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Bioassays of *Glomus fasciculatum* Against Pathogenic Fungi *Sclerotium rolfsii* In *Glycine max* L. Merril. var. Argomulyo

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ABSTRACT

The aims of this research were to find out the influence of *Glomus fasciculatum* dosage against the infection of *Sclerotium rolfsii* which causes wilting diseases on soybean (Glycine max L. Merril.var. Argomulyo) and its effects on the soybean growth. Several dosage of mycorrhizal were used in this experiment .i.e : 0 gram of mycorrhizal with and without fungal pathogen, 10 grams, 20 grams, 30 grams, 40 grams, and 50 grams. The result of this research shows that *G. fasciculatum* has a positive effect to inhibit *S. rolfsii* based on long stems, number of leaves (health and wounded), dry plants weight (pods, root's,and dry crown's) and intensity of the disease attack for emphasized attack by *S. rolfsii*.

KEYWORDS: : *Glomus fasciculatum*, *Sclerotium rolfsii*, soybean.

INTRODUCTION

Soybean (*Glycine max* L. Merrill) is annual crops, and important legumes commodity that widely cultivated as main of nutrition in Indonesia beside rice. Improvement of Population and GDP increased it demand [1], involved import as a solution to fill it supply due to the low of domestic productivity. Recently Indonesia was soybeans importing country. Soybean import dependence seriously threatened Indonesia food security. On the other hand, the lack of fulfillment of soybean in Indonesia due to the presence of various diseases on soybean plants among, one of them was disease wither.

Plant disease is one constraint in enhancing soybean crop production in Indonesia. Argomulyo is soy varieties with two tons per ha in the yield [2]. The arrangement of the body consists of two kinds of the soybean plant organ namely vegetative and generative organs. Vegetative organ (15 - 30 days), covering the root stem, and leaves while the generative organs (35 - 90 days), covering fruit flowers and seeds. The soybean plant has two grow phase namely vegetative and reproductive. Vegetative phase ranging from germination until flowering while reproductive phase ranging from the establishment of flower to seeds. The soybean plant in Indonesia grows on high temperature (>30° C), and most of it flowering began at 5 – 7 weeks [2].

Pathogen fungus (*Sclerotiumrolfsii*) is a causative pathogen wilted legume crops such as soybean. A strike rate of more than 5% in the field could damage economically, and lead low field or completely failed harvests. Losses due to fungal pathogens on soybean plants In Indonesia was vary, in West Nusa Tenggara's assault on soybean commodity intensity reaches 55% [4]. These pathogens include Boletus occupants of land with a white mycelium seen at the base of the stem. Sklerotia (fungi spores) fungus is formed on a white growing mycelium and hardened brownish later, with 0.5 - 2 mm as the diameter and commonly attack on 2 - 3 weeks in plants. Sklerotium can last up to 6 - 7 years in the ground, wrinkled in the dry weather, and will germinate quickly in wets environment. The optimum temperature for the development of the disease is optimally on 22°C – 29°C and 3 – 6pH[5].

Some of the results showed that plants with the addition of mycorrhizal vesicular arbuscular (MVA) is generally more resistant to disease and high productivity. Therefore, combating the disease wither on the stem and the root can be used mycorrhizal as a prevention efforts to reduce the negative impact of the disease wither by*S. Rolfsii.* InfectedMA (Mycorrhizal Arbuscular) mushroomsoybean roots content of more glyceoline (phenols compound which can reduce highly pathogeniceffect) due to the accumulation of phytoalexin influences compared to uninfected mushrooms MA and phenol compounds increase is differentat each plant [6]. The soybean plant which given additional *G. Fasciculatum*is used in this research, to reduce the negative effects of pathogenic *S.rolfsii*with certain level treatment dose. Mixed inoculum of mycorrhizal is composed of the land pieces, the host plant roots, the mycelia and also spores.

METHODS

The research was carried out in October 2012 to March 2013 at the Laboratory of Botany and Mycology, Biology ITS Surabaya as well as Green House Nurseries JL. Surabaya Kendalsari belonging to the Department of Hygiene and gardening Surabaya.

