INTEGRATED APPLICATION OF PLANT GROWTH-PROMOTING BACTERIA, STRAW MULCH AND SOYBEAN RESISTANT CULTIVARS TO CONTROL THE SCLEROTIUM BLIGHT DISEASE (*Sclerotium rolfsii*) OF SOYBEAN*

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ABSTRACT

One of the factors affected the increasing of soybean production in Indonesia is a disease caused by fungal pathogen, i.e. sclerotium blight disease caused by *Sclerotium rolfsii*. Integrated control was not applied yet intensively by the farmers. Straw mulch were used to control bean fly (*Ophiomyia phaseoli*) but not for sclerotium blight disease. Some growth-promoting bacteria have been applied to enhance the growth of soybean plants but it still limited in the green house. There are few reports related with integrated control of basal stem rot of soybean especially application in the field. This experiment was conducted to evaluate the application of varieties, straw- mulch, and plant growth-promoting bacteria in integrated way to control the incidence of basal stem rot caused by *S. rolfsii* in soybean. Two soybean cultivars and two isolates of PGPB were used in this experiment. Research result showed there were no significantly different effects of the interaction of varieties, straw mulch, and PGPB. Significant single effects toward disease incidence and AUDPC values were only shown by the application PGPB and straw mulch. The value of Area Under Disease Progress Curve (AUDPC) on plots covered with straw mulch (829.9 units) were significantly higher compared with those on plots without straw mulch (458.9 units). Plants in plots treated with PGPB have AUDPC values 464.3 units which significantly lower compared with the plots without application of PGPB (824.7 units). Disease incidence and the AUDPC value on Anjasmoro and Gepak Kuning varieties were not significantly different.

Keywords: Integrated Pests Management, *Bacillus subtilis*, *Pseudomonas fluorescens*, Anjasmoro, Gepak Kuning.

INTRODUCTION

Soybean is one of important commodities in Indonesia. Demand of this commodity is increase yearly in line with the increasing of human population.

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* Paper was presented in International Symposium and Congress of Indonesian Micological Society (MIKOINA), 10-11 September 2013

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Domestic demand of soybean was up to 2 million tons per year or more than 18 kg/capita/year, whereas production was only 800 000 tons or only 40% of demand (Balitkabi 2008).

One of the factors affected the increasing of soybean production is the disease caused by fungal pathogen, i.e. sclerotium blight disease caused by *Sclerotium rolfsii* (Rupe 1999). Control activities toward this disease have been conducted. Blum and Rodriguez-Kabana (2004) reported that soil amended with high amounts of benzaldehyde (0.4 ml kg-1 of soil) and velvetbean (100 g kg-1) inhibited *S. rolfsii* mycelial growth and sclerotium germination. However, low amounts of benzaldehyde (0.1 ml kg-1), kudzu (25 g kg-1), and pine-bark (25 g kg-1) stimulated mycelial growth and sclerotium germination. Kudzu (25-100 g kg-1) and velvetbean (25-100 g kg-1) inhibited the formation of sclerotia. Mishra et al. (2011) reported that mixed formulation of *Trichoderma harzianum* and *Pseudomonas fluorescence* revealed a general trend towards greater suppression and enhanced consistency against *Rhizoctonia solani*, *S. rolfsii*, and *Macrophomina phaseolina*.

Control activities toward this disease have been conducted but usually control methods were applied partially. Integrated control was not applied yet intensively by the farmers because soybeans were planted as inter-cropping plant after rice was harvested. But if the disease was not controlled properly, it will be possible that once it caused severe disease on the long period, because the pathogen could survive in the soil for long period.

Some farmers have already growing the resistant varieties but they did not use mulch. The other farmers use straw mulch to control bean fly (*Ophiomyia phaseoli*) but not for sclerotium blight disease. Some growth-promoting bacteria have been applied to enhance the growth of soybean plants but it still limited in the green house. There are few reports related with integrated control of basal stem rot of soybean especially application in the field. This experiment was conducted to evaluate the application of varieties, straw- mulch, and plant growth-promoting bacteria in integrated way to control the incidence of basal stem rot caused by *S. rolfsii* in soybean.

MATERIAL AND METHODS

Soybean Seeds

Two soybean varieties were used in this experiment, i.e. Anjasmoro variety and Gepak Kuning variety. Anjasmoro variety is belonged to the collection of Balai Penelitian Tanaman Kacang-kacangan dan Umbi-umbian (Balitkabi), East Java, while Gepak Kuning variety is belonged to the collection of Kebun Benih Palawija Bujomartani (Bujomartani Cereal Seed Farm), Sidoharjo, Central Java.

Seeds diameter of Anjasmoro variety were higher compared with those of Gepak Kuning, with the range of weight per 100 seeds was 14,8 – 15,3 g for Anjasmoro and 8,25 g for Gepak Kuning. Both of the varieties were not examined yet their resistance to the sclerotium blight disease caused by *S. rolfsii*.

