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SCREEN HOUSE AND FIELD INVESTIGATIONS OF Arbuscular mycorrhiza AND ORGANIC FERTILIZER FOR THE CONTROL OF THE ROOT – KNOT NEMATODE, *Meloidogyne incognita* INFECTING COWPEA IN SOUTH WESTERN, NIGERIA

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ABSTRACT

This study was undertaken to determine the potential of individual and combined effects of *Glomus mosseae,* a mycorrhiza fungus and organic fertilizer for the management of *Meloidogyne incognita,* a root knot nematode infection of cowpea (IT90K-277-2) under Screen house and field conditions. The standardised method of evaluating crop germplasm for resistance to *M. incognita* including crop yield was employed. *M. incognita* caused significant reduction in the yield components of the cowpea variety both in the screen house and under field conditions. Single and combined treatments of *Glomus mosseae* and organic fertilizer significantly increased the pod weight, grain yield and number of pods per plant of cowpea plants as compared to nematode infected plants. Single treatments of *Glomus mosseae* and organic fertilizer significantly suppressed root galling; inhibited nematode reproduction and nematode population both in the screen house and under field conditions. The mixture of *G. mosseae* and organic fertilizer as a treatment was more effective than individual treatments in suppression of *M. incognita.* This study shows that *G. mosseae* has potential in the management of Root knot nematodes of cowpea and should be exploited with organic fertilizer serving as a viable carrier in Nigeria.

Keywords: Biological control, Root – knot nematode, Mycorrhiza, Organic amendment, cowpea, *Meloidogyne incognita*, *Glomus mosseae*

INTRODUCTION

The *Meloidogyne* spp. are cosmopolitan pests which occur in association with wide range of crops in the temperate, tropical and subtropical regions of the world where they reduce crop yield in quantity and quality (Adesiyan *et al*., 1990). Root knot nematodes are serious pest of cowpea (*Vigna unguiculata* L. Walp) on a worldwide basis with *Meloidogyne incognita* found as a predominant pest on cowpea in south western Nigeria and most crop growing regions of the world (Caveness, 1992; Sasser *et al*., 1984). Losses on cowpea attributable to *Meloidogyne* species in some West African countries have been put at between 10% and 89% including total crop losses in some cases (Adesiyan *et al*., 1990).

The enormous crop losses caused by this nematode warrant efficient control measures to increase the chances of protecting the

world population from hunger particularly in the tropics where cowpea is one of the major grain legumes. In Nigeria and the entire African savanna zone, cowpea grain serves as a good plant protein source to livelihood of millions (Quin, 1997). Cowpea grains contain on the average, 23-25% protein and 50-70% starch. It is rich in lysine with high levels of methionine and tryptophan compared to other legumes (Boultler *et al*., 1975). Chemical control of nematodes, though very effective, has proved too expensive for resource poor farmers in the developing countries where the cost of control exceeds profit made from crop sale. However attempts to keep plant parasitic nematode population low at a safe level could be achieved by a combination of several control methods.

The potential of using certain biocontrol agents and soil additives such as soil amendments had been extensively studied (Zaki and Bhatti, 1990; Caswell *et al*., 1991; Maareg and Badr, 2000; Odeyemi I, 2004). *Arbuscular mycorrhizal* (AM) Fungi and soil borne pathogens commonly occurred together in the root or rhizosphere of the same plant. However, studies have shown that AM fungi decreases the incidence of diseases, limit nematode development and activities in plant roots and minimises growth suppression of host plants by root knot nematode (Hussey and Roncadori, 1982; Carling *et al*., 1996). The parasitation of plants by endoparasitic nematodes can be suppressed by the establishment of AM fungi. The penetration rate of parasitic nematodes can be decreased; their development inside the root may be retarded, or the degree of damage caused by the nematode lowered by AM fungi (Rajeswari – Sundarababu *et al,* 2001, Cheng *et al.,* 2001, Talavera *et al*., 2001, Sankaranarayana *et al*., 2001, Stroebel *et al*.,1992).

