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Biological Control Stem Rot Diseases (*Sclerotium rolfsii*) on Peanut (*Arachis hypogaea* L.) using Arbuscular Mycorrhizal Fungi (AMF) Indigenous

Fradilla Swandi, Eri Sulyanti*, and Darnetty
Department of Plant Protection, Faculty of Agriculture, Andalas University, Limau Manis Padang. Indonesia

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*Corresponding author:
E-mail: erisulyanti13@gmail.com

ABSTRACT

Arbuscular Mycorrhizal Fungi (AMF) are known to have potential as biological agents controlling plant pathogens. This study aims to obtain indigenous AMF isolates that can suppress the attack of *Sclerotium rolfsii* which causes stem rot disease in peanut. The method used is an experimental method with a Completely Randomized Design with 5 treatments, namely A : AMF *Glomus* sp-3 + *S. rolfsii*; B: AMF *Acaulospora* sp + *S. rolfsii*; C: AMF *Gigaspora* sp + *S. rolfsii*; D: Combined AMF *Glomus* sp-3, *Acaulospora* sp, and *Gigaspora* sp + *S. rolfsii*; E: Without AMF + *S. rolfsii* (Control). Each treatment was repeated 5 times. The data were analyzed using Analysis of Variance (ANOVA) using the Statistix 8 program and the Least Significance Different (LSD) test at a 5% significance level. The results showed that the isolates of *Acaulospora* sp and *Gigaspora* sp were able to increase the resistance of peanut plants to stem rot disease (suppressing the incidence andseverity of the disease up to 100%)

INTRODUCTION

Stem rot caused by *Sclerotium rolfsii* fungus is the most detrimental disease by reducing pod yield of 60 % (Kator *et al.,* 2015). This fungus can attack almost all parts of the peanut plant, especially those in direct contact with the soil. This disease is relatively difficult to control because pathogens form sclerotia that can survive in the soil for a long time, have diverse hosts, and can attack all stages of growth (Le, 2011; Soesanto, 2013; and Xu *et al*., 2010).

Control of *S.* rolfsii has been carried out using synthetic fungicides, but the action is not appropriate because it can pollute the environment, kill natural enemies and microorganisms that degrade toxic chemical compounds, and harm public health. Therefore it is necessary to have environmentally friendly control measures. Some biological control techniques that have been tested are using the bacteria *Pseudomonas* and *Bacillus* (Le, 2011), and endophytic fungi *Trichoderma harzianum* and *Beauveria bassiana* (Munawara and Haryadi, 2020) but no biological agents have been found that are truly effective in controlling *S. rolfsii*. Therefore it is necessary to have other alternatives, one of which is by using the Arbuscular Mycorrhizal Fungi (FMA) indigenous (Rahman et al, 2017).

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FMA is found in almost all habitats throughout the world and can associate with many plants (INVAM, 2020). Indigenous inoculants are inoculants sourced from AMF spores isolated from the rhizosphere of certain plants, then applied to the same plant. Indigenous inoculants have a higher level of conformity compared to introduction because inoculants have adapted to similar plants. Suharti et al., (2011) stated that FMA indigenous isolates were able to increase the ginger plant's resistance to bacterial wilt disease of *Ralstonia solanacearum* race 4 reaching 100%. Sulyanti (2012) also reported that the FMA indigenous of the genus *Glomus* was able to increase the resistance of banana seedlings to the *Fusarium oxysporum* f.sp cubense race 4 attack. The study aims to obtain indigenous AMF isolates identified in previous studies that can suppress the attack of Sclerotium rolfsii, which causes stem rot disease in peanuts.

MATERIALS AND METHOD

This research has been carried out from December 2019 to April 2020 in the greenhouse and the Phytopathology Laboratory, Faculty of Agriculture and Chemical and Natural Materials Laboratory, Pharmacy Faculty of Andalas University.

Local cultivar (*Arachis hypogeae* L.) was used as plant material. *S. rolfsii* isolated from naturally infected peanut plants was maintained on the Potatoes Dextrose Agar (PDA) were then cultured on Corn Meal Sand (CMS) media. This study used four indigenous AMF isolates namely A (*Glomus* sp-3), B (*Acaulospora* sp), C (*Gigaspora* sp), D (Combined *Glomus* sp-3, *Acaulospora* sp, and *Gigaspora*).