Plant Growth Promoting Bacteria (PGPB)

Two isolates of PGPB were examined in this experiment, i.e. *Pseudomonas fluorescens* RH4003 and *Bacillus subtilis* AB89. Both of the bacteria are belonged to the collection of the Laboratory of Plant Bacteriology, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University. *P. fluorescens* RH4003 was isolated from the rhizosphere of healthy tomato plant, while *B. subtilis* AB89 was isolated from the rice phyllosphere. Both of the bacteria were effective to control the bacterial wilt of tomato caused by *Ralstonia solanacearum* (Nawangsih 2006).

Bacteria were re-cultured by streaking on the King's B agar plates (KB). Inocula of PGPB were produced in the King's B Broth (KBB) by inoculated 5 loops full of 24-48h old bacteria from KB into 500 ml of KBB. The liquid media were than incubated in room temperature (28°C) for 48h and 200 rpm. Suspensions of bacteria were diluted with 9.5l of water prior to application in the field.

Soybean Planting and straw-mulch application

The experiment was conducted in the farmer's filed in Ciburuy Vilage, Distric of Cigombong, Bogor Region, West Java Province. The area was laid on altitude 495m above sea level, on 6°43' South Latitude (SL) and 106°48.32' East Longitude (EL).

RESULT AND DISCUSSION

Application of cultivars, straw-mulch, and biocontrol agents (PGPB) in combination was not significantly affected the disease incidence of sclerotium blight disease of soybean. Summary of the effects of the treatments toward the incidence and AUDPC values of sclerotium blight disease of soybean was presented in Table 1. Significant effect to the disease incidence and AUDPC value were shown by the single application of straw-mulch or biocontrol agent. Combination application of cultivar and straw-mulch was not significantly affected the incidence of sclerotium blight disease in weekly observation, except in 49 DAP (Days after Planting) and to the AUDPC values. Effects of treatments, singly or in combination, were presented in Table 2, 3, 4, and Table 5.

Table 1 Summary of the effects of treatments toward the incidence and AUDPC values of sclerotium blight disease

Treatments	Df[1]	Disease Incidence (%)					AUDPC[3]
		42 DAP[2]	49 DAP	56 DAP	63 DAP	70 DAP	
Blocks	2	NS[5]	NS	NS	NS	NS	NS
Cultivars	1	NS	NS	NS	NS	NS	NS
Mulch	1	S[6]	S	S	S	S	S
Biocontrol	1	S	S	S	S	S	S
Var*Mul[4]	1	NS	S	NS	NS	NS	S
Var*Bio	1	NS	NS	NS	NS	NS	NS
Mul*Bio	1	NS	NS	NS	NS	NS	NS
Var*Mul*Bio	1	NS	NS	NS	NS	NS	NS

[1] Db = Degree of freedom
[2] DAP = Days after Planting
[3] AUDPC = *Area Under Disease Progress Curve*
[4] * = interaction
[5] NS = Not significantly different
[6] S = significantly different

Data on Table 2 showed that both interaction of cultivar Anjasmoro or Gepak Kuning without straw-mulch caused lower AUDPC value compared with those with straw-mulch. Table 3 showed that both of cultivars were not significantly different affected the disease incidence and AUDPC values. Significant effect was performed by the single application of straw-mulch and biocontrol agents (PGPB) as shown in Table 4 and Table 5, respectively. Application of straw-mulch seems increasing the incidence of sclerotium blight disease in soybean. In soybean, straw-mulch usually applied in order to control bean fly (*Ophiomyia phaseoli*).

Van Den Berg and Lestari (2001) reported that the use of rice straw for mulching increased yield across sites by 41%, but variations between sites was large, suggesting that the effect of mulching was highly location-specific. Mulching increasing yield doubled at some sites whereas no effect was observed at several other sites. Application of straw-ash increasing the yield up to 15%.

Both cultivars of soybean, i.e. Anjasmoro and Gepak Kuning have no different resistance to the sclerotium blight disease. There was yet no report about the level of resistance of both cultivars. Deptan (2010) only reported that Anjasmoro cultivar was moderately resistant to leaf rust, while Gepak Kuning was reported resistant to damping off and stem end rot diseases.

Application of mulch seems to fostering the disease incidence and AUDPC value of sclerotium blight. Table 4 shows that the AUDPC value of plants covered with straw mulch was significantly higher compared with those without straw mulch. *S. rolfsii* is a saprophytic pathogen, that application of organic matter, especially straw mulch, will enhance the growth of the fungi.

Application of biocontrol agents (with PGPB) was able to suppress the AUDPC value of sclerotium blight up to 43.7% compared with the AUDPC value of plants without PGPB (Table 5). *S. rolfsii* is a wide spread pathogen in the world with wide range of hosts. Some biocontrol agents have been studied and used to control this pathogen. *Trichoderma harzinum* is the most studied biocontrol agents. The other biocontrols were: actinomycetes, *Pseudomonas*, *Bacillus*, etc. Paramageetham and Babu (2012) isolated 35 isolates of fluorescent *Pseudomonad* strains; among them 19 were able to inhibit the growth of *S. rolfsii* in *in vitro* condition.