Amending the soil with organic material as often resulted in improved plant growth. However, these may be due as much to improved plant growing conditions to biotic competition with nematodes. Population densities of *M. incognita* were lowered on cowpea when one ton/ha of dried pulverized kolanut (*Cola nitida*) pods was applied to the ridges fabrics after planting (Oyedunmade *et al*., 1995). Amending the soil with six tons/ha of partially decayed, flaked, dried cocoa pod husks reduced root knot galling by 27% in the field trials and increased measured grain yield by 7%. In field trials although the lowest population densities of *M. incognita* were found in plot treated with the nematicide cabofuran, the highest net revenue per hectare and the best crop growth were obtained by adding 10 tons/ha of cocoa pods husks or cassava peel to the soil (Egunjobi and Olaitan 1986, Egunjobi 1985). These, have encouraged the formulation of organic fertilizers. Also, the efficacy of organic amendment is known to increase many times when used with microbial antagonists (Rodriguez-kabana *et al*. 1990, Khan and Hussain, 1988).

The present study was, therefore, carried out to examine the single and combined effect of *Glomus mosseae*, a mycorrhizal fungus and the newly formulated organic fertilizer from Oyo State Ministry of Agriculture, in the control of root knot nematode, *Meloidogyne incognita* infecting cowpea.

MATERIALS AND METHODS

Mycorrhiza culture

The inoculum of *G. mosseae* obtained from the International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria was main-

tained and increased on maize. Fifty grams of mycorrhiza inoculum of *G. mosseae* made up of soil, spores, hyphae and root fragments of infected host was placed in the planting hole when planting the Maize (Oba super II) seeds in sterilised soils placed in plastic buckets (used as pots) and watered daily for eight weeks. A week to termination of the maize plants, watering was stopped to allow high infection of host root. The number of *G. mosseae* spores present in the inoculum was estimated by putting fifty grams of soil from the composite sample in one liter of water in a beaker, after breaking up the large soil clumps by hand and the mixture was stirred for 10 to 15 seconds. The coarse particles were allowed to settle in the water for 5 to 10 seconds after which the suspension was decanted through stacked sieves with coarse sieve on top (420 to 250 μm) , medium size opening sieve(250 –105 μm) in the middle and a fine pore sieve (105 to 45 μm) at the bottom. This was repeated to increase the likelihood that a majority of the spores in the soil have been removed. The content on the lowest sieve was backwashed into a petri plate after which the spores were examined under a stereomicroscope and counted (Daniels and Skipper, 1982).

Nematode inoculum culture

The inoculum of *Meloidogyne incognita* was obtained from heavily galled roots of *Celosia argentea* maintained in a pot culture in the screen house of the Department of Crop Protection University of Agriculture, Abeokuta, Nigeria. Eggs were obtained from the roots of *C. argentea*, using Hussey and Barker (1973) extraction method by shaking segments of clean roots for five minutes in 0.52% sodium hypochlorite solution in 250 ml conical flask. The resulting egg suspension was quickly poured into a 200-mesh sieve nested upon a 500-mesh sieve. Eggs caught in the 500-mesh sieve were then rinsed under gentle stream of cool tap water for four minutes and enumerated in a Doncaster (1962) counting dish under stereo microscope.

Organic fertilizer

The organic fertilizer made from industrial and municipal waste was obtained from the Oyo State Ministry of Agriculture, Ibadan, Oyo State, Nigeria. The chemical property of the organic fertilizer before use is contained in Table1.

Table 1: Chemical properties of Organic fertilizer produced by Oyo State Ministry of Agriculture

Property	Value
pH (water)	7.93
pH(KCl)	7.47
O.C (g/kg)	19.6
N (g/kg)	5.0
Avail. P (Brayl) (mg/kg)	0.01
K (mg/kg)	0.15
Ca (mg/kg)	0.06
Na (mg/kg)	0.4
Mg (Mg/kg)	57.5
Fe (mg/kg)	33.0

Soil Source and Soil Sterilization

Sandy-loam topsoil (87.0% sand, 9.8% silt, 3.2% clay, pH 5.86 at 29.5°C) used for the screen house study was sourced from the OARPTIN experimental field of the University of Agriculture Abeokuta, Nigeria. The soil was thoroughly mixed and heat sterilized using an electric soil sterilizer at 65^{0}C for 90 minutes. The sterilised soil was stored in jute sacks and rested for six weeks to restore stability.

