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Research Article

Arbuscular Mycorrhiza (AM) Suppressed Fungal Disease Incidence, Severity and Population of Nematode on Soybean (*Glycine max*) L.

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Abstract

Background and Objective: There has been intriguing nutrient soil conservation status associated with mycorrhizal fungus associations with plants. The influence of arbuscular mycorrhiza fungus (AMF) *Glomus mosseae* on disease incidence and severity, nematode population and residual nutrient on soybean was investigated under both screenhouse and field conditions. This was with the view to providing information on the bio protective potential of arbuscular mycorrhiza fungi. **Materials and Methods:** Inoculation of *G. mosseae, S. rolfsii* and *M. incognita* singly and simultaneously near root zones of soybean seedlings was carried out 2 weeks after planting. Symptomological characteristics were taken two weeks after inoculation till maturity. Soils samples were analyzed for nutrient and nematode population before planting and at harvest. **Results:** Soybean plants inoculated with either *Sclerotium rolfsii* or *Meloidogyne incognita* (pathogens) alone expressed higher disease incidence than mycorrhizal plants. The combined application of *G. mosseae*, *S. rolfsii and M. incognita* caused a significant reduction in nematode population while plants inoculated with *M. incognita* alone had the highest population in both sites of studies. Residual phosphorus and nitrogen were obtained in soil treated with mycorrhiza. **Conclusion:** This study shows that mycorrhizal inoculation could be used as a bio-protective agent to suppress the incidence and severity of *S. rolfsii* and *M. incognita* infection in soybean.

Key words: Mycorrhizal fungi, bioremediation, pathogens, soil conservation, environmental safety

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mycorrhizas are widespread symbiotic associations between soil fungi and plants which are present in 92% of all land plant families[1]. The benefits of AMF infection in plants include efficient nutrient uptake[2] especially in infertile soils[3] improved plant growth[4,5] and disease protection against soil-borne pathogens[6]. Arbuscular mycorrhizal (AM) fungi has been found to have dramatic but stimulatory effects on phosphorus nutrition, water transport, N_2 fixation[7], growth and seed production of soybeans[8]. An additional benefit of mycorrhizal symbiosis often reported for soybean and other crops is the suppression of sedentary nematode parasites typically accompanied by improved host tolerance to the nematode[9]. The ubiquitous nature of arbuscular-mycorrhizal fungi (AMF) has been confirmed by available information and the importance of its inoculation has been demonstrated overwhelmingly in recent decades[10-12].

Thus, the exploration of biological control approaches with AMF is therefore necessary to protect man and his environment from the hazardous effects associated with the use of synthetic pesticides in crop production. In addition, the approach will constitute valuable tool in sustainable agriculture. Against this background, this study was conducted to evaluate the effects of *G. mosseae* on disease incidence and severity of *S. rolfsii* and *M. incognita* in soybean.

MATERIALS AND METHODS

Propagation of *Glomus mosseae* (mycorrhiza) inoculum: Mycorrhizal inoculum was multiplied on *Zea mays* using the bucket method. After 3 months of growth, the upper part of the plant was removed and the colonized root were chopped and mixed with dry soil. The mean fungal spore density observed from assessment of soil sample was 800 spore/200 g.

***Sclerotium rolfsii* inoculum:** Five-day old pure cultures of the *S. rolfsii* were comminuted in a warring blender after removing the aerial mycelium. About 1000 mL of sterile distilled water was added to the culture and 16 mL of the suspension served as inoculum for *S. rolfsii*.

***Meloidogyne incognita* cultures:** Chopped root pieces of *Celosia* infected with *M. incognita* were shaken vigorously with 0.5% sodium hypochlorite solution to extract eggs of the nematode[13].

Screen house experiment: Seeds of soybeans were sown in 15 cm diameter pots filled with steam sterilized topsoil and the pots, were arranged in a Completely Randomized Block Design. Seedlings were thinned to 4/pot, 6 days after emergence. Each pot was inoculated with *G. mosseae*, *S. rolfsii* and *M. incognita* singly and simultaneously near the root zone of soybean seedlings 2 weeks after planting. One thousand eggs of *M. incognita* were inoculated close to the roots of plants in the screenhouse. About 30 g of the soil /root fragment mixture served as fresh mycorrhizal inoculum.

