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RESEARCH ARTICLE

BIO-MANAGEMENT OF ROOT KNOT NEMATODE *MELOIDOGYNE JAVANICA* IN SUGARCANE BY COMBINED APPLICATION OF ARBUSCULAR MYCORRHIZAL FUNGI AND NEMATOPHAGOUS FUNGI

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Abstract

A glass house experiment was conducted to study the integrated use of two arbuscular mycorrhizal fungi (AMF) *Glomus fasciculatum* and *Glomus mosseae* and three nematophagous fungi viz. *Arthrobotrys oligospora, Paecilomyces lilacinus* and *Pochonia chlamydosporia* for the management of root knot nematode *Meloidogyne javanica* in sugarcane. Increased shoot and root growth was observed due to either individual or combined inoculations of bioagents in nematode-free plants as compared to nematode alone control plants. Among the treatments, *G. fasciculatum* + *P. lilacinus* combination recorded maximum shoot weight followed by *G. mosseae* alone but were on par with each other. Reduction in *M. javanica* population was recorded in all the bioagent inoculated plants compared to nematode alone used as control. Reduction in *M. javanica* due to AMF or bioagents or combination of both organisms ranged between 29 to 47 per cent. Maximum reduction of *M. javanica* was recorded in *G. fasciculatum* + *P. lilacinus* combination. Compatible association between AMF and other bioagents was observed. Increased mycorrhizal colonization (2 to 17% increase) was recorded in combined treatments of AMF and nematophagous fungi than in plants treated with AMF alone. The combined application of *G. fasciculatum* with *P. lilacinus* for root knot nematode control indicated the compatibility of the components and their potential for integration in root knot nematode management in sugarcane crop system.

Keywords: Sugarcane, *Meloidogyne javanica,* biological control, AMF, nematophagous fungi

Introduction

Plant parasitic nematodes are one of the important biotic constraints in sugarcane production in subtropical and tropical regions of the world. About 208 species of nematodes belonging to 48 genera have been reported in sugarcane. Among these, the root-knot nematode *Meloidogyne javanica*, a sedentary endoparasite, causes severe galling of roots in sugarcane with changes in the cell morphology (Nirmala and Mehta 1994). *Meloidogyne javanica* induces the formation of giant cells in cortical region accompanied by cell wall lysis, cellular hypertrophy and damage to the cells of epidermis and those in cortical and vascular regions. Nematode control using chemical nematicides has become environmentally unsafe and economically unviable due to removal of efficient fumigant nematicides from world market on environmental grounds. There is a need to develop environmentally and economically sound alternatives to nematicides for sustainable nematode management in sugarcane ecosystem. Biological control of plant parasitic nematodes using fungi and bacteria has been found to be a feasible option. Among the various kinds of organisms engaged in biological control of nematodes, arbuscular mycorrhizal fungi (AMF) and

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nematophagous fungi, such as *Paecilomyces lilacinus, Pochonia chlamydosporia* and *Arthrobotrys oligospora* are now attracting considerable attention as potential biocontrol agents. The interaction between AMF and nematodes has been studied and reduction in nematode population demonstrated. Significant reduction of root-knot nematodes or reduced formation of root galls was reported in different plants inoculated with *Glomus mosseae, Glomus fasciculatus* or *Glomus macrocarpus* (Sikrora and Schonbeck 1975; Kellam and Schenck 1980; Sankaranarayanan and Rajeswari Sundarababu 1994 & 1998). *Paecilomyces lilacinus* infects the eggs and females of root-knot nematodes and destroys the embryo within five days (Jatala 1986) and is a successful biocontrol agent under different conditions (Siddiqui and Mahmood 1996; Rumbos et al. 2006). *Pochonia Chlamydosporia* infects and destroys the eggs of root-knot and cyst nematodes (Kerry 2000; Van Damme et al. 2005). *Arthrobotrys oligospora* is a nematode trapping fungus which forms three dimensional traps to capture nematodes in the soil and is considered a potential bioagent against *M. incognita* juveniles (Dhawan et al. 2004). These fungi have shown biocontrol activity against *M. javanica* on sugarcane in our earlier studies (Anonymous 2004 & 2006). In general, a single biocontrol agent is used against a single pathogen (Wilson and Backman 1999) which may sometimes lead to inconsistent performance by the biocontrol agent, because a single agent is not active in all soil environments or against all pathogens that attack the host plant. On the other hand, dual inoculation with biocontrol agents having different mechanisms of action is known to provide greater biocontrol against plant pathogens on different crops than inoculation with a single agent (Sankaranarayanan and Rajeswari Sundarababu 2001; Guetsky et al. 2002).

