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The Potential of Arbuscular Mycorrhizal Fungi (AM Fungi) as Biocontrol Agent Against Stem Rot Diseases caused by *Sclerotium rolfsii* in Peanut (*Arachis hypogaea* L.)

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Abstract

The arbuscular mycorrhizal fungi (AM Fungi) can be associated with almost all types of plants to control plant disease. In this study, AM Fungi was used as a biocontrol agent against stem rot disease caused by *Sclerotium rolfsii* in the peanut plants. 40 g AM Fungi inoculum (25 spores/g in concentration) was introduced to peanut seedling at planting time, while 50 g *Sclerotium rolfsii* was inoculated at 8 weeks after planting. The experiment was arranged in the greenhouse using a Completely Randomized Design (CRD) with 7 treatments and 5 repeats. The data were analyzed using analysis of variance (ANOVA) using STAT program 8 and the LSD test at a 5% significance level. The AM Fungi treatments showed significantly reduce the percentage of disease severity in infected peanut plants around 30.77% - 100% and can extend the incubation period of the disease. AM Fungi increased root colonization (20,00 - 46.67%) with a middle to high category. The AM Fungi C isolate (isolated from Solok regency) was the best biocontrol agent against *S. rolfsii* (100 %), followed by A isolate (isolated from Payakumbuh city) and D isolate (isolated from Padang Pariaman regency) 84,62% respectively. AM Fungi also increased Salicylic acid content 1,6 times (70.72 ppm) compared to control (45.59 ppm). From the data, we concluded that the application of AM Fungi as a biocontrol agent plays an important role in plant resistance and exhibits a greater potential to protect peanut plants against *S. rolfsii*.

Keywords: controlling, bio fungicides, salicylic acid

1. Introduction

Peanut is one of the essential plant commodities that have a fairly high nutritional value. The main contents of peanut, oil (42%) and protein (22%) (Hardiningsih 2012). *Sclerotium rolfsii* Sacc. is an important soil-borne pathogen and causes disease in numerous crops including peanut (Punja, 1988). The loss of yield caused by *S. rolfsii* infection is 25%, but sometimes it reaches 80-90%. The disease causes damage to the root and stem of the plant. The disease is relatively difficult to eradicate because the fungus has a diverse host and the sclerotia can survive in the soil for a prolonged period (Punja *et al*., 1985). Usually, pathogenic fungi are controlled by synthetic fungicides, but it's not effective although repetitive application. Excessive use of synthetic fungicides also pests natural enemies and microorganisms, contaminate the water and the environment and adversely affect the health of the local community (Sumartini, 2012).

Arbuscular mycorrhizal fungi (AM Fungi) known as symbiotic organisms that could be associated with any type of plants, and found almost in all types of soil. Several AM Fungi species had been found to control soil-borne pathogens such as *Aphonomyces, Cylindrocladium, Fusarium, Macropomina, Phytophthora, Pythium, Rhizoctonia, Sclerotium,* and *Verticillium* (Akthar and Siddiqui, 2008). The AM Fungi has a very important role to improve the nutrients absorption capacity, increase drought and pathogen resistance, and increase crop productivity (Delvian, 2006).

Twenty-four AM Fungi isolates have been collected from the healthy banana rhizosphere in West Sumatera. The sampling area is banana central production area and endemic fusarium wilt areas. All isolate can control Fusarium wilt in the greenhouse (Sulyanti *et al*., 2011, 2014, 2016). Setiawan *et al*., (2014) reported that AM Fungi application in soybean plants can suppress *S. rolfsii* attack by about 75% compared without mycorrhizal. Ozgonen *et al*., (2010) report that AM Fungi was effectively used to control the stem rot disease caused by *S. rolfsii*. *Glomus caledonium* AM Fungi can reduce the ratio of infected plants by 84%, and reduce the disease severity by 63.3%, while *Glomus clarum* can reduce the disease severity by 64.7%. The study aimed to evaluate the mycorrhizal activity as a biocontrol agent against stem rot diseases caused by *Sclerotium rolfsii* on peanut under greenhouse conditions.

2. Materials and Methods

A. Time and Location of Research

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

B. Research Materials

Tala 1 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 six AM Fungi isolates (multispore) from various regions, namely isolate A (isolated from Payakumbuh city-1), B (isolated from Payakumbuh city-2), C (isolated from Solok regency), D (isolated from Padang Pariaman regency), E (isolated from Bogor city-1) and F (isolated from Bogor city-2).

