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Full Length Research Paper

The effects of arbuscular mycorrhizal fungi on yield and stem rot caused by *Sclerotium rolfsii* Sacc. in peanut

Hulya Ozgonen[1]*, D. Soner Akgul[2] and Ali Erkilic[3]

[1]Department of Plant Protection, Agricultural Faculty, Suleyman Demirel University, 32260, Isparta, Turkey.
[2]Manisa Viniculture Research Institute Manisa, Turkey.
[3]Department of Plant Protection, Agricultural Faculty, Cukurova University, 01330, Adana, Turkey.

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The effects of *arbuscular mycorrhizae* fungi (AMF) against stem rot disease caused by *Sclerotium rolfsii* Sacc. in peanut were investigated. The mycorrhizal fungi used were *Glomus etunicatum*, *Glomus mosseae*, *Glomus clarum*, *Glomus caledonium*, *Glomus fasciculatum*, *Gigaspora margarita.* In pot experiments, mycorrhizal fungi decreased infected plant ratio between 17.5 - 84.0%. *G. caledonium* showed the highest effect by 84.0%. The AMF decreased the disease severity between 37.8 - 64.7%. The effect on disease severity of *G. caledonium* and *G. clarum* were 63.3 and 64.7%, respectively. In field trials, the effect on disease locus of mycorrhizal fungi ranged between 30.6 and 47.2%. *G. caledonium* showed the lowest effect with 30.6% while the other mycorrhizal species had the same effect. *G. etunicatum* and *G. caledonium* increased the yield by 24.3%. The results show that AMF fungi could effectively be used against stem rot caused by *S. rolfsii*.

Key words: Arbuscular mycorrhizal fungi, *Glomus* sp., *Sclerotium rolfsii*, peanut, yield.

INTRODUCTION

Sclerotium rolfsii Sacc. is an important soil-borne pathogen and causes disease in numerous crops including peanut (Punja, 1988; Krupa and Dummergues, 1979). The loss of yield caused by pathogen infection generally is 25%, but sometimes it reaches 80 - 90% in some cases (Grichar and Bosweel, 1987). The disease causes damage on root and stem of plant. The pathogen produces sclerotia which overwinter in soil and on plant debris and can survive in a long period causing disease in the following season (Punja, 1985). Thus, the control of the disease is very difficult. Different control methods have been investigated before including cultural practices (Gurkin and Jenkins, 1985; Punja et al., 1986), chemical control (Bowen et al., 1992; Minton et al., 1993; Damicone and Jackson, 1994, Culbreath et al., 1992), biological control (Elad et al., 1984; Papavizas and Lewis, 1989; Papavizas and Collins, 1990; Latunde-Dada, 1993; Bechemal and Chet (1996) and integrated control (Bicici et al., 1994; Cilliers et al., 2003).

In recent years, mycorrhizal fungi as symbiotic organisms have been used against plant pathogens successfully. Several studies indicated that, AMF influenced fungal diseases caused by root pathogens (Krishna and Bagyaraj, 1983, Caron et al., 1986; Matsubara et al., 1995; Trotta et al., 1996; Karagiannidis et al., 2002). Most of the studies concluded that, disease severity could be reduced by root colonization of AMF via several mechanisms including increasing the mineral absorption and plant growth (Baath and Hayman, 1983; Davies and Linderman, 1991; Smith and Read, 1997), phenolic compounds (Devi and Reddy, 2002) and pathogenesis-related proteins (Dumas-Gaudot et al., 1996; Dassi et al., 1998; Pozo et al., 1999). The objective of this study was to investigate the interaction of AMF and the pathogen *S. rolfsii* on peanut and yield.

MATERIALS AND METHODS

Plant material, pathogen and mycorrhizal fungi

American type peanut (*Arachis hypogeae* L.) was used as plant material. *S. rolfsii* isolated from naturally infected peanut plants was

*Corresponding author. E-mail: hozgonen@ziraat.sdu.edu.tr.

