EFFECT OF *GLOMUS MOSSEAE* (ARBUSCULAR MYCORRHIZAL FUNGUS) ON HOST – PARASITE RELATIONSHIP OF *MELOIDOGYNE INCOGNITA* (SOUTHERN ROOT-KNOT NEMATODE) ON FOUR IMPROVED COWPEA VARIETIES

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Abstract: Two pot experiments and a field study were conducted in a Randomized Complete Block Design (RCBD). The experiments were conducted to determine the effect of *Glomus mosseae*, a mycorrhiza fungus, on the reaction of four improved cowpea varieties to *Meloidogyne incognita*. Cowpea plants were inoculated with a single or a combination of 5 000 eggs of *M. incognita* and 50 g of *G. mosseae* inoculum containing 5 spores/g of soil. The standardized method of screening and reporting resistance of crop germplasm to root-knot nematodes at 60 days after planting, and the modified version of including yield for resistance rating at harvest were used for this study. Root galling due to *M. incognita* infection was significantly lower on all the cowpea varieties treated with *G. mosseae* and more significantly on IT90K-277-2 and IT89KD-288 in the screenhouse. *G. mosseae*, suppressed root-knot nematode reproduction on all the varieties compared to cowpea plants infected only by *M. incognita* both in the screenhouse and field experiments. Also, *G. mosseae* mitigated the damage attributable to the root-knot nematode on all these varieties. Using Gall Index (GI), reproduction factor and yield, *G. mosseae* was effective in improving the resistance of the cowpea varieties to *M. incognita*. IT90K-76 cowpea variety was consistently resistant to the root-knot nematode, while IT90K-277-2 was tolerant with *M. incognita* infection but resistant with *G. mosseae* treatment. IT90K-941-1 variety was resistant in the screenhouse. The results of this study also confirmed *G. mosseae* as a potential bio-control agent for *M. incognita* on these cowpea varieties.

Key words: cowpea, *Meloidogyne incognita*, *Glomus mosseae*, root-knot nematode, bio-control

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the major grain legumes in Nigeria and in all the African savanna zones. It serves as a good source of plant protein and for the livelihood of millions (Quin 1997). Cowpea grains contains on the average, 23–25% protein and 50–70% starch. It is rich in lysine with high levels of methione and tryptophan, compared to other legumes (Boultler *et al*. 1975). The low yield of cowpea in production, particularly in Nigeria where 240–300 kg/ha are typical, can be attributed to serious biotic stresses. These stresses include root-knot nematodes, which often affect cowpea throughout its lifecycle (Rachie 1985). Root-knot nematodes are serious pests of cowpea on a worldwide basis (Karajeh 2008). *Meloidogyne incognita* is a major pest found on cowpea in Nigeria and most growing regions of the world (Sasser *et al*. 1984; Caveness 1992).

In some West African countries, cowpea losses, attributable to the *Meloidogyne* species, have been put at between 10 and 89% including total crop losses in a few cases. *M. incognita*, due to its frequency of occurrence and high level of infestation, is considered the most predominant species attacking cowpea in Nigeria (Adesiyan *et al*. 1990). The use of resistant varieties holds out the most promising effective and economic control of root-knot nematodes for resource-poor farmers in Nigeria. Although cowpea breeders have produced varieties that are resistant to many pests and pathogen, most of these varieties do not have grains that command a premium price on the market (Afolami 2002; Faye *et al*. 2002). Resistant varieties can have an unacceptable cooking period or unacceptable taste characteristics, or both. These reasons show that there is a need to reduce crop losses due to nematode attacks on already adopted varieties and a need to increase crop yields. There has been an enhancement in the exploitation of bio-control methods for integrated management of plant parasitic nematodes using ubiquitous antagonistic organisms. Such methods are meant to minimize environmental pollution from chemical control and to keep the root-knot nematode management processes more economical for cowpea farmers in Nigeria (Cabamillas and Baker 1989; Thompson 1998; Maareg and Badr 2000; Korayem *et al.* 2008; Yousef *et al*. 2008; Oclarit *et al*. 2009). Beside these, arbuscular mycorrhizal

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fungi (AMF) are ubiquitous in the soil and are commonly symbiotic with the root systems of many crops supporting shoot growth and phosphorus nutrition in cowpea (Bethlenfalvay and Liderman 1992; Barea and Jefferies 1995). Also, the roots of these crops are often inhabited by AMF and root-knot nematodes because they are obligate feeders and can affect plant health and each others activities (Hussey and Roncadori 1982; Carling *et al*. 1996). The present study was designed to explore the antagonistic potential of *Glomus mosseae* on host-parasite interactions of *M. incognita* and cowpea.