Screen house studies

The potted experiment consisting of single and combined inoculation of 5,000 eggs per plant of *M. incognita*, 5 spores/g soil of *G. mosseae* and 1% w/w of organic fertilizer on cowpea IT90K-277-2 variety (already screened susceptible to *M. incognita*; Odeyemi, 2004) obtained from the International Institute of Tropical Agriculture, Ibadan (IITA) was conducted in the Screen house of the Department of Crop Protection, University of Agriculture, Abeokuta, Nigeria. The experiment was laid out in a Completely Randomized Design with six replicates per treatment. 5kg of sterilized sandy loam was measured into each of the 5Ltr buckets used as plastic pots and 50 g of mycorrhiza inoculum containing 5 spores/g of soil was thoroughly mixed with the soil. Organic fertilizer was mixed with the soil two weeks before planting and watered to facilitate mineralization. Three seeds of cowpea IT90K-277-2 were planted per pot. The cowpea seedlings were thinned to a plant per pot one week after emergence. The cowpea seedlings were thereafter, inoculated with 5,000 eggs of *M. incognita*. The inoculation point was covered with sterile soil and wetted lightly to enable freshly hatched second-stage juveniles to locate and penetrate cowpea roots (Ihekwuemere *et al*., 1995). The Noninoculated cowpea plants were only grown in sterile soil only.

Eight weeks after inoculation, three replicates each of the treatments were destructively sampled by gently uprooting the cowpea plants from the soil in the pots to observe their root for root gall initiation and to determine the rate at which the nematode multiplied in the roots of cowpea plants. Each root system was washed in water and mopped dry with white tissue paper. Roots were subsequently weighed, cut into about 2-cm pieces, and thoroughly mixed. A 10 g subsample was then taken for egg and juvenile extraction using the Hussey and Barker (1973) sodium hypochlorite method. The total number of juveniles and eggs from each root system was estimated from these subsamples in each case. Similarly, the total number of juveniles from soil was estimated from a 250 g subsample using the Whitehead and Hemming (1965) modified tray method. Soil was placed in two sieves sandwitched with double-ply extractor tissue paper and placed in a plastic bowl containing 500 ml water and left for 24 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 inside diameter siphon tube inserted to the spout, until the siphon process breaks up automatically at a factory fixed level just above the concentrated nematode suspension. Five 1 ml aliquots of the suspensions were observed under the stereomicroscope, and nematode populations counted. Final nematode population was determined by the addition of larval and egg populations for each treatment. Final and initial nematode populations were used to determine the reproduction factor of the nematode, using the for-

mula, R = Pf ÷ Pi, where R is reproduction factor, Pi is initial population and Pf is final population. The three remaining replicates of each treatment provided data for the second phase of the experiment: number of pods per plant, pod weight and grain yield.

Field studies

The field experiments were undertaken in late August during 2006 and 2007 cropping season in the experimental field of OARPTIN, University of Agriculture, Abeokuta, Nigeria (7°.15¹N and 3°. 25¹E). *Celosia argentea* had been grown intensively in this field for over five years; consequently *Meloidogyne incognita*, a pest of Celosia *argentea* occur at high levels every year. The experiments were laid out in a Randomised Complete Block Design with three replications. An initial sampling to determine soil population of *M. incognita* was done. The field was planted on a unit treatment plot size of 3 m x 2 m at 60 cm x 25 cm planting spacing with three seeds per stand which was later thinned one week after planting to 2 seedlings per hole. Organic fertilizer was incorporated at 10 tons/ha before planting. Also, 50 g of mycorrhiza inoculum containing 5 spores/g of soil was placed in the planting hole when planting the seeds (Atayese *et al.,* 1993). Weeding was done on the 21st of September 2006 and 30th of September in 2007. Insect pest was controlled using shepaphus™ at 40ml/20 liter of water. It was sprayed at 10 days interval beginning from 2 weeks after planting.