Table 1. The effects of mycorrhizal fungi on the development of the stem rot of peanut caused by *S. rolfsii*.

Treatments	Seed germination (%)	Healty plant (%)	Dead plant (%)	% Effect
Gc + Sr	63.3	47.4	52.6	24.0
Gcl + Sr	60.3	88.9	11.1	84.0
Ge + Sr	46.7	42.9	57.1	17.5
Gf + Sr	63.3	52.6	47.4	31.6
Gm + Sr	50.0	60.0	40.0	42.2
Gim + Sr	66.7	75.0	25.0	63.9
Sr	43.3	30.8	69.2	-
Control (-Myc, -Sr)	60.0	100.0	0.0	-

maintained on potato dextrose agar at 24°C. Six mycorrhizal fungi including *Glomus clarum*, *Glomus caledonium*, *Glomus etunicatum*, *Glomus fasciculatum*, *Glomus mosseae* and *Gigaspora margarita* were used in the experiments. The mycorrhizal fungi maintained on maize (*Zea mays* L.) were used as inoculum for each mycorrhizal fungi.

Determination of the effects of mycorrhizal fungi on diseases severity of *Sclerotium rolfsii*

Pot experiments

The soil used in the experiments was sandy loam. Soil that was twice autoclaved for one hour was placed in 15 cm-diameter pots. In order to determine the effects of mycorrhizal fungi on diseases severity, two different pot experiments were conducted. In the first experiment, mycorrhizal fungi inoculum containing about 1000 spores/10 g soil and infected root pieces were placed 2 - 3 cm below the seeds for each mycorrhizal fungi and 3 seeds were sown in each pot (Menge and Timmer, 1982). At the same time, pathogen inoculum mixture of 25 g sclerotia and 40 g sand was applied to pots at equal portion as 500 mg sclerotia in each pot. Second experiment was conducted in the same manner, but roots were left to be colonized by mycorrhizal fungi and pathogen inoculations were done 4 weeks after mycorrhisal inoculation. Treatments for both experiments were as follow: *G. etunicatum* + *S. rolfsii* (Ge + Sr), *G. mosseae* + *S. rolfsii* (Gm + Sr), *G. clarum* + *S. rolfsii* (Gc + Sr), *G. caledonium* + *S. rolfsii* (Gcl + Sr), *G. fasciculatum* + *S. rolfsii* (Gf + Sr), *G. margarita* + *S. rolfsii* (Gim), pathogen *S. rolfsii* (+Sr) and without mycorrhizal fungi and pathogen infected plant (C i.d -Myc, -Sr). All pots were maintained in growth room at 24 ± 2°C and 12h photoperiod. Experiments were conducted with 4 replications and 5 plants in each.

In the first experiment, numbers of emerged, infected and healthy plants were determined by counting. In the second experiment, four weeks after sowing, the colonization percentages of peanut roots of six mycorrhizal fungi were determined. The roots were cleared and stained by procedure described by Koske and Gemma (1989) and the percentage of root colonization was estimated by gridline intersect method according to Giovannetti and Mosse (1980). Disease was estimated using 0 - 6 scale which graded between 0 = no infection and 6 = complete dead plant (Timper et al., 2001). Disease index and disease severity were calculated and differences between treatments were evaluated by LSD test and significance level was accepted as 0.05 (Gomez and Gomez, 1983).

Field experiment

To determine the effects of mycorrhizal fungi on *S. rolfsii* and peanut yield, a trial was conducted in naturally infested field in Adana. Plots were prepared with 4 x 6 m, 70 cm between rows and five lines in each plot with soil properties of 53.6% clay, 21.4% silt, 25.0% sand and pH: 7.6. Mycorrhizal fungi inoculum was applied as a band to each seed row prior to sowing (Menge and Timmer, 1982). Control plots had no mycorrhizal inoculum. Experiments were designed as randomized complete block design with four replications (six mycorrhizal fungi species and control). In the experiments, 20 - 25 kg/da diamonium phosphat (DAP, 18-46-0) was applied before sowing as subsoil fertilizer. After sowing, rainy irrigation was performed for supplying plant outlet in the experiment area and ammonium sulphate was applied on the account of 7 kg pure nitrogen in first irrigation during plant development and 8 kg ammonium nitrate during second irrigation.