MATERIALS AND METHODS

Source of inoculum and increase

G. mosseae inoculum obtained from the International Institute for Tropical Agriculture, Ibadan, Nigeria (IITA) was cultured on maize in a pot culture. Number of spores per gram of soil was estimated using the methods of Philips and Hayman (1970). *M. incognita* inoculum was raised on *Celosia argentea* in the screenhouse of the Department of Crop Protection, University of Agriculture, Abeokuta, Nigeria.

Pot experiment

Treatments in this study consisted of: individual, and joint inoculation of each organism on cowpea varieties (IT90K-76, IT90K-277-2, IT90K-941-1 and IT89KD-288). Each treatment was replicated six times and arranged in a randomized complete block design in the screen house of the Department of Crop Protection, University of Agriculture, Abeokuta, Nigeria. The experiment was repeated. Sandy loam soil, heat-sterilized for 90 minutes at 65°C with the aid of electric sterilizer and stored for six weeks in jute sacs to restore soil stability before planting, was homogenised and weighed (5 kg) into seven litre plastic buckets used as pots. Three seeds each of a cowpea variety were place in a planting hole and covered with soil. There were 3 seeds per pot. The planting hole was filled with 50 g of *G. mosseae* inoculum made up of soil, 5 spores/g of soil, hyphae, and root fragments of infected maize. One week after emergence, the plants were thinned to one seedling per pot. Two weeks after emergence, the cowpea seedlings were inoculated with 5 000 pure culture of *M. incognita* eggs obtained from heavily galled roots of *C. argentea* using Hussey and Barker (1973) extraction method. This method involved shaking pieces of the clean roots in 0.52% sodium hypochlorite solution in a 500 ml conical flask covered tightly with a rubber bung. The egg suspension was poured into a 200-mesh sieve nested upon a 500-mesh sieve. The eggs collected in the 500-mesh sieve were then rinsed under a gentle stream of cool tap water. Next, the eggs were counted in a Doncaster (1962) counting dish under a stereo microscope. At 60 days after inoculation, three replicates each per treatment, were carefully assayed to avoiding damage to the roots. Galls on each root system were counted after which eggs per root of each root system were assayed as previously described. The number of nematode juveniles in the soil of each treatment unit was determined using the Whitehead and Hemming (1965) modified tray method. Root-knot nematode juveniles were extracted from 250 g sub-samples of respective soil from each treatment. Soil was placed in two plastic sieves sandwiched with double-ply extractor tissue paper and placed in a bowl containing 250 ml of water and left for 18 h. The sieves were removed from the bowls and the nematode suspensions poured into 500 ml nalgene bottles, adjusted to the fill level. After five hours, excess supernatant water was siphoned out with the aid of 3 cm inner diameter siphon tube fixed to the spout. This was done until the siphon process broke up automatically at a factory-fixed level just above the concentrated nematode suspension. The suspensions were observed under the stereomicroscope, and nematode populations counted. Final nematode population was determined by the addition of juvenile and egg populations for each treatment. Final and initial nematode populations were used to determine the reproduction factor of the nematode, using the Oostenbrink (1966) formula: R = Pf ÷ Pi, where: R is reproduction factor, Pi is initial population and Pf is final population (Adegbite *et al*. 2008). At harvest, the remaining three replicates per treatment were uprooted. Following the uprooting, the number of galls per plant were counted, and the seeds in the pods per plant were obtained and weighed using a Metler electronic weighing balance.