Experiment design in this study was Completely Random Design (CRD) with 5 treatments and 5 replication, so there were 25 experimental units (each experimental unit consisted of 3 units). Data were analyzed using Statistix 8 program. Analysis of variance (ANOVA) was used to determine the treatment effects and the differences between treatments were determined using LSD Test on 5%. Treatments were as follow A : AMF *Glomus* sp -3 + *S. rolfsii*; B: AMF *Acaulospora* sp + *S. rolfsii*; C: AMF *Gigaspora* sp + *S. rolfsii*; D: Combined AMF *Glomus* sp-3, *Acaulospora* sp, and *Gigaspora* sp + *S. rolfsii*; E: Without AMF + *S. rolfsii* (Control).

The planting medium is a mixture of ultisol soil with manure (2:1 v/ v). The mixture medium then was mashed and sieved. The mixture is sterilized for 2 hours at 100^{o}C and then dried at room temperature for 1 day. The medium was put into 45 x 50 cm polybags. AM Fungi inoculum (100 spores/plant) was introduced at planting time (seeds are ± 7 days old after germinated). Synthetic fertilizer was applied to the planting medium with a half recommendation dose (Urea 0.1 g / polybag, TSP 0.2 g / polybag, and KCl 0.2 g / polybag). Eight weeks after introducing AMF, all plant was inoculated with 50 g inoculum of *S. rolfsii.*

The incubation period, disease incidence, and disease severity

The incubation period was observed every day after the inoculation of *S. rolfsii* until the plant showed the first symptom. The disease incidence and severity is carried out when the plants exhibit the first symptoms at intervals of 1 time a week until the plants are 90 days old using the following formula:

$$I = \frac{n}{N} \times 100\% \qquad S = \frac{\sum_{0-s}^{ni\ yi}}{ZN} \times 100\%$$

Where i = Disease incidence, n = number of the infected plant, N = Total number of observed plant, s = Disease severity, ni = Number of disease plants having the same degree of infection, yi = Degree of infection, and Z = Highest degree of infection.

Disease severity was rated on a scale from 0 to 4, with 0 = no disease symptoms, 1 = disease symptoms without visible fungal outgrowth, 2 = disease symptoms with visible fungal outgrowth, 3 = partial wilting of the plant, and 4 = complete wilting and plant death (Le, *et al.,* 2011)

Root colonization by AM Fungi

Eight weeks after introducing AMF, the colonization percentages in peanut roots of four AMF isolates were determined. The roots were cleared and stained following a protocol described by Nusantara (2011) and the percentage of root colonization was estimated

by the gridline intersect method (O'Connor *et al.* (2001) *cit* Nusantara *et al.* (2015)). Colonization was estimated using 0 –30 % scale, which 0% = no colonized, <10 %= low, 10-30 %= moderate, and >30 %= high colonized on root (O'Connor *et al.,* (2001) *cit* Nusantara *et al,.* (2015)).

The level of salicylic acid test on peanut **root that colonized by AM Fungi**

Salicylic acid content was analyzed using a protocol described by Rasmussen *et al*., (1991) with minor modification. 5 grams of peanut plant roots were crushed, then extracted with methanol. The extract was centrifuged for 15 minutes in 6,000 rpm, then the supernatant was collected. Salicylic acid content was analyzed using High-Performance Liquid Chromatography (HPLC). Salicylic acid concentration is expressed in micrograms per gram of fresh weight. The salicylic acid content in treated plants compared to control plants. The high content of salicylic acid in the treated plants shows an increase in plant resistance.

RESULTS AND DISCUSSION

Incubation period

Peanut plants treated with Arbuscular Mycorrhizal Fungi (AMF) indigenous isolates were able to slow the emergence of the disease (prolonging the incubation period of *S. rolfsii*) with an incubation period of 21 - 32 days compared to control (9-14 days) and treatment of *Acaulospora* sp and *Gigaspora* sp showed no symptoms at all until the last day of observation (Table 1).