Data were taken eight weeks after planting on three randomly selected cowpea plants per treatment plot. The number of galls per root system was estimated. *Meloidogyne incognita* eggs were collected from the roots according to the procedure of Hussey and Barker (1973) and reproduction factor estimated using the Oostenbrink's 1966 formula. At harvest, data were collected on number of pods per plant, pod weight and grain yield per treatment plot using Metler electronic weighing balance.

Data analysis

Data collected were subjected to analysis of variance (ANOVA). Data collected on number of eggs recovered from roots and number of juveniles from root and soil of the nematode were natural logarithm transformed before subjecting it to analysis of variance. Significant means based on ANOVA were separated using Least Significant Difference (LSD).

RESULTS

Pathogenicity of M. incognita on cowpea

M. incognita infection significantly reduced the yield components of the cowpea variety in the screen house (Table 2). The number of pods per plant, pod weight and grain yield were reduced by 32.00, 36.03 and 34.17% respectively in the screen house when compared with nematode free cowpea plants. Further analysis showed that number of pods per plant, pod weight and grain yield were significantly lowest on cowpea plant inoculated with *M. incognita* only compared to other treatments (Table 3). In the field experiments, the root knot nematode significantly reduced the number of pods per plant, pod weight and grain yield of the infected cowpea plants as compared to other treatments in the two planting seasons (Table 4).

Single and concomitant effect of G. mosseae and organic fertilizer on M. incognita and yield of cowpea

G. mosseae inoculation improved significantly the pod weight and grain yield of cowpea plants with respect to the control in the screen house studies and in the field studies with respect to cowpea plants infected by the

Table 2: Effect of *M. incognita* on some yield parameters of cowpea (Pot Experiment)

Parameter	Inoculated	Control	LSD
Number of pods/ plant	7.44	11.03	2.46*
Pod weight	15.69	24.83	6.40*
Grain yield	10.87	16.97	5.20*

*= Significant at $p<0.05$

Table 3: Interactive effect of *G. mosseae, M. incognita* and Organic fertilizer on some yield parameters of cowpea (Pot Experiment)

Treatment	Pods/plant	Pod weight (g)	Grain yield (g)
Control	11.16	24.27	14.67
Nematode infected (N)	6.61	19.27	7.92
N x G. mosseae (G)	10.77	23.97	16.67
N x Organic fertilizer (O)	11.50	28.27	16.07
N x G x O	12.11	27.44	21.2
LSD	2.60*	2.98*	5.20*

*= Significant at $p<0.05$

Table 4: Effect of *G. mosseae, M. incognita* and Organic fertilizer on some yield parameters of cowpea (Field Experiment)

Treatment	Pods/plant		Pod weight (Kg / ha)		Grain yield (Kg / ha)	
	2006	2007	2006	2007	2006	2007
Nematode infected (N)	34.00	30.00	354.16	316.66	164.43	146.66
N x *G. mosseae* (G)	45.60	42.34	389.16	366.25	182.29	166.87
N x Organic fertilizer (O)	47.00	42.12	383.33	362.91	183.75	166.25
N x G x O	48.50	47.28	402.08	391.67	185.00	167.75
LSD	4.51*	5.60*	27.98*	39.44*	14.51*	19.51*

*= Significant at $p<0.05$

Table 5: Effect of *G. mosseae* inoculation on some yield parameters of cowpea (Pot Experiment)

Parameter	Inoculated	Control	LSD
Number of pods/ plant	10.75	11.11	2.05ns
Pod weight	23.93	28.37	1.40*
Grain yield	14.62	19.63	2.88*

Ns = not significant (p> 0.05) ,*= Significant at p<0.05

Table 6: Effect of Organic fertilizer treatment on some yield parameters of cowpea (Pot Experiment)

Parameter	Control	Organic fert.	LSD
Number of pods/ plant	10.58	11.46	3.64ns
Pod weight	22.94	28.50	3.88*
Grain yield	13.47	22.40	5.26*