Field study

The field study was conducted between January and March 2005, in the vegetable field previously grown with *C. argentea* for three seasons, at the FADAMA of the University of Agriculture, Abeokuta, Nigeria. The experiment was laid out in a Randomized Complete Block Design with three replications. An initial sampling to determine soil population of *M. incognita* after land clearing and field layout, was done as described earlier. The field was planted on a unit treatment plot. The size of the plot was 3 m x 2 m at 60 cm x 25 cm. The field was planted with 3 seeds placed in holes. The holes were filled with 50 g of *G. mosseae* inoculum per pot, and covered with soil. The inoculum was made up of soil, 5 spores/g of soil, hyphae and root fragments of infected maize. One week after planting the cowpea seedlings were thinned to 2 seedlings per hole. At 60 days after planting, destructive sampling of three randomly selected plants per plot on the field, was done to assess the number of galls per plant and number of *M. incognita* eggs per root system. The rest of the plants were grown to maturity for yield assessment. Root knot nematode juveniles were extracted from 250 g sub-samples of respective soil from each treatment. The reproduction factor and final population were also determined. Data collected were subjected to analysis of variance (ANOVA) and means separated using Duncan's test using SAS 8.0 software at the significance level $p \leq 0.05$.

RESULTS

Effect of *G. mosseae* on root galling and *M. incognita* reproduction, on cowpea varieties in a greenhouse and in field experiments

Tables 1 and 2 show the effect of *G. mosseae* on root galling and *M. incognita* reproduction in the greenhouse and field experiments. At 60 days after inoculation, *G. mosseae* reduced root galling and *M. incognita* reproduction on all the cowpea varieties in all the experiments. Also at harvest, *G. mosseae* reduced galling and *M. incognita* reproduction on all the varieties and significantly on infected plants of IT90K-277-2 cowpea variety in the field.

Host status of cowpea varieties to *M. incognita* as influenced by *G. mosseae*

Table 3 shows the resistance rating of the cowpea varieties to *M. incognita* and as influenced by *G. mosseae* based on actual yield (Afolami 2000). In the first pot experiment, *G. mosseae* had no effect on the resistance rating of infected plants of IT90K-76 cowpea variety-rated resistant. IT90K-277-2 cowpea variety was rated tolerant and IT90K-941-1 cowpea variety was rated resistant. In fact, the results show that *G. mosseae* improved the resistance rating of IT89KD-288 cowpea variety to *M. incognita,* which was rated tolerant as compared to nematode infected plants which were rated susceptible. However, *G. mosseae* inoculation had no effect on the resistance rating of all the cowpea varieties in the second pot experiment.

In the field, *G. mosseae* improved the resistance rating of the *M. incognita* infected plants of IT90K-277-2 and IT90K-941-1 cowpea varieties. The *M. incognita* infected plants of IT90K-277-2 and IT90K-941-1 cowpea varieties were rated resistant, as compared to nematode infected plants rated tolerant in IT90K-277-2 and hypersusceptible on IT90K-941-1 cowpea varieties.

Table 1. Influence of *G. mosseae* on root galling and *M. incognita* reproduction, on four improved cowpea varieties (at 60 days after inoculation)

Variety	Treatment	Pot experiment 1		Pot experiment 2		Field experiment	
		number of galls/plant	nematode reproduction	number of galls/plant	nematode reproduction	number of galls/plant	nematode reproduction
IT90K-76	*M. incognita*	4.66 c	0.87 cd	3.50 bc	0.87 bc	4.00 b	0.84 b
	M. incognita + *G. mosseae*	1.00 d	0.82 cd	2.66 c	0.32 c	2.00 b	0.45 b
IT90K-277-2	*M. incognita*	4.66 c	3.34 a	2.33 c	0.63 bc	27.00 a	22.32 a
	M. incognita + *G. mosseae*	2.33 d	2.61 b	5.00 ab	0.46 c	1.00 b	0.47 b
IT90K-941-1	*M. incognita*	6.66 b	1.05 c	2.33 c	0.39 c	6.00 b	0.98 b
	M. incognita + *G. mosseae*	3.33 bc	0.41 d	2.00 c	0.32 c	3.00 b	0.32 b
IT89KD-288	*M. incognita*	17.33 a	1.29 c	7.33 a	1.35 a	5.00 b	0.66 b
	M. incognita + *G. mosseae*	16.66 a	1.17 c	4.83 bc	1.00 ab	2.00 b	0.23 b
±SE		0.82	0.74	0.67	0.01	1.48	0.001
CV		10.00	10.86	9.49	27.06	5.04	23.49