C. Experiment details and statistics analysis

Experiment design in this study was Completely Random Design (CRD) with 7 treatments and 5 replication, so there were 35 experimental units (each experimental unit consisted of 3 units). Data were analyzed using STAT program 8. 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: AM Fungi isolates A + *S. rolfsii,* AM Fungi isolates B + *S. rolfsii*, AM Fungi isolates C + *S. rolfsii*, AM Fungi isolates D + *S. rolfsii*, AM Fungi isolates E + *S. rolfsii*, AM Fungi isolates F + *S. rolfsii* and Without AM Fungi isolates and *S. rolfsii* (control).

D. Experiment procedure

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 (1000 spores/pot) was introduced at planting time (seeds are ± 3 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 AM Fungi, all plant was inoculated with 50 g inoculum of *S. rolfsii.*

E. Observations

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:

$$S = \frac{\sum_{0-5}^{ni\ yi}}{Z.N}\ x\ 100\%$$

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.

Table 1. Observation scale of disease severity symptoms on peanut plants

Disease rating	Description
0	No disease symptoms
1	Disease symptoms without visible outgrowth of the fungus
2	Disease symptoms with visible outgrowth
3	Partial wilting of the plant
4	Complete wilting and plant death

Stem rot disease severity was measured on a scale of 0 - 4 according to Le *et al*., (2012) (Table 1).

Root colonization by AM Fungi

Eight weeks after introducing AM Fungi, the colonization percentages in peanut roots of six AM Fungi 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.

3. Results and Discussion

A. Incubation period

Incubation periods of *S. rolfsii* on peanuts varied around 6-19 days longer than controls (3-5 days). Introductions of AM Fungi made plants stronger and not easily infected by *S. rolfsii*. Not all of the test plants (replications) showed symptoms until the end of observations. In particular, isolates C does not show symptoms until the last day of observation (Table 2).

The introduction of AM Fungi can affect the incubation period of the disease due to competition of food sources between pathogens and AM Fungi, so pathogens need a longer time to infect plant roots. This is following the statement by Graham (2001) that obligately biotrophic relationship of the AM fungi in the root cortex and the regulation of colonization by carbon supply are strongly suggestive that mycorrhizas interact directly with root pathogens that have similar trophic requirements. The potential exists for resource competition between the symbiotic fungus and pathogen, leading to the reduction of each other's colonization and reproduction when they co-inhabit roots.

The introduction of AM Fungi isolates C caused no symptoms at all. This is maybe caused by the compatibility between AM Fungi species with the host plant and the soil used. Azcon-Anguilar *et al*., (2002) reported that the effectiveness of AM Fungi depends on the match between the type of AM Fungi, the host plant and soil types, and the interaction between all three of these factors. Plants and soil types, particularly the level of acidity, and soil fertility levels give different responses to the AM Fungi. A strong interdependence between the AM Fungi with its host will produce a synergistic relationship giving rise to a high response compared to plants without mycorrhiza. The main requirement is the establishment of the association AM Fungi functional suitability, which is determined by the physiological activity of plants, genetics, morphology, and the character of the plant roots, in addition to external factors such as temperature, light, soil fertility, and pesticide.

B. Diseases severity of *S. rolfsii*

Statistical analysis (transformed to $\sqrt{x+0.5}$) showed that the effectiveness percentage of AM Fungi against *S. rolfsii* varied from 30.77 to 100%. The AM Fungi isolates D, A, and C on peanut plants provide a significantly different effect than the control with the effectiveness of 84.62 to 100%, while the introduction of AM Fungi isolates B, E, and F are not significantly different from the control (Table 3).

AM Fungi isolates D, A, and C have higher effectiveness in controlling *s. rolfsii.* It was suspected because isolates D, A, and C had healthy, and intact spores while isolates B, E, and F had several spores that are broken and not intact anymore. AM Fungi can protect the root from *S. rolfsii* attack because they colonized the roots and caused morphologically

Table 2. The incubation period of *S.rolfsii* on peanut plants

Treatment	Incubation period (dai) / replications				
	1	**2**	**3**	**4**	**5**
A (Payakumbuh 1)	-	19	-	-	-
B (Payakumbuh 2)	-	5	8	-	-
C (Solok)	-	-	-	-	-
D (Padang Pariaman)	16	-	-	-	-
E (Bogor 1)	6	16	-	-	-
F (Bogor 2)	-	-	8	17	-
G (control)	3	3	5	4	3

The data was transformed into $\sqrt{x + 0.5}$. Remark (-) = no symptoms until the last day of observation, dai = day after inoculation of *S. rolfsii*

Figure 1. Peanut plant. A. Healthy plant B. Plants attacked by *S. rolfsii* (dead plant) C. Withered plant D. Plant with stem rot disease (source: personal documentation).

changes in roots, such as the occurrence lignification at endodermis cells so that they inhibited the penetration of *S. rolfsii*. The plants are infected by mycorrhizal mantle or sheath to form mycorrhizal fungal mycelia which acts as a physical barrier of penetration of pathogens (Siddiqui and Pichtel, 2008; Akthar and Siddiqui, 2008). AM Fungi affect plant extend life and plant health where the growth is better than plants without mycorrhizae (control) (Figure 1A). The stem rot symptoms caused by *S. rolfsii* are the presence of dry rot at the base of the plant stem, then the leaves turn yellow, and wilt (Figure 1C), and eventually the plant will die (Figure 1B). Around the base of the plant's stem will be encountered signs of the disease as well as the white to brown fungal mycelium round (Figure 1D). These signs and symptoms are similar to a study by Rahayu (2003).