Ns = not significant (P> 0.05) ,*= Significant at P<0.05

Table 7: Effect of *G. mosseae M* and Organic fertilizer treatment on root galling, *M. incognita* reproduction and population on cowpea

Treatment	Galls/ root system			Nematode Reproduc-tion			Nematode population		
	Pot	Field		Pot	Field		Pot	Field	
		2006	2007		2006	2007		2006	2007
Nematode in-fected (N)	33.50	45.66	56.98	3.40	4.55	4.06	4.13	3.41	3.97
N x G. mosseae (G)	18.06	12.45	14.34	1.20	0.68	0.77	3.06	2.13	2.88
N x Organic fertilizer (O)	10.04	10.00	12.01	0.88	1.00	1.02	3.08	3.02	3.00
N x G x O	10.01	7.45	13.34	0.97	0.66	0.98	2.72	2.54	2.68
LSD	6.24*	5.44*	12.78 *	1.04*	2.47*	1.34*	1.01*	0.97*	0.82*

*= Significant at p<0.05

M. incognita only (Table 5, 3 and 4). However *G. mosseae* inoculation had no significant effect on number of pods per plant of cowpea both in the screen house and on the field. Organic fertilizer application significantly increased the yield components of the cowpea variety in the screen house and on the field (Table 6). Grain yield, pod weight were significantly higher on cowpea plants infected with the nematode and treated with organic fertilizer than on cowpea plants infected by the nematode only both in the screen house and on the field (Table 3 and 4). Interactive effect of *G. mosseae* inoculation and 10 tons/ha of organic fertilizer on yield components of cowpea was significant (Table 3 and 4). Highest number of pods per plant, pod weight and grain yield were observed on cowpea despite nematode infection when both *G. mosseae* and organic fertilizer were combined as treatment relative to single treatments with *G. mosseae*, organic fertilizer and the control plants in the screen house (Table 3) and relative to cowpea plants infected by the nematode only on the field (Table 4). *G. mosseae* effect on Root –knot nematode reproduction, number of galls per root system and nematode population was significant both in the screen house and in the field studies (Table7). Root galling, nematode reproduction and nematode population were significantly suppressed on cowpea plant inoculated with *G. mosseae* and nematode than on cowpea plants infected by the nematode only both in the screen house and on the field. Also, Organic fertilizer application at 10 tons/ha significantly reduced root galling, nematode, reproduction and population on cowpea plants both in the screen house and on the field as compared to cowpea plant infected by *M. incognita* only (Table 7). However, highest significant suppression of number of galls, nematode reproduction and nematode population were observed on cowpea plants infected by the nematode and treated with combination of *G. mosseae* and organic fertilizer (Table 7). *Glomus mosseae* significantly reduced root galling and inhibit nematode reproduction and more evidently in mixture with 10 tons/ha organic fertilizer application (Table7).

DISCUSSION

In this study, *G. mosseae* with or without organic fertilizer was effective in lowering root galling, reducing fecundity of *M. incognita* and increasing cowpea yield components. The effect of *G. mosseae* on nematode root galling, reproduction, population and yield components of cowpea was more pronounced when the mycorrhizal fungus was combined with organic fertilizer. Similar studies by Amer and Zaki (2002), Radwad *et al*. (2004); Youssef *et al*. (2008) confirm the finding of this study. Also Rodriguez – kabana *et al*. (1987); Cheng *et al*. (2000) employed biological control agents in combination with organic materials thus suggesting organic materials enhance biological activities against target nematodes by providing the needed nutrients for initial growth of biological agents and the production of ammonia that affects plant parasitic nematodes. Breakdown of organic materials may release toxic and nematicidal substances that contribute to nematode control. Additionally, organic residues have been associated with a build-up of free – living nematodes that contribute to the control of root – knot nematodes (Esmard *et al*., 1998). *G. mosseae* as a potential biocontrol for root – knot nematodes may become part of integrated nematode management in view of the fact that organic materials could serve as carriers to facilitate culturing and distribution of these nematode antagonists.

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