Application of indigenous AMF can prolong the incubation period of the disease (21 - 32 days) compared to control (9-14 days) allegedly due to competition of food sources between pathogens and AMF, where AMF *Acaulospora* sp and *Gigaspora* sp are the highest in colonizing plant roots (Table 4) so at the time needed for pathogens to infect the roots of plants is longer. Living plant roots release various compounds in the form of exudates which have an important role in providing signals to encourage FMA infection in the roots. Colonization of plant roots by FMA can cause changes in the quality and quantity of root exudates. These changes also affect the microbial community in the plant rhizosphere, thus allowing a decrease in root pathogens. Changes in root exudate can also change the microbial community, attract antagonistic agents, and in mycorrhizae, the root exudate can affect spore germination, hyphal growth, branching, and development on the root surface (Akhtar and Siddiqui, 2008; Jung et al., 2012).

Incidence and disease severity of *S. rolfsii*

The incidence and diseases severity of *S. rolfsii* in peanut plants treated with various types of AMF isolates can be seen in Tables 2 and 3. Application of various types of AMF isolates has varied effects on the incidence of disease with the effectiveness of 40-100% and also gives results that are significantly different from the severity of the disease with the lowest severity found in the treatment with *Acaulospora* sp and *Gigaspora* sp which is 0.00% with the effectiveness of 100.00%.

Application of AMF has different effects on the rate of disease progression in peanut (Figure 1). Plants treated with *Acaulospora* sp and *Gigaspora* sp showed no symptoms at all while AMF combines and *Glomus* sp-3 showed that the rate of disease development tended to

Table 1. The incubation period of *S. rolfsii* on peanut plants

Treatment	Incubation period Of S. rolfsii (day) on replication				
	1	2	3	4	5
A (*Glomus* sp-3)	28	21	32	-	-
B (*Acaulospora* sp)	-	-	-	-	-
C (*Gigaspora* sp)	-	-	-	-	-
D (Combined)	-	31	-	-	23
E (Control)	13	13	14	9	11

Note: (-): there are no signs and symptoms of the disease.

Table 2. Disease incidence of *S. rolfsii* at 6 week after inoculation (Transformation to Arcsin (x + 0.5))

Treatment	incidence	Transformation to Arcsin (x + 0.5)	Effectiveness (%)
B (*Acaulospora* sp)	0.00 c	0.5236 c	100
C (*Gigaspora* sp)	0.00 c	0.5236 c	100
D (Combined)	40.00 bc	0.6243 bc	60
A (*Glomus* sp-3)	60.00 ab	0.6747 ab	40
E (Control)	100.00 a	0.7754 a	0
Cv = 13.97			

Note: The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test.

be lower and stable from the first week to the last week of observation, but instead the control plants showed the rate of disease development which tends to increase from the first week to the last observation. This proves that the four indigenous AMF isolates can adapt and develop rapidly in colonizing roots, to induce the resistance of peanut plants to *S.rolfsii* fungal attack. Symptoms of the *S.rolfsii* attack in each treatment can be seen in Figure 2.

The application of AMF is also able to reduce the incidence and disease severity with the effectiveness of 40-100% (Table 2) and 61.54-100% (Table 3). Application of *Acaulospora* sp and *Gigaspora* sp can reduce the incidence and severity of the disease with effectiveness reaching 100%. This is because the AMF is able to induce plant resistance to *S. rolfsii* attacks. The resistance of this plant is triggered by the increase in secondary metabolite compounds (salicylic acid) after being given an inducing agent in the form of indigenous AMF. Punja and Utkhtede (2003) stated that plants are able to defend themselves against pathogenic attack by producing secondary metabolites. The production of secondary metabolites is triggered by elicitors or inducing agents. The ability of AMF is