Table 2 Effects of interaction among soybean cultivars and straw-mulch to the incidence and AUDPC of sclerotium blight disease of soybean in the field

Treatments	Disease Incidence (%)					AUDPC[2]
	42 DAP[1]	49 DAP	56 DAP	63 DAP	70 DAP	
IA[3]	11.1 a[4]	20.8 a	27.4 a	31.8 a	35.2 a	802.2 a
IB	5.8 b	13.3 b	17.1 b	21.1 b	23.5 b	505.5 b
IIA	12.0 a	22.6 a	31.1 a	33.4 a	34.5 a	857.6 a
IIB	5.1 b	10.4 b	14.6 b	17.0 b	18.1 b	412.4 b

[1] DAP = Days After Planting
[2] AUDPC = *Area Under Disease Progress Curve*
[3] I = Anjasmoro, II = Gepak Kuning, A = with straw-mulch, B = without straw-mulch
[4] Means followed by the same letter of the same column, do not significantly different according to Duncan Multiple Range Test ($P<0.05$)

Table 3 Effects of soybean cultivars toward the incidence and AUDPC values of sclerotium blight disease in the field

Treatments	Disease Incidence (%)					AUDPC[2]
	42 DAP[1]	49 DAP	56 DAP	63 DAP	70 DAP	
Anjasmoro	6.5 a[3]	12.2 a	16.7 a	18.1 a	18.9 a	653.9 a
Gepak Kuning	10.6 a	21.3 a	28.4 a	33.6 a	36.7 a	635.0 a

[1] DAP = Days After Planting
[2] AUDPC = *Area Under Disease Progress Curve*
[3] Means followed by the same letter of the same column, do not significantly different according to Duncan Multiple Range Test ($P<0.05$)

Table 4 Effects of straw-mulch toward the incidence and AUDPC values of sclerotium blight disease in the field

Treatments	Disease Incidence (%)					AUDPC[2]
	42 DAP[1]	49 DAP	56 DAP	63 DAP	70 DAP	
With mulch	11.6 b[3]	21.7 a	29.2 a	32.6 a	34.9 a	829.9 a
Without mulch	5.5 b	11.8 b	15.8 b	19.1 b	20.8 b	458.9 b

[1] DAP = Days After Planting
[2] AUDPC = *Area Under Disease Progress Curve*
[3] Means followed by the same letter of the same column, do not significantly different according to Duncan Multiple Range Test ($P<0.05$)

Table 5 Effects of PGPB toward the incidence and AUDPC values of sclerotium blight disease in the field

Treatments	Disease Incidence (%) Plant Growth (DAP)[1]					AUDPC[2]
	42	49	56	63	70	
With PGPB	6.5 b[3]	12.2 b	16.7 b	18.1 b	18.9 b	464.3 b
Without PGPB	10.6 b	21.3 a	28.4 a	33.6 a	36.7 a	824.7 a

[1] DAP = Days After Planting
[2] AUDPC = *Area Under Disease Progress Curve*
[3] Means followed by the same letter of the same column, do not significantly different according to Duncan Multiple Range Test ($P<0.05$)

Straw mulching has been applied in rice cultivation. Devasinghe et al. (2011) wrote that rice straw mulching prevents weed growth and supplies organic matter for heterotrophic N-fixing microorganisms. Jiang-tao et al. (2006) reported that straw mulching treatment decreased the evapotranspiration and delayed the soil drying process after irrigation as compared with treatment without straw mulching. Straw mulching significantly increased leaf area per plant, main root length, gross root length and root dry weight per plant of rice. Balwinder-Singh et al. (2010) reported that the mulch lowered total soil evaporation over the crop growth season by 35 and 40 mm in relatively high and low rainfall years, respectively. In both years, there was significantly higher tiller survival and grain weight with mulching, and this led to significantly higher grain and total biomass yields in 2006-07, probably because the non-mulched treatment suffered from water deficit stress for a period after maximum tillering that year.

CONCLUSION

Application of cultivars, straw-mulch, and biocontrol agents in combination of three of them was not significantly reduced the incidence of sclerotium blight disease of soybean. Combination of cultivars and straw-mulch significantly reduced the AUDPC value of the disease. Application of straw-mulch was significantly increasing the disease incidence and AUDPC value of sclerotium blight of soybean, while application of biocontrol agents was significantly reduced the disease incidence and AUDPC value. Disease incidence and the AUDPC value on Anjasmoro and Gepak Kuning varieties were not significantly different.

ACKNOWLEDGEMENT

This research was funded by IMHERE B2C-IPB, Program: STRENGTHENING AGRICULTURAL RESEARCH FOR FOOD SECURITY AND SOVEREIGNTY, Sub Program: Pengembangan Sistem Pengendalian Hama Terpadu Biointensif untuk Mendukung Ketahanan dan Kedaulatan Pangan, Activity: PENELITIAN KOMPONEN DAN TEKNOLOGI PENUNJANG PELAKSANAAN PENGENDALIAN HAMA TERPADU BIOINTENSIF.

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