Disease incidence was determined by counting diseased leaves expressing it as a percentage of the total number of plants observed. The scoring was based on easily distinguishable symptomatological characteristics using a modified 0-5 scale as described by Tarr[14]:

Where:

0 = All leaves without symptoms
1 = 1- 25% total number with symptoms
2 = 26-50% total leaf number with symptoms
3 = 51-75% total leaf number with symptoms

Field experiment: Field trials were conducted between 2009 and 2010 at the Teaching and Research Farm of Obafemi Awolowo University Ile-Ife, located at 7° 28 N, 4°33 E at 22 m above the sea level in the tropical rainforest area of Nigeria. A plot of 10.2×27.5 m was cleared, ploughed and harrowed. The experiment was laid out in a Randomized Complete Block Design. The field was divided into four blocks of 1.8×1.5 m with an alley of 0.5 m between plots and 1 m between blocks. Seeds were drilled at the spacing of 0.36 cm and 5 m with 5 rows in each plot. The three middle rows were inoculated and sampled while outer row serves as guard rows. The time, method of inoculation and data collected were similar to that of screen house with slight modification in nematode treated plots. While eggs of *M. incognita* were used in the screenhouse, equal quantity of chopped galled *Celosia* infected roots infected with *M. incognita* was used for the field inoculation.

Statistical analysis: All data were transformed using natural logarithm ($x = \log_e y+1$) and subjected to analysis of variance. Treatment means were separated using Duncan's Multiple Range Tests (DMRT) at 5% level of probability.

RESULTS

The incidence and severity of disease was higher in plants inoculated with single pathogens alone in both the screen house and under field conditions (Table 1 and 2, transformed values on Table 3). In the first screen house experiment at 4 weeks after inoculation (WAI) mild to moderate chlorotic symptoms were expressed on all soybean plants except in un -inoculated soybean plants. At 6 WAI more severe basal stem lesions were observed in plants treated with single pathogen alone than in those simultaneously inoculated with the pathogens, in the second trial, at 4 WAI, chlorotic symptoms were moderate on TGX1903-7F and TGX1449+*Glomus moseae*+*Sclerotium rolfsii*. Basal stem lesion was moderate on TGX 1449+*Sclerotium rolfsii* but only mild on TGX1903-7F+*Sclerotium rolfsii*. The uninoculated control treatment only showed mild symptoms of chlorosis. At 6 WAI soya bean plants treated with *S. rolfsii* alone showed moderate symptoms of basal stem lesion. Chlorotic symptoms were observed on plants as early as 6 WAI in both field experiments except uninoculated control plants which showed no symptoms of chlorosis. At 6 WAI, moderate dieback was observed in addition to severe necrotic lesions in plants inoculated with pathogens alone whereas plants treated with *Glomus* either singly or simultaneously showed no symptoms of dieback. Similar trend was observed in the 2 soybean cultivars and for nematode population. The results presented in Table 4 and 5 show that varieties treated with the combination of *M. incognita* and *G. mosseae* had the highest percentage reduction in population of *M. incognita* (93.33%) and (90.90%) under screen house conditions in the first and second trials, respectively. The results in Table 5 show that in the first field experiment, TGX 1449 inoculated with *M. incognita* alone had significantly higher percentage increase in nematode population (181.76%). Results show that the effects of mycorrhiza on residual soil nutrient (NPK). The soil residual N

Table 1: Disease incidence of basal stem rots of soybean at 6 weeks after inoculation under screen house conditions

	Disease symptoms					
	1st planting			2nd planting		
Treatments	Chlorosis	Basal stem lesion	Die back	Chlorosis	Basal stem lesion	Die back
TGX1903-7F+Sr	2	3	2	1	3	1
TGX1903-7F+Gm+Sr	1	1	1	1	2	0
TGX1903-7F+Gm+Sr+Mi	1	1	2	1	2	1
TGX1449+Sr	1	3	2	1	3	1
TGX 1449+Gm+Sr	2	0	1	2	0	0
TGX1449+Gm+Sr+Mi	1	0	0	2	0	0
TGX1903-7F+Gm	1	0	0	0	0	0
TGX1449+Gm	1	0	0	0	0	0
TGX1903-7F	0	0	0	0	0	0
TGX1449	0	0	0	0	0	0

