 = soil sprayed with Tebuconazole at *in vitro* concentration (100 g/ha)
F_2 = soil sprayed with Tebuconazole at field concentration (210 g/ha)
T_0 = soil not inoculated with *T. aureoviride* BIO-5
T_1 = soil inoculated with *T. aureoviride* BIO-5
A = soil sprayed with the fungicide at the same day of seed planting
B = soil sprayed with the fungicide 7 days after planting

CONCLUSIONS

T. aureoviride BIO-5 or Tebuconazole could be used to control *S. rolfsii.* They also could increase seed production. The fungicide was more effective when applied at the same day of seed planting than at 7 DAP.

The use of the fungicide at *in vitro* concentration combined with the use of the antagonist was more effective in decreasing the percentage of infected plants and increasing the seed production than the use of the fungicide at field concentration at the same day of seed planting.

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