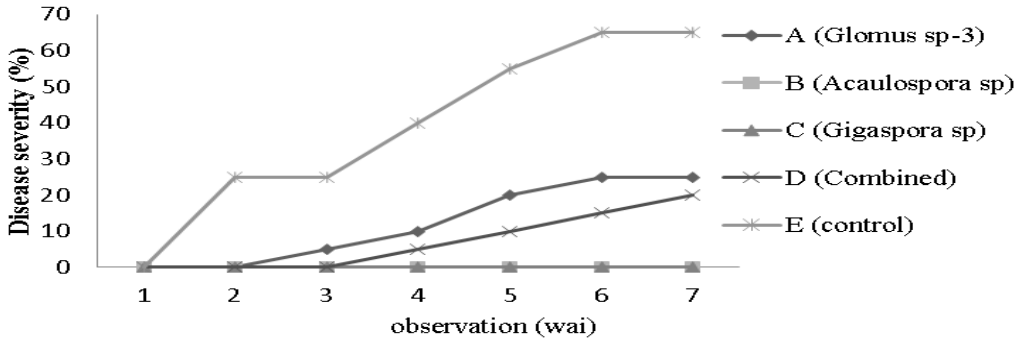

Figure 1. The rate of development of the base stem rot disease of peanut in each treatment.

Table 3. Disease severityof *S. rolfsii* at 6 week after inoculation (Transformation to Arcsin (x + 0.5))

Treatment	severity (%)	Transformation to Arcsin (x + 0.5)	Effectiveness (%)
B (*Acaulospora* sp)	0.00	0.5236 b	100
C (*Gigaspora* sp)	0.00	0.5236 b	100
D (Campuran)	20.00	0.5721 b	69.23
A (*Glomus* sp-3)	25.00	0.5839 b	61.54
E (kontrol)	65.00	0.6825 a	0.00
Cv = 9.54			

Note: The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test.

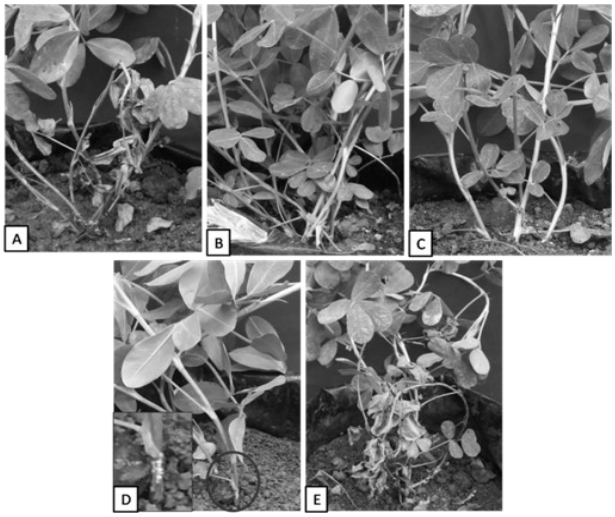

Figure 2. Comparison of *S.rolfsii* attack symptoms in peanut plants inoculated with indigenous AMF and control. A. *Glomus* sp-3 (partially withered plants), B. *Acaulospora* sp (no symptoms), C. *Gigaspora* sp (no symptoms), D. Combined (white patches present), E. Control (partially withered plants).

suspected because the AMF used is an indigenous AMF derived from peanut plants so that when given back to the same host plant (peanuts) a match will occur. This is supported by the statement of Suharti et al., (2011) that the indigenous AMF returned to the original plant will be more effective in increasing plant resistance to pathogen attack. In addition, Azcon-Aguilar et al., (2002) stated that the effectiveness of AMF was highly dependent on the suitability between AMF types, host plants, soil types, and interactions between the three factors. Strong interdependence between AMF and its hosts will produce a synergistic relationship that results in a high response compared to plants without AMF.

The AMF colonization on root

Colonization level of AMF at the root of peanut plants varies from moderate to high. AMF with a high rate of root colonization at 4 wai is found in *Acaulospora* sp while at 8 wai followed by *Glomus* sp-3 and *Gigaspora* sp isolates and at 12 wai all treatments showed high colonization rates (Table 4). The structure of the colonization of AMF can be seen in Figure 3.