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Analysis of physical and chemical properties of Soil

The physical and chemical analysis properties conducted in Department of Land of Brawijaya University. Soil samples in analysis is a mixture of ground and sand by 2: 1comparison,it will sterilize using formalin 5%beforehand. Soil samples in each sample media cropping contains about 250 grams and doses treatment analyzed[7]. Physical properties measured is soil texture and temperature ground. While the chemistry of soil measured are NPK, pH of land, and water level[8].

The Propagation of Pathogenic Fungi *S. rolfsii*

S. rolfsii is a pure isolates propagated within Petri dish which is obtained from Corn Sweeteners and Fiber Research Institute (BALITTAS). Pure isolates are growing on a medium Potato Dextrose Agar (PDA) in a Petri dish that has been previously sterilized using an autoclave at a 121˚C temperature and 1,5 atm vapor pressure for 15 minutes. Propagation of *S. rolfsii*conducted using pure isolates of sklerotia and placed on the middle part of the Petri dish that contains medium PDA using tweezers.

PDA Medium incubated at room temperature for up to amount of its sklerotia enough to be harvested. Then, *S. rolfsii*which have grown on medium PDA propagated again on chaff medium before applied to the plant. Chaff medium made from a mixture of 30 grams chaff and 30 ml of water, which put into heat-resistant plastic bags. Media are sterilized using an autoclave at a temperature of 121^{o}C with 1,5atm vapor pressure for 20 minutes before they used. Every husks medium inoculated with 5 pieces sklerotia and mycelia as much as a quarter of Petri dish from *S. rolfsii*propagation results. *S. rolfsii*isolates were bred for 4 weeks at room temperature before it is inoculated into the planting medium [9].

Preparation of cultivation medium

The cultivation medium with mixture of soil and sand with a ratio of 2: 1 are used. The cultivation medium is inserted in heat-resistant plastic as much as 3 kg, then sterilized with5%formaldehyde. Each 3 kg were given 75 ml5% formalin. Then itshake and wrapped with plastic for 7 days and it will be open for aerating in 7 days [10].

A preparation of the cultivation and inoculation Mycorrhizal

Mycorrhizal *G. fasciculatum* type is used in the form of mixture inoculum. The soybean seed and mycorrhizal put into poly bag that has been contains 3 kg cropping media. Each poly bag contains one soybean seed and labeled. The seed and mycorrhizal inoculum inoculated simultaneously in the press cropping by inserted inside a hole with the depth of 2-3 cm using a small spade. This hole then closed back to the ground. Furthermore, put the soybean seed as deep as 1 cm from the upper surface of the ground on the same hole when mycorrhizal inserted [7]. There were seven kinds of treatment; without granting mycorrhizal and without the provision of pathogenic as control, mycorrhizal 0 grams (without granting mycorrhizal only), mycorrhizal as much as 10 mycorrhizal, 20 grams mycorrhizal, 30 grams mycorrhizal, 40 grams mycorrhizal, and mycorrhizal as many as 50 grams. The provision of basic NPK fertilizer in 1 gram/plant proportion applied in medium [11].

Pathogenic fungi *S. Rolfsii* **inoculation**

Pathogenic mushroom *S. rolfsii*who has propagated is used. After four time planting, fungi is inoculated. It inoculated by punching holes in the area around the plant, and sprinkled the chaff media by as much as 30 grams that have contained *S. rolfsii*into the hole. Ground covered with sand use a trowel small [12]. Watering on the land surface around plants in poly bag used hand sprayer is conducted every day.

The observation parameters

Length of the stem and Leaves number

Length of the stem Leaves number observed for 12 times in every week. Length measured used yarn and a ruler from the lowest limit growth to the top limit of plant growth, which is the last leaf that grows. Leaves number accounted to health and sick leaves [8].

The weight of dried plants

Drought weight measurements carried out on the root and canopy, after harvesting crops in the 12 weeks after planting. Parts of the plant are separated so that retrieved 2 parts of the plant that were the root and canopy. They washed with water in the beaker glass and rinse again using aquadesand placed in between the filter paper using tweezers to absorb the remaining water washing then weighing by using analytical balance. They dried at a 70˚C temperature in the oven for 2 days and weighted use analytic balance after totally dry thoroughly [8].