0: No symptoms, 1: Mild symptoms, 2: Moderate symptoms, 3: Severe symptoms, Gm: *Glomus mosseae*, Sr: *Sclerotium rolfsii*, Mi: *Meloidogyne incognita*

Table 2: Disease incidence of basal stem rots of soybean on the field at 6 weeks after inoculation on the field

	Disease symptoms					
	1st planting			2nd planting		
Treatments	Chlorosis	Basal stem lesion	Die back	Chlorosis	Basal stem lesion	Die back
TGX1903-7F+Sr	2	3	2	2	3	2
TGX1903-7F+Gm+Sr	1	0	0	1	0	0
TGX1903-7F+Gm+Sr+Mi	1	0	0	1	0	0
TGX1449+Sr	2	3	0	2	3	2
TGX 1449+Gm+Sr	1	0	0	2	0	0
TGX1449+Gm+Sr+Mi	1	0	0	1	0	0
TGX1903-7F+Gm	0	0	0	0	0	1
TGX1449+Gm	0	0	0	0	0	0
TGX1903-7F	0	0	0	0	0	0
TGX1449	0	0	0	0	0	1

0: No symptoms, 1: Mild symptoms, 2: Moderate symptoms, 3: Severe symptoms, Gm: *Glomus mosseae*, Sr: *Sclerotium rolfsii*, Mi: *Meloidogyne incognita* was increased in both field plantings. The highest value was observed in TGX1903-7F+*Meloidogyne incognita* while the least value was in TGX1449+*Glomus mosseae*+*Sclerotium rolfsii*+*Meloidogyne incognita*. Soil N level in the screen house trials increased only in TGX1903-7F+Srand TGX1449 (control treatments). The residual soil P decreased in all field plantings. TGX1449+*Glomus mosseae*+*Sclerotium rolfsii*+*Meloidogyne incognita* gave highest soil P level while the least value was obtained in TGX1449+*Meloidogyne incognita*. P level only increased in the screen house in the following treatments TGX1903-7F+*Sclerotium rolfsii*, TGX1903-7F+*Glomus mosseae*+*Sclerotium rolfsii*+*Meloidogyne incognita* while it decreased in all other treatments. Residual nutrient in screen house and field experiment were presented in Table 6 and 7, respectively.

Table 3: Effects of *Glomus mosseae* on soybean cultivars inoculated with *Meloidogyne incognita* and *Sclerotium rolfsii*

Treatments	Disease incidence	Disease severity
TGX 1903-7F+Mi	75.700	1.9688
TGX 1903-7F+Gm+Mi	3.4906	1.1563
TGX 1903-7F+Gm+Sr+Mi	3.275	1.1563
TGX 1449+Mi	72.372	1.9688
TGX+1449+Gm+Mi	2.2156	1.0938
TGX+1449+Gm+Sr+Mi	4.9781	1.1875
Mean	27.005	1.4219
LSD	10.366	0.2046

Sr: *Sclerotium rolfsii*, Mi: *Meloidogyne incognita*, Gm: *Glomus mosseae*

Residual K level varied with the various the treatments on the field while it was generally reduced in all the screen house treatments except in TGX1903-7F+*Glomus mosseae* which remained unchanged from the initial level.

DISCUSSION

Disease development and symbiotic establishment of the mycorrhizal fungus is a function of host-pathogen-interaction and time. The influence of mycorrhizal fungus on disease incidence and severity had been attributed to the fact that mycorrhiza usually affect the physiology of the host plant positively by creating or inducing a form of mechanical barrier against the invading pathogen[2]. Findings from both screen house and field experiments in the current study showed that soybean plants inoculated with pathogens alone expressed higher disease incidence and severity while mycorrhizal plants were more resistant to the test pathogens. Similarly, disease severity of pepper caused *Fusarium oxysporum*[2], soybean infected with *S. rolfsii* was observed on inoculation with AM fungi[15]. Nematode population suppression by *G. mosseae* was also observed in this study. In earlier studies, the parasitization of plants by nematode was found to be influenced by the establishment of AM fungi by Todd *et al.*[9], Carling *et al.*[16], Siddiqui and Mahmood[17] and Pinochet *et al.*[18]. The population

Table 4: Effects of *Glomus mosseae*, *Sclerotium rolfsii* on soil population of *Meloidogyne incognita* (number/200 mL soil) infested soybean plants under screen house conditions