Figures are means of 3 replicates in experiments 1 and 2 but nine replicates on field experiment; values with the same alphabetical letter(s) within each experiment do not differ significantly based on Duncan's Multiple Range Test

Table 2. Influence of *G. mosseae* on root galling and *M. incognita* reproduction, on five cowpea varieties at harvest

Variety	Treatment	Pot experiment 1		Pot experiment 2		Field experiment	
		number of galls/plant	nematode reproduction	number of galls/plant	nematode reproduction	number of galls/plant	nematode reproduction
IT90K-76	*M. incognita*	4.21 c	0.91 c	4.11 b	0.92 b	3.00 b	0.85 b
	M. incognita + *G. mosseae*	1.14 d	0.51 cd	2.66 c	0.41 c	1.00 b	0.50 b
IT90K-277-2	*M. incognita*	3.72 cd	3.20 a	2.52 c	1.61 a	29.00 a	2.31 a
	M. incognita + *G. mosseae*	2.24 d	2.10 b	4.00 b	1.41 a	2.00 b	0.47 b
IT90K-941-1	*M. incognita*	5.42 c	0.90 c	2.40 c	0.41 c	4.00 b	0.91 b
	M. incognita + *G. mosseae*	1.12 d	0.40 d	2.11 c	0.40 c	2.00 b	0.31 b
IT89KD-288	*M. incognita*	18.42 a	1.18 c	12.81 a	1.32 a	4.00 b	0.61 b
	M. incognita + *G. mosseae*	10.11 b	1.10 c	5.42 b	1.00 b	1.00 b	0.61 b
±SE		0.89	0.97	1.24	0.76	0.24	0.02
CV		34.40	35.04	24.38	30.48	24.01	25.49

Explanation: see table 1

Table 3. Resistance ratings of cowpea varieties to *M. incognita* as influenced by *G. mosseae* based on actual yield

Variety	Pot experiment 1							Pot experiment						Filed experiment					
	nematode Infection	GI	R factor	yield			degree of resistance	GI	R factor	yield			degree of resistance	GI	R factor	yield			degree of resistance
				[a] infected	control [b]	[a–b]				[a] infected	control [b]	[a–b]				[a] infected	control [b]	[a–b]	
IT90K–76	–	2	0.91	13.26	15.45	–2.19 ns	resistant	2	0.92	7.59	4.24	3.35**	resistant	2	0.85	439	461	–22ns	resistant
	with mycorrhiza	1	0.51	13.20	15.45	–2.25 ns	tolerant	2	0.41	6.94	4.24	2.70**	resistant	1	0.50	548	376	172*	resistant
IT90K–277–2	–	2	3.20	25.85	17.18	8.67 ns	tolerant	2	1.61	10.13	5.98	4.1**	tolerant	3	2.31	596	386	210*	tolerant
	wth mycorrhiza	2	2.10	27.94	17.18	10.766 ns	tolerant	2	1.41	10.49	5.98	4.15**	tolerant	1	0.47	515	304	211*	resistant
IT89KD–288	–	3	1.18	17.37	29.34	–11.97*	susceptible	3	1..32	3.90	7.46	–3.56*	susceptible	1	0.61	214	359	–145*	hypersu-sceptible
	with mycorrhiza	3	1.17	24.68	29.34	–4.66 ns	tolerant	2	1.00	10.32	7.46	2.86*	tolerant	1	0.61	282	359	–98ns	resistant
IT90K–941–1	–	2	0.90	9.02	9.49	–0.47 ns	resistant	2	0.40	6.07	3.78	2.29 ns	resistant	2	0.91	369	496	127*	hyper-sceptible
	with mycorrhiza	2	0.40	9.64	9.49	–0.15 ns	resistant	1	0.41	5.08	3.78	1.30 ns	resistant	2	0.31	401	311	90 ns	resistant

ns – not significant
* significant at p = 0.05
GI – Gall Index

DISUSSION

Arbuscular mycorrhizal (AM) fungi as biological control for many pathogens are obvious. The protective effects of AM inoculation may be both systemic and localized and there is evidence supporting both types of induced resistance (Linderman 1994). It is evident that an increased capacity for nutrient acquisition resulting from mycorrhiza association could help; resulting in stronger plant health. Better plant health could be done through a more specific increase in protection [improved resistance and/or tolerance against biotic and abiotic stresses (Koch *et al*. 1997)].