The percentage of Mycorrhizal*G. Fasciculatum infection*

Semi-permanent smear preparation slices roots is made and Mycorrhizal infection in roots of soybean quantified from 10 observed semi-permanent smear preparation slices roots under microscope observation. Infected roots remarkable as a vesicular or arbuscula appearance within plant cortex. Observation conducted in a month.

Roots slice into 1 cm used scalpel after cleaned and washing. 10% KOH is increased into the roots after the second washing and admitted in a tube for 60 minute of 95˚C within oven. $H_2O_2$is added into the tube after throwing KOH and washing with water. HCl 5% also used to washed after H_2O_2. Coloring used *Lactophenol Tryphan Blue* (LTB) in 30 minute under 70˚C temperature in oven. After heating and watering with water, *lactogliserol* is employed once [8].

Roots slices compiled on object glasses and covered by cover glasses. 10 slices are taken and selected randomly from them. They observed under microscope and account the percentage of infection.Mycorrhizal namely viable if only they reached 50% percentage of infection, and the percentage of infection is measured under this formula[13] :

$$\% \text{ infection} = \frac{\Sigma \text{ infected roots}}{\Sigma \text{ total roots}} \text{ x } 100\%$$

The percentage of *S. rolfsii*

The percentage of infection calculation to be done to know the infection of *S. rolfsii* on soybean plants which obtained from observations on root semi permanent preparations smear. Percent infection of *S.rolfsii* was calculated from the number of infected roots from the 10observed root pieces in each plant. Percent infection of *S.rolfsii* was calculated based on the formula [14],

$$\% \text{ infection} = \frac{JAT}{JSP} \text{ X } 100\%$$

Description:
JAT = number of infected roots of *S. rolfsii*
JSP = number of slices roots plant

The intensity of the disease attacks

The determination of the intensity of diseases attack based on the ratio between number of sick plant or compartment plant sick with total plant or number of all plant bodies likes leaves, stem, fruits, roots, or other plant bodies that shows it symptoms. Intensity measurement accounted regarding attacks level on soybean leaves. The measurement is applied according the formula [8]:

$$I = \frac{\Sigma\,(nxV)}{Z \text{ x } N} \text{ x } 100\%$$

Description:
I = attacks intensity
n = attackednumber of leaves
V =scale on attacked leaves
Z =the highest scale on attacked leaves
N = number of observed leaves

Research design

The Complete Random Design (RAL) as research design is used and mycorrhizal treatment dose that includes 7 level dose i.e. without granting of mycorrhizal and pathogenic unannounced as a negative control, without granting of mycorrhizal fungal pathogens but was given as a positive control, mycorrhizal as much as 10 grams, mycorrhizal as much as 20 grams, mycorrhizal as much as 30 grams, 40 grams mycorrhizal and mycorrhizal as much as 50 grams, whereas each treatment was repeated four times.

RESULTS AND DISCUSSION

Chemical Analysis of cultivation Media

4,26% – 5,5% Nitrogen; 0,26% – 0,5% Phosphorus element; and Potassium as much as 1,71% – 2,5% are required as nutrient levels which needed by soybean [15]. This below table is a results analysis of cultivation media.

Table 1. Table of analysis results on cultivation media at previous condition and the afterward.

Parameters	Previous condition	Afterward						
		1	2	3	4	5	6	7
N (%)	0,0167	0,06	0,06	0,07	0,08	0,09	0,11	0,17
P (mg/kg)	9,046	4,53	4,59	6,38	7,15	9,33	9,70	11,82
K (me/100g)	0,23	0,36	0,35	0,40	0,40	0,49	0,61	0,74
pH	6,3	6	4,2	4,5	3,4	3,7	3,8	4,3
Humidity (%)	21,47	60%	65%	70%	80%	90%	85%	92%

Description:

1 = 0 Grams mycorrhizal and without granting of pathogenic (control negative).
2 = 0 Grams mycorrhizal and without granting of pathogenic (control positve).
3 = 10 Grams mycorrhizal and without granting of pathogenic.
4 = 20 Grams mycorrhizal and without granting of pathogenic.
5 = 30 Grams mycorrhizal and without granting of pathogenic.
6 = 40 Grams mycorrhizal and without granting of pathogenic.
7 = 50 Grams mycorrhizal and without granting of pathogenic.