Before harvest, stem rot incidence was evaluated based on the number of disease locus, where a locus represented one or more plants in 30 cm of row infected with *S. rolfsii* (Timper et al., 2001). At the time of harvest, plots were harvested separately and yield was evaluated. The data were analyzed by analysis of variance. Means were compared using LSD test and significance level was accepted as 0.05 (Gomez and Gomez, 1983).

RESULTS

The effects of mycorrhizal fungi on disease severity of *S. rolfsii*

Pot experiment

In the first experiment at the condition of application of mycorrhizal inoculum and pathogen at the same time, the ratios of seed emergence, healthy and diseased plant were shown in Table 1.

The ratio of seed germination was 60% in control plants. In contrast, it was reduced to 43.3% in inoculation with pathogen only. It can be thought that, the difference of plant ratio was resulted from early infection of pathogen. As a matter of fact, the effect on disease was increased with increased post emergence plant ratio when post emergence plant ratio changed between 46.7 and 66.7 in mycorrhizal treatments and the effect of mycorrhizal fungi (%) were examined together. For instance, post emergence plant ratio in *G. caledonium* treatment was the same as in negative control. In this treatment, the effect (%) on pathogen infection was 84% at post emergence stage. Similarly, the rate of emergence and effect (%) on pathogen infection were

Table 2. The effects of mycorrhizal fungi on disease severity of stem rot.

Treatments	Colonization (%)	Disease			% Effect
		İndex	Severity (%)		
Gc + Sr	75	0.50	16.7	a*	64.7
Gcl + Sr	72	0.46	15.5	a	63.3
Ge + Sr	65	0.89	29.8	b	37.8
Gf + Sr	70	0.53	17.6	ab	61.2
Gm + Sr	69	0.61	20.2	ab	54.9
Gim + Sr	65	0.82	27.2	ab	41.3
Sr		1.43	47.6	c	-

*Means fallowed by different letters were significantly different according to the LSD test (P = 0.05).

Table 3. The effect of mycorrhizal fungi on diseases loci of *S. rolfsii* and yield under field conditions.

Treatments	Disease locus		% Effect	Disease Locus		% Effect	Yield (kg / plot)		Increasing ratio (%)
	1. Evaluation			2. Evaluation					
Gc + Sr	4.0	a*	50.0	6.3	a	47.2	6.3	b	15.9
Gcl + Sr	6.3	bc	20.8	8.3	b	30.6	7.0	a	24.3
Ge + Sr	4.3	ab	45.8	6.7	a	44.4	7.0	a	24.3
Gf + Sr	5.7	ab	29.2	6.7	a	44.4	6.2	b	14.5
Gm + Sr	6.0	abc	25.0	7.0	ab	41.7	6.0	b	11.7
Gim + Sr	4.3	ab	45.8	6.3	a	47.2	5.8	bc	8.6
Sr	8.0	c		12.0	c	-	5.3	c	-

*Means fallowed by different letters were significantly different according to the LSD test (P = 0.05).

66.7 and 63.9%, respectively. However, the ratio of emergence was 46.7% in *G. etunicatum* treatment same as pathogen treatment only. The ratio of prevention of infection was lower with 17.5% in this treatment.

In the second experiment preinoculation with mycorrhizal fungi prevented the early infection of *S. rolfsii*. The disease severity was 47.6% in positive control while it was changed between 15.5 and 29.8% in mycorrhizal fungi treatments (Table 2). The highest effect on disease severity was seen in *G. caledonium* (15.5%) and *G. clarum* (16.7%) and the effect on disease was found as 63.3 and 64.7%, respectively. *G. etunicatum* showed the lowest effect (37.8%) on disease.

Field experiment

In the field experiment, the data of disease locus and yield were collected and evaluated and results summarized in Table 3.