Furthermore, mycorrhiza may decrease or increase nematode penetration, development and reproduction. If nematode reproduction is decreased, the plant and its fungal symbiont can be described as having induced resistance or it can be described as a poorer host to the parasitic nematode than non-mycorrhizal plant. Mycorrhizal fungi also are capable of directly interacting with sedentary states of plant – parasitic nematodes. The reaction of plant growth to these interactions may be positive, negative or neutral. Yield, therefore, is dependent dynamically upon how much damage the nematode is doing and how much benefit the plant is deriving from the fungus. If growth is enhanced, the plant (again with its fungal symbiont) can be described as more tolerant to the nematode (Hussey and Roncadori 1982; Smith 1987).

In this study, *G. mosseae* significantly suppressed nematode reproduction and damage on IT89KD-288, *G. mosseae* improved plant tolerance to *M. incognita* in the greenhouse. In addition, the resistance of IT90K-76, IT90K-277-2 and IT90K-941-1 cowpea varieties to *M. incognita* was improved by *G. mosseae* showing: reduction in nematode reproduction, reduction of galling and significant increase in yield on IT90K-76 and IT90-277-2 in the field. The findings in this study conform to the postulate of Sivaprasad *et al*. 1990 and findings of Florini (1997). Thus, it is suggested that *G. mosseae* can be used as a potential bio-control agent for *M. incognita* on these cowpea varieties.

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POLISH SUMMARY

DZIAŁANIE *GLOMUS MOSSEAE* (ARBUSKULARNY GRZYB MYKORYZOWY) NA WZAJEMNE STOSUNKI ROŚLINY ŻYWICIELSKIEJ I PASOŻYTA *MELOIDOGYNE INCOGNITA* (GUZAK POŁUDNIOWY) NA CZTERECH ULEPSZONYCH ODMIANACH WSPIĘGI CHIŃSKIEJ

Przeprowadzono dwa doświadczenia wazonowe i badania polowe metodą kompletnych bloków losowych (RCBD), w celu określenia działania *Globus mosseae*, grzyba mikoryzowego, na reakcję czterech ulepszonych odmian wspięgi chińskiej na *Meloidogyne incognita*. Rośliny wspięgi zaszczepiono pojedynczymi jajami lub ich kombinacją w ilości: 5 000 jaj *M. incognita* i 50 g inokulum *G. mosseae* zawierającym 5 zarodników na 1 g ziemi oraz 50 g *G. mosseae* zawierającym 5 zarodników w 1 g ziemi. Wykorzystano standardową metodę skryningu oraz podawania informacji dotyczącej odporności plazmy zarodkowej na guzaka południowego w 60 dni po sadzeniu roślin. W badaniach tych wykorzystano zmodyfikowaną wersję metody z włączeniem danych o plonie do określenia odporności w czasie zbiorów. Powstawanie narośli w wyniku infekcji wywoływanej przez *M. incognita*, było istotnie niższe na wszystkich odmianach wspięgi traktowanych *G. mosseae* oraz istotniejsze na IT90K-277-2 i IT89KD-288 w pomieszczeniu skryningowym. *G. mosseae* ograniczał reprodukcję mątwika na wszystkich odmianach roślin wspięgi zainfekowanych tylko przez *M. incognita* zarówno w pomieszczeniu skryningowym, jak i w doświadczeniach polowych. *G. mosseae* maskował szkodę przypisywaną mątwikowi *M. incognita* na wszystkich badanych odmianach. Wykorzystując indeks powstawania narośli (GI), czynnik reprodukcji i dane o plonie, stwierdzono, że *G. mosseae* był skuteczny w poprawianiu odporności na *M. incognita* na odmianach wspięgi. Odmiana IT90K-76 była trwale odporna na mątwika *M. incognita*, podczas gdy IT90K-277-2 była tolerancyjna na porażenie przez *M. incognita*, ale była odporna, gdy potraktowano ją *G. mosseae*. Odmiana IT90K-941-1 była odporna w pomieszczeniu skryningowym. Wyniki przeprowadzonych badań potwierdziły, że *G. mosseae* jest potencjalnym czynnikiem biologicznego zwalczania *M. incognita* na powyższych odmianach wspięgi.