The test results show that at 3,4 - 5 pH the land belonged acid due to mycorrhizal fungi and pathogens were overgrown. Both are include fungi whose life took out a variety of compounds from it metabolic processes that cause the cultivation medium condition become acidic [15]. It has a humidity ranges from 60% - 95%, that suitable for growing soybeans. Such a condition is optimum seed planted until charging pods. Water shortage in its growth will cause stunted plants till death when drought has exceeded the limits of toleration [17].

On the number of Nitrogen and potassium had given treatment shows increased. It is allegedly mycorrhizal capable of producing various compounds of its metabolic process. Those compounds may constitute an organic acid, ammonium (NH_4^+), and various enzymes. In a decline off phosphorous element shown at the treatment 1 to 4 analysis. On this research allegorist that soybean plant is absorbing Nitrogen, Phosphorous, and potassium element derived from synthetic fertilizers it lead their number on the ground depopulated. The absorption efficiency of mycorrhizal acts as a fertilizer in the soil so that nutrients are absorbed by the plant according to the crop nutrient requirements [8]. On treatment 5 to 7 increased the amount of phosphorous element. It is caused of mycorrhizal inoculated on plants are issuing a metabolite compounds accumulate in the soil where the compound can be used for the formation of the Phosphorous element.

The influence of granting mycorrhizal G. fasciculatum in soybean Argomulyo varieties

Mycorrizhal with 5 spores densityper gram are used in this research. These are length of stem and leaves number average which tested with Duncan test:

Table 2. Length of the stem and health leaves number of soybean at 4 week after planted before pathogenic fungus inoculated.

Treatment	Length of the stem	Leaves number
(-) mycorrizhal and (-) pathogen	82,75 ab	12,5 a
(-) mycorrizhal and (+) pathogen	70,5 a	12,25 a
10 gr mycorrizhal and (+)pathogen	93,25 bc	13,25 a
20 gr mycorrizhal and (+) pathogen	94,25 bc	13,75 a
30 gr mycorrizhal and (+) pathogen	99 bc	13,75 a
40 gr mycorrizhal and (+) pathogen	106,5 c	20,5 b
50 gr mycorrizhal and (+) pathogen	122 c	22,25 b

Means with the same letter were not significantly different from each other (Duncan test in 5% level).

Table 2 shown the length of the stem averages which given significant differ on plant with mychorrizal and without mychorrizal. The highest growth soybean is 50 grammychorrizal inoculated and the lowest is control without mychorrizal. Similar result also found in leaves number. Those can happen because uninfected plant with mychorrizal elemental nutrient absorbance was not optimal and cultivation media which used have contains lower nutrient so that plant growth were not much better rather than plant with inoculated mychorrizal.

Mycorrizhal might help water absorbance that cause length of the stem and leaves number optimally growth and it increase as long as much dose of mychorrizal had given. It able to absorbs water, yet external hyphae thathelped to create larger water absorbance area covered by the roots[7]. Figure1 shows improvement of length of the stem seen at the stem and leaves number related to mychorizal dose that had given. Spores of mychorrizal were cropping and form functional structure that help them in gaining water and nutrients in the ground. They also could stimulate growth hormones such as cytokinins and auxin. Cytokinins and auxin lead cell differentiation and elongation, whereas stem cell of the plant so get increasing plant high or even more leaves number [2].

The influence of granting *G. Fasciculatum* **against Pathogen fungus** *S. Rolfsii* **in soybean Argomulyo varieties**

Mycorrizhal with 5 spores density per gram are used in this research. The results reached based on the parameter; lenght of the stem, health leaves number, droughtweight roots, canopy and bean that linear with mychorrizal dose and percentage of infection excepted infected leaves number parameter. Observation as long weeks 12 as harvested time tested with Duncan test in 5% level;

Table 3. Growth of length of the stem, health and infected leaves number and drought weight plant (roots, canopy, and bean) inn weeks 12 after cultivation.