	1st trial		2nd trial	
Treatments	At harvest	Change (%)	At harvest	Change (%)
TGX 1903-7F+Mi	372.00^{a}	-78.20^{a}	334.00^{a}	-77.73^{a}
TGX 1903-7F+Gm+Mi	100.00^{c}	-93.33^{c}	158.75^{d}	-89.42^{d}
TGX 1903-7F+Gm+Sr+Mi	118.75^{c}	-92.08^{c}	171.75^{cd}	-88.55^{d}
TGX 1449+Mi	194.75^{b}	-87.02^{b}	291.75^{b}	-80.55^{b}
TGX+1449+Gm+Mi	125.75^{c}	-91.62^{c}	136.50^{e}	-90.90^{e}
TGX+1449+Gm+Sr+Mi	110.75^{c}	-92.62^{c}	189.25^{c}	87.38^{c}

Each value is a mean of 4 replicates, values with the same letter in the same column are not significantly different (p = 0.05) by DMRT, negative values indicate decrease in nematode population, Gm: *Glomus mosseae*, Sr: *Sclerotium rolfsii*, Mi: *Meloidogyne incognita*

Table 5: Effects of *Glomus mosseae*, *Sclerotium rolfsii* on soil population (number/200 mL soil) of *Meloidogyne incognita* infested soybean plots under field conditions

	1st trial			2nd trial		
Treatments	Before planting	At harvest	Change (%)	Before planting	At harvest	Change (%)
TGX 1903-7F+Mi	199.25^{a}	405.25^{a}	103.44^{b}	187.50^{a}	432.7^{ba}	133.00^{a}
TGX 1903-7F+Gm+Mi	130.75^{d}	285.75^{c}	116.56^{b}	204.50^{a}	313.75^{c}	54.07^{c}
TGX1903-7F+Gm+r+Mi	188.75^{ba}	382.75^{ba}	105.42^{b}	178.75^{a}	327.00^{c}	83.696^{c}
TGX 1449+Mi	114.50^{d}	321.75^{bc}	181.76^{a}	224.75^{a}	447.00^{a}	103.93^{ba}
TGX+1449+Gm+Mi	170.75^{bc}	337.75^{bc}	97.19^{b}	194.50^{a}	407.50^{b}	110.08^{ba}
TGX+1449+Gm+Sr+Mi	158.50c	379.00^{ba}	140.97^{b}	225.25^{a}	402.70^{b}	81.026^{c}

Each value is a mean of 4 replicates, values with the same letter in the same column are not significantly different (p = 0.05) by DMRT, Gm: *Glomus mosseae*, Sr: *Sclerotium rolfsii*, Mi: *Meloidogyne incognita*

Table 6: Effects of *Glomus mosseae, Sclerotium rolfsii* and *Meloidogyne incognita* on residual soil nutrient under screen house conditions

	Final		
Treatments	Nitrogen	Phosphorus	Potassium
TGX 1903-7F+Gm	1.23[a]	18.40[c]	0.34[j]
TGX 1449 Gm	0.28[c]	26.20[d]	0.50[d]
TGX 1903-7F+Sr	0.46[b]	29.30[a]	0.50[d]
TGX 1449 Sr	0.21[e]	20.30[k]	0.49[e]
TGX 1903 Mi	0.21[e]	27.80[c]	0.52[c]
TGX 1449 Mi	0.28[c]	25.00[a]	0.56[b]
TGX 1903-7F+Gm+Mi	0.18[f]	22.70[g]	0.42[f]
TGX 1449 Gm+Mi	0.14[g]	22.10[i]	0.42[f]
TGX 1903-7F+Gm+Sr	0.25[d]	20.90[j]	0.42[f]
TGX 1449 Gm+Sr	0.14[g]	23.20[f]	0.38[h]
TGX 1903-7F+Gm+Sr+Mi	0.25[d]	28.60[b]	0.60[a]
TGX 1449 Gm+Sr+Mi	0.21[e]	25.00[e]	0.40[g]
TGX 1903-7F	0.11[h]	22.60[h]	0.50[d]
TGX 1449	0.46[b]	26.20[d]	0.36[i]

Values with the same letters in the same column coded final are not significantly different (p = 0.05) by DMRT, Gm: *Glomus mosseae*, Sr: *Sclerotium rolfsii*, Mi: *Meloidogyne incognita*, initial N: 0.39, initial. P: 26.22, initial K: 0.60