C. The AM Fungi colonization on root

Colonization level of AM Fungi isolates at the root of peanut plants varies from moderate to high. AM Fungi with root colonization levels higher category consecutively isolate C, A, and D meanwhile isolates B, E, and F included in the medium category (Table 4).

Roots colonized by the mycorrhizal plants characterized by the presence of one of the internal structures of the AM Fungi as hyphae, vesicles, external hyphae, or spores (Figure 2). The AM Fungi that colonized roots will influence plant resistance to pathogen infection. According to Garcia-Garrido and Ocampo (2002), plant defense mechanisms by AM Fungi are activated early in the process that began with the mycorrhizal symbiosis penetration until the formation of colonization established, the faster the AM Fungi colonize the roots, the higher the possibility of a protective effect against the pathogen. Nusantara *et al*., (2015), reported that AM Fungi has 4 functional roles, namely as a bio protector because it can protect plants from biotic stresses such as plant pathogens, as a bio processor because it is able to help plants absorb nutrients and water from locations that are not reachable by root hairs, as a bio activator because it is able to increase carbon storage in the rhizosphere to increase the activity of microorganisms in carrying out biogeochemical processes, and bioagregator because it is able to increase soil aggregation.

Table 3. Disease severity of *S. rolfsii*

Treatment	Severity (%)	Effectiveness (%)
A (Payakumbuh 1)	10.00 bc	84.62
B (Payakumbuh 2)	40.00 abc	38.46
C (Solok)	0.00 c	100.00
D (Padang Pariaman)	10.00 bc	84.62
E (Bogor 1)	45.00 ab	30.77
F (Bogor 2)	30.00 abc	53.85
G (control)	65.00 a	-
CV = 20.41		

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

Table 4. Percentage of roots colonized with AM Fungi

Treatment	4 wai (%)	8 wai (%)	Category
A (Payakumbuh 1)	36.67	40.00	High
B (Payakumbuh 2)	16.67	20.00	Moderate
C (Solok)	36.67	46.67	High
D (Padang Pariaman)	33.33	40.00	High
E (Bogor 1)	13.33	20.00	Moderate
F (Bogor 2)	16.67	23.33	Moderate
G (control)	0.00	0.00	Not Colonized

wai = weeks after the introduction of the AM Fungi

The existence of external hyphae growth is an important source of inoculum for the continued colonization of the same root system in producing spores formed in the soil whose function is to transfer nutrients from the soil to plants.

D. Salicylic acid content

The salicylic acid content in the roots of peanut plants with AM Fungi treatment can be seen in Figure 3. The salicylic acid content in peanut root after inoculation of *S. rolfsii* is high, compared to before inoculation in all treatments including controls. The increasing of salicylic acid content in all treatments was higher than controls, except treatment with isolate A. This is suspected because the plant, in general, has salicylic acid but, when the plant was attacked by pathogens or antagonist agents, salicylic acid content was increased and then can trigger plant resistance. However, the AM Fungi isolate A was different with others isolate, which suspected because the existence of other compounds besides salicylic acid which can trigger plant resistance as conveyed by Pozo *et al*., (2009) and Vlot *et al*., (2009) that introduction of AMF can influence the physiological and biochemical responses of plants through increased production of chemical compounds such as jasmonic acid, ethylene, chitinase, phytoalexin, and salicylic acid.

Salicylic acid has an important role in the signaling pathway that triggers systemic acquired resistance enhancement and is associated with the accumulation of Pathogenesis-related proteins (PR proteins), such as PR1 (Lyon, 2007).