The first evaluation was done a month before the harvest. Disease locus was found in control plot while it was found as 4.0 with the lowest degree in *G. clarum* treatment. This was followed by *G. etunicatum* and *G. margarita* as the same value (4.3) that is, the effect on disease severity of pathogen was 50 and 45.8%, respectively. Second evaluation was done fifteen days after first evaluation and disease loci were reached 12.0. Except *G. caledonium* disease loci were changed between 6.3 - 7.3 among the other species and also the effects (%) of this species was found between 38.9 - 47.2%. The effect of *G. caledonium* was found as 30.6% with lower degree at this time.

Yield was increased by 24.3% in *G. etunicatum* and *G. caledonium*. The lowest increasing ratio was found in *G. margarita* treatment by 8.6%.

DISCUSSION

The present study establishes that, mycorrhizal fungi reduce the severity of disease caused by *S. rolfsii*. Colonization of root of peanut plants by AMF varied between 65 - 75%. In *G. clarum* inoculated plants, the percentage of colonization was 75% while the lowest colonization rates had *G. etunicatum* and *G. margarita*.

Liu (1995) investigated the effect of mycorrhizal fungi on verticillium wilt of cotton and the role of disease resistance. At the condition of simultaneously inoculations of mycorrhizal fungi and pathogen, the inoculation rate was decreased mutually and there were vital competition and antagonism between two organisms. However, disease severity was decreased by mycorrhizal fungal inoculation under suitable condition and wilt

severity was lower compared to control. Among the tested mycorrhizal fungi, *Glomus versiforme* induced the disease resistance against verticillium wilt effectively.

In present study, plants root allowed with mycorrhizal colonization sufficiently and plants was protected from early infection of *S. rolfsii* by mycorrhizal fungi. In another study, *G. fasciculatum* decreased the number of sclerotia and infection rate of root rot of peanut caused by *S. rolfsii*, effectively, under the pot conditions (Krishna and Bagyaraj, 1983). In pot experiment, *G. fasciculatum* decreased the ability of sclerotia formation of *S. rolfsii* by 40%. The positive effect of mycorrhizal fungi on some fungal pathogen was determined under field conditions, also Rabie (1998) revealed the effectiveness of *G. mosseae* against *Botrytis faba* of broad bean, the disease severity was 83% in plant inoculated with only pathogen, while plants precolonised with *G. mosseae* had 32.3% disease severity ratio.

Compared with the pot experiment, it appeared that the lowest effect was caused by exposing to pathogen infection or high pressure of disease until the harvest. Actually, the infection of *S. rolfsii* in natural conditions was actualized after the flowering of plants. Therefore, the rate of effect obtained from natural conditions could be evaluated as the higher effect level compared to pot experiment. The reason of main effect was that the mycorrhizal colonization establishment accomplished until flowering. In addition, almost all mycorrhizal fungi showed similar effect.

Among mycorrhizal fungi, *G. caledonium* and *G. etunicatum* increased the yield by 24.3%. The lowest increasing ratio had *G. margarita* (8.6%). However, the effect of that mycorrhizal fungus on diseases severity was 47.2% with pretty much degree. The reason for this is not reflected the yield that, plants infected with *S. rolfsii* and even completely dead plants during harvest had infected seed capsules under soil; however after separating from capsules, seeds were not formed, weak formed or had low market value cause of infection. If we were found the possibility of determination of amount of yield to introduce to market, the effect of mycorrhizal fungi on yield would be higher in practice actually. For instant, obtained yield raise was ensured by mycorrhizal colonization than decreasing diseases intensity. Some studies reported also the effect of mycorrhizal fungi on development of plant yield. In eggplant, after inoculation of *G. etunicatum* and *Gigaspora margarita*, yield was increased by 48.2 and 66%, respectively, under field conditions and the weight of eggplant fruit increased also, by 53.7 and 70.7%, respectively (Matsubara et al., 1995). These findings showed that mycorrhizal fungi inoculation increased the yield and gave best results for control of *S. rolfsii*.

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