Treatment	Lenght of the stem (cm)	Leaves number		Drought weight (gram)		
		Health	Infected	Root	Canopy	Bean
(-) mycorrizhal and (-) pathogen	188,375 b	44,25 b	0 a	0,1975 ab	1,745 ab	0 a
(-) mycorrizhal and (+) pathogen	83,5a	27,25 a	27,25 d	0,0975 a	1,1325 a	0 a
10 gr mycorrizhal and (+) pathogen	190,625 b	46,5 b	25,75 cd	0,3075 abc	2,2875 ab	0 a
20 gr mycorrizhal and (+) pathogen	230,125 bc	48,5 b	20 bcd	0,41 bcd	3,1075 abc	0 a
30 gr mycorrizhal and (+) pathogen	279,75 c	50,75 b	19,25 bc	0,5075 cd	4,2625 bc	0,1825 b
40 gr mycorrizhal and (+) pathogen	289 c	52,75 b	16 b	0,615 de	5,0425 cd	0,43 b
50 gr mycorrizhal and (+) pathogen	301,625 c	58 b	14,75 b	0,815 e	7,0675 d	0,6825 b

Means with the same letter were not significantly different from each other (Duncan test in 5% level).

On Table 3, average yield increased is noticeable in the point of; length of the stem, number of healthy leaves, drought weight plants is directly proportional to the magnitude of the mycorrizhal dose on this study of 50 grams. At the plants who were given the mycorrizhal treatment with the largest dose at this research i.e. 50 g produced the highest average among them on the long stem of 301,625 cm; the number of healthy leaves of 58 pieces; drought weight root of 0,815 grams; drought weight of 7,0675grams of headers; and drought weight of 0,6825grams of pods. However different results occur in the number of infected leaves where the average infected leaves number were inversely proportional to the dose addition mycorrizhal. At the plant who were given a dose of mycorrizhal 50 gram, average leaves amount the pain most small i.e. 14.75 fruit. Wilt on crops of soybeans of pathogenic fungus-infected is caused due to water transport and nutrients impaired. *S. rolfsii*attacked plants with mycelia infection on root and the host plant stem by issuing a hydrolytic and oxidative enzyme damaging functional structure of the plants roots to absorb carbon, sugar, polysaccharides, lipids, amino acids, polypeptide, sulfur, and phosphorus as a food source [18]. Yet of this water transport to all parts of the plant by root also was disrupted.

On a drought weight of beans, there are some plants that did not grow bean.Podsonly grow at the treatment by given the addition of pathogenic fungi and mycorrizhal dose of 30 grams, 40 grams, and 50 grams. At the plant who were given a dose of 10 grams of mycorrizhal and 20 grams, nitrogen content was lower, it happens because the mycorrizhal dose given is either low, where the percentage of mycorrizhal infections at a dose of 10 grams is 55% and 20 gram doses is 65%. Pod formation is influenced by carbohydrates which is the end result of photosynthesis so that the higher rate of photosynthesis, then the higher carbohydrate produced. If plants infected by a pathogen, then photosynthesis on crops alsowould be interrupted. Therefore, it can inhibit the phosphorylation process and prevents the synthesis of chlorophyll. In the end, leaf surface who conducted the process of photosynthesis is reduced and the production of carbohydrates would not enough for using in bean or pods formation. Mycorrizhalexerted an influence against the wilt diseases *S. rolfsii*on soybean plant also determine on the percentage of infection mycorrizhal *G. Fasciculatum* and pathogen fungus*S. rolfsii*.

Table 3. The observation of mychorrizal infection percentage, pathogen fungi, and the intensity of disease attack on soybean at week 12 after cultivation time

Treatment	% infection of*G.fasciculatum*	% infection of*S. rolfsii*	Attacks intensity
(-) mycorrizhal and (-) pathogen	0% a	0% a	0000 a
(-) mycorrizhal and (+) pathogen	0% a	83% f	90,3375 f
10 gr mycorrizhal and (+) pathogen	55% b	68% ef	80,075 e
20 gr mycorrizhal and (+) pathogen	65% bc	58% de	71,59 d
30 gr mycorrizhal and (+) pathogen	73% c	48% cd	52,06 c
40 gr mycorrizhal and (+) pathogen	78% c	38% bc	43,500 b
50 gr mycorrizhal and (+) pathogen	95% d	28% b	38,0933 b

Means with the same letter were not significantly different from each other (Duncan test in 5% level).