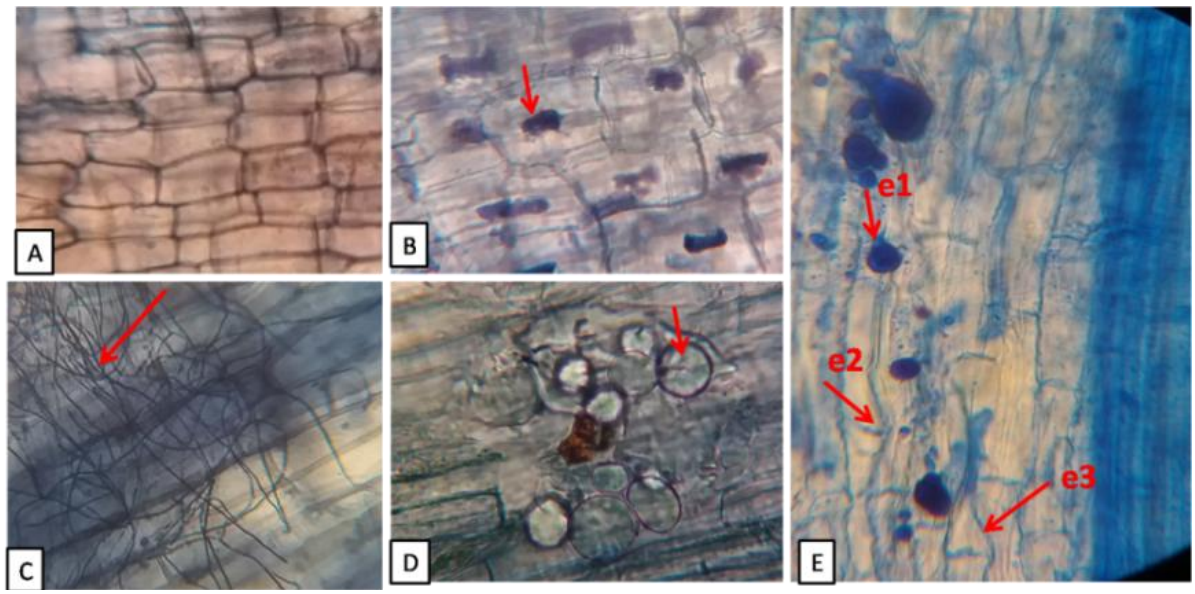

Figure 2. Structure of mycorrhizal colonization in plant roots. A. Plant roots not colonized by mycorrhiza (control), B-E. Plant roots colonized by mycorrhizal. B. Arbuscular, C. External hyphae, D. Vesicles, E1. Vesicles, E2. Intercellular hyphae, E3. Intracellular hyphae (source: personal documentation).

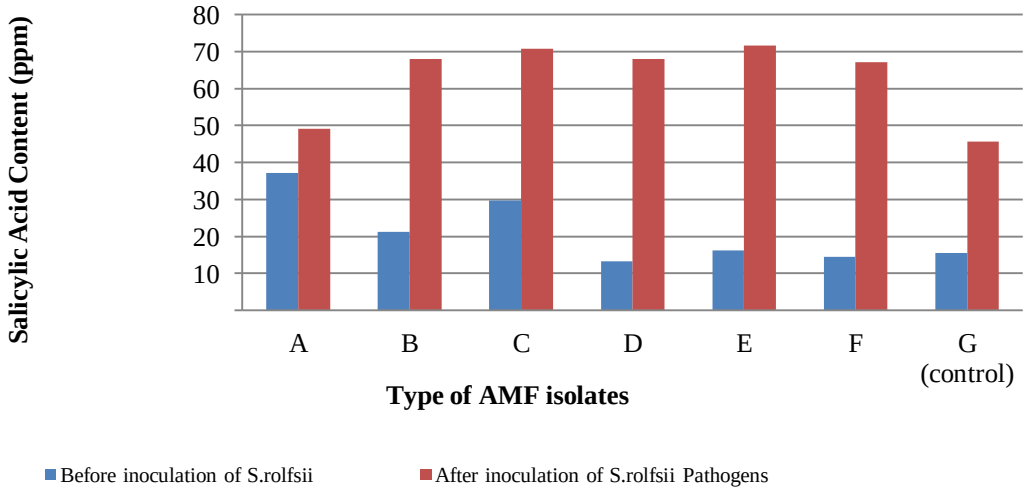

Figure 3. Effect of AM Fungi to the salicylic acid content before and after inoculation with the *S. rolfsii*.

According to Heil and Bostock (2002), salicylic acid plays a role in the systemic pathway for enhancing the level of elicitation and signaling. At the elicitation level, salicylic acid is synthesized in response to mechanical damage, necrosis, and stress oxidative, then transported systemically. At the signaling level, salicylic acid acts as a regulator of signaling pathways for gene expression related to cell wall components, phytoalexin, PR protein, and phenol compounds.

4. Conclusions

The introduction of mycorrhizal isolates (AM Fungi) can reduce the severity of stem rot disease caused by *S. rolfsii* with an effectiveness percentage of about 30.77% - 100%. AM Fungi isolate C has higher mycorrhizal colonization (46.67%). AM Fungi isolate C is the best biocontrol agent against *S. rolfsii* in the peanut plant, followed by the isolate A and D.

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