Table 7: Effects of *Glomus mosseae, Sclerotium rolfsii* and *Meloidogyne incognita* on residual soil nutrient under field conditions

	Final		
Treatments	Nitrogen	Phosphorus	Potassium
TGX 1903-7F+Gm	0.32[i]	16.09[c]	0.27[d]
TGX 1449 Gm	0.53[d]	0.60[b]	0.19[i]
TGX 1903-7F+Sr	0.25[j]	0.52[e]	0.24[f]
TGX 1449 Sr	0.56[c]	0.46[g]	0.24[f]
TGX 1903 Mi	0.63[a]	0.49[f]	0.23[g]
TGX 1449 Mi	0.53[d]	0.49[f]	0.26[e]
TGX 1903-7F+Gm+Mi	0.60[b]	0.21[k]	0.23[g]
TGX 1449 Gm+Mi	0.52[e]	0.39[h]	0.23[g]
TGX 1903-7F+Gm+Sr	0.46[g]	6.55[c]	0.23[g]
TGX 1449 Gm+Sr	0.49[f]	12.51[f]	0.23[g]
TGX 1903-7F+Gm+Sr+Mi	0.49[f]	20.26[b]	0.34[a]
TGX 1449 Gm+Sr+Mi	0.21[k]	27.15[a]	0.22[h]
TGX 1903-7F	0.39[h]	7.57[k]	0.28[c]
TGX 1449	0.46[g]	8.34[j]	0.29[b]

Values with the same letters in the same column coded final are not significantly different (p = 0.05) by DMRT, Gm: *Glomus mosseae*, Sr: *Sclerotium rolfsii*, Mi: *Meloidogyne incognita*, initial N: 0.39, initial. P: 26.22, initial K: 0.60

suppression of and (or) improved host tolerance to the root knot nematode (*M. incognita*) by AMF may vary with fungal species and phosphorus nutrition[9]. The increased residual N and P associated with *Glomus mosseae* in this study supports the findings of Chen *et al.*[19]. It has also been reported that *Glomus mosseae* mycorrhizal plants improves phosphorus uptake of the plant[9,19]. Leaf chlorosis was a prominent above ground symptom observed in this study especially in plants inoculated with *M. incognita* alone. *In vitro* bioactivity of *S. rolfsii* against *M. incognita* was reported by Adekunle and Akinsanmi[20] hence this compatible combination can be responsible for lower pathogenic infection. This suggests that root colonization of *G. mosseae* has a negative effect on the development of nematodes. According to Diedhiou *et al.*[21], the pre-inoculation of tomato plants with *Glomus coronatum* or Fo62 (non-pathogenic *Fusarium* strain) stimulated plant growth and reduced *M. incognita* infestation. Similarly Dos Anjos *et al.*[6] reported the protection of *Scutellospora heterograma* (mycorrhizal fungus) of passion fruit against *M. incognita*. The suggested mechanisms of plant protection against soil borne pathogens includes lignifications of cell wall, production of other polysaccharides to prevent penetration of plants (Sharma and Johri[7] and Alban *et al.*[11]). An evidence of localized biochemical defense of *Glomus fasciculatum* colonization against root knot nematode on tomato plant had also been reported by Nagesh and Reddy[22]. The main effects of AM fungi on nematodes seem to be in competition for space nutrient and as well as prevention of juvenile penetration. Since the protective ability of AM fungi against soil-borne diseases is as a result of complex interacting factors at a specific time and situation which include, effect of nematode, fungi, plant species, environmental conditions, time of mycorrhization, period of exposure, while generalization of each unique interaction seems difficult, the research for compatible combinations will be important in agriculture and ecology because of the benefits they provide to the majority of plant cultivars and the conservation of the environment by acting as bio-protectors, bioremediation, bio- fertilizers and bio-control agents.

SIGNIFICANCE STATEMENT

This study discovered that inoculation of soybeans with *Glomus mosseae* (arbuscular mycorrhiza) reduced disease incidence caused by *Sclerotium rolfsii*, suppressed populations of *Meloidogyne incognita* (nematode) and increased the residual soil nitrogen and phosphorus. Thus, a new theory on the interactive effect of *Glomus mosseae* on the test pathogens of soybean and residual soil nutrient may be arrived at.

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