In Table 3, it can be seen that the plant was given the highest mycorrizhal dose treatment in this study which is 50 g, produced high percentage of infection by *G. fasciculatum*(95%). While on the percentage of infection of *S. Rolfsii* and has low intensity, accounted as much as 28% (infection) and 38,0933% (the intensity of the attacks). Plants that were not given mycorrizhal but given the pathogen has the most massive attack's intensity which is 90,34%. At the treatment 10 grams mycorrizhal up to 40 grams resulted 80,08%; 71,59%; 52,06%; and 43,05%. This occurs due to the interaction of plant roots with mycorrizhal, increases the activity of enzymes chitinase that effectively withstand the attack of pathogenic fungi[19].

Here is a microscopic roots images of infected mycorrizhal *G. fasciculatum* :

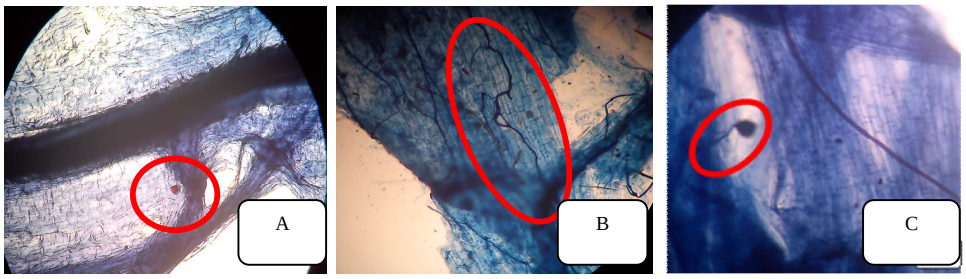

Figure 5. Microscopic observation with 400 magnification mycorrizhal *G. fasciculatum*such of spore (a), hyphae (b), and vesicles (c).

Andhere is a microscopic roots images of infected pathogenic fungi*S. rolfsii* :

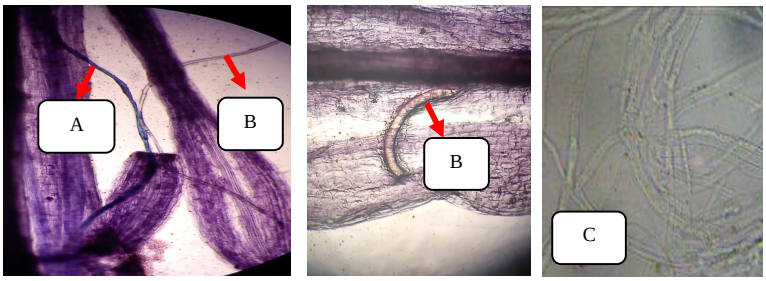

Figure 6. Microscopic observation with 400magnification hyphae that is colored blue is hyphae mycorrizhal *G. fasciculatum*(a), white colored hyphae is *S. rolfsii*(b), photograph literature hyphae *S. rolfsii*[20].

From the results of this research gained that as much higher dose of mycorrizhal as lower the impact caused by *S.rolfsii*[21]. Mycorrizhal can increase the growth of plants using the roots of plants protection of pathogenic and toxic elements. Therefore the soybean was given a dose of the treatment of pathogenic fungi with mycorrizhal, these plants can still grow with long stems, leaf number, and optimal plant dought weight though has been stricken with the disease wither *S.rolfsii*. Chitinase enzyme can inhibit the growth of pathogenic hyphae whichcomposed of the polymer chitin, so that the pathogenic fungal hyphae cell wall will undergo lysis.

CONCLUSION

Granting mycorrizhal *G. fasciculatum* with 50 gram dosehad influenced soybean growth in such paramaters likes lenght of the stem, health and infected leaves number, weight drought plant, (roots, canopy, and beans) and the intensity of disease attacks.

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