Observation of root colonization (Table 4) shows that AMF is able to colonize plant roots with a high category (43.33 - 60.00%). Root colonization by AMF is beneficial for plants to be protected from pathogens. Symbiosis AMF with plant roots is able to activate plant resistance both locally and systemically. One of the resilience shown by plants is an increase in phenol compounds or antibiotic substances in plant roots due to increased activity of the enzyme Phenylalanine Ammonium Lyase (PAL) which functions in inducing plant resistance to pathogenic attack (Pozo et al. 2009 and Prasasti et al. 2013). Ability was higher than AMF *Acaulospora* sp and *Gigaspora* sp in colonizing roots allegedly because these two types of AMF have compatibility with host plants and also the environment in which they live. Tahat and

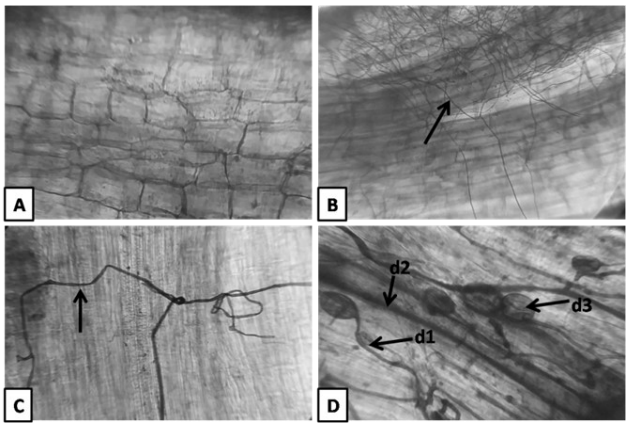

Figure 3. Structure of AMF colonization in plant roots. A. Plant roots not colonized by AMF (control), B-C. External hyphae, D1. Intracellular hyphae, D2. Intercellular hyphae, D3. Vesicles.

Table 4. Percentage of roots colonized by AMF at 4 , 8, and 12 week after inoculation (WAI)

Treatment	4 WAI (%)	8 WAI (%)	12 WAI (%)
A (*Glomus* sp-3)	26.67 (M)	43.33 (H)	50.00 (H)
B (*Acaulospora* sp)	37.67 (H)	46.67 (H)	60.00 (H)
C (*Gigaspora* sp)	30.00 (M)	40.00 (H)	56.67 (H)
D (Combined)	23.33 (M)	26.67 (M)	43.33 (H)
E (Control)	0	0	0

Note : M = Moderate, H = High.

Table 5. The salicylic acid content of peanut roots at 8 week after inoculation (WAI)

Treatment	Salicylic acid content (ppm)	Effectiveness (%)
C (*Gigaspora* sp)	53.56 a	49.09
B (*Acaulospora* sp)	44.31 b	38.46
A (*Glomus* sp-3)	39.61 c	31.15.
D (Combined)	37.62 d	27.51
E (Control)	27.27 e	0.00
Cv = 0.96		

Note: The numbers followed by the same lowercase letters in the same column indicate no significant results among treatments at 5% level by LSD test.

Sijam (2015) state the association of mycorrhizae and host plants is influenced by environmental factors such as temperature, intensity light, and humidity, and soil conditions.

Salicylic acid content

The content of salicylic acid in the roots of peanut plants with indigenous AMF treatment can be seen in Table 5. The content of salicylic acid in the roots with the treatment of FMA indigenous isolates is higher than of control plants with the effectiveness of 27.51- 49 09%.

The incubation period of *S. rolfsii* in peanut plants was also shown by high levels of salicylic acid in the roots of peanut plants (Table 5). Table 5 shows the levels of salicylic acid in the roots of plants that were given AMF significantly different than in the controls. This shows that application of AMF can increase levels of salicylic acid in plant roots so that plants are more resistant to pathogenic attack as a result the incubation period of the pathogen is longer even there are treatments that show no symptoms at all until the last day of observation (Table 1). The introduction of AMF can affect the physiological and biochemical responses of plants through increased production of chemical compounds such as ethylene, chitinase, phytoalexin, jasmonic acid, and salicylic acid which increase plant resistance to pathogenic infections (Pozo et al., 2009 and Vlot et al., 2009). Salicylic acid is one of the compounds that induce the formation of pathogenesis-related (PR) proteins and increases plant resistance to pathogenic infections (Chen et al., 2010; Vlot et al., 2009).

CONCLUSION

Isolates of *Acaulospora* sp and *Gigaspora* sp were able to increase the resistance of peanut plants to stem rot disease (suppressing the incidence and severity of the disease) reaching 100%. *Acaulospora* sp has higher AMF colonization (60.00%) and *Gigaspora* sp has higher salicylic acid content of 53.56 ppm with the effectiveness of 49.09%.

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