In the present study, the AMF *G. fasciculatum* and *G. mosseae* were used alone and in combination with three nematophagous fungi *A. oligospora, P. lilacinus* and *P. chlamydosporia* to find out the suitable combination for biological management of root-knot nematode *Meloidogyne javanica* in sugarcane.

Materials and methods

Cultivar selection and pot mixture preparation: Single budded setts of sugarcane (cv. Co 86032) were planted in earthen pots (20 cm dia., volume 2kg) filled with sterilized (autoclaved at 121°C and 1.5 atm for 2 h) mixture of red soil : sand : farm yard manure (2:2:1 v/v). Planting was done one month before the commencement of the experiment. Tomato (cv. Co 3) seedlings required for nematode culture were similarly raised in two kg capacity earthen pots containing autoclaved pot mixture; three seedlings were maintained in a pot.

Nematode culture: The inoculum required for raising pure culture of root knot nematode *M. javanica* was obtained from tomato plants maintained at Nematology glass house, Sugarcane Breeding Institute, Coimbatore. Roots with conspicuous galls were selected, washed gently but thoroughly in water and examined for the presence of egg masses under stereo microscope. Galls which showed the protruding bodies of the mature females covered with gelatinous matrix were dissected and the egg masses were then kept individually in embryo cups half filled with water. Perineal pattern of the females was prepared for confirmation of the species as *M. javanica*. The egg masses collected were utilized for raising pure cultures on tomato seedlings. The larvae that emerged from each individual egg mass were collected and inoculated in the soil at the base of the tomato plants by making small holes. The pots were maintained in the glass house and regularly irrigated with tap water that was passed through 325 mesh sieve. The plants were uprooted gently 45 days after nematode inoculation, carefully washed in water and examined for well developed egg masses. Such egg masses were removed and transferred to petri dishes containing adequate amount of water and incubated at room temperature in the laboratory; the larvae that hatched were used in the present study.

Inoculum preparation and application

AMF inoculum: The starter inoculum of arbuscular mycorrhizal fungi *G. fasciculatum* and *G. mosseae* maintained in sorghum plants in Nematology glass house, Sugarcane Breeding Institute, Coimbatore was used in the study. The starter cultures consisted of live AMF spores, root tissues of the previous host and soil. The starter cultures of *G. fasciculatum* and *G. mosseae* were transferred separately to earthen pots containing red soil, sand and FYM (2:2:1). Pearl millet (cv. WCC 75) seeds were sown and thinned down to eight plants/pot after germination. Spore count and mycorrhizal colonization were assessed 60 days after planting. About 20g of AMF inoculum containing spores (35 to 40 spores/g), hyphal fragments and root bits along with soil was used per pot in the experiment.

Nematophagous fungal inoculum: The nematophagous fungi *A. oligospora, P. lilacinus* and *P. chlamydosporia* were isolated from the rhizosphere of sugarcane and maintained in the culture collection of Nematology laboratory, Sugarcane Breeding Institute, Coimbatore. One hundred gram sorghum grain was soaked in water for one hour in 500 ml conical flasks and autoclaved at 15 psi. After cooling, the flasks were inoculated with a 5 mm disc of each fungus and incubated at 24°C in dark for 25 days. After incubation, the fungal growing substrates were transferred to sterile polyethylene bags under aseptic condition and the bags were shaken for uniform distribution. The nematophagous fungi were applied as per the following dosage; *A. oligospora* @$18x10^8$ CFU/pot; *P. lilacinus* @ $21x10^8$ CFU/pot and *P. chlamydosporia* @$11x10^8$. CFU/pot.

AMF fungi and nematophagous fungi were applied in the sugarcane root zone by removing 10 cm layer of soil without damaging the sugarcane root system and the soil was replaced. One week later, second stage juveniles of *M. javanica* were inoculated @ 2000 juveniles/pot at the root zone by making small holes in the soil around the plant stem. The plants were supplemented weekly with Hoagland's nutrient solution (Hoagland and Arnon 1950) lacking phosphorus.

Observations: The experiment was conducted in the glass house in a completely randomized blocked design with 24 treatments and three replications. Data on shoot length, shoot weight, root length and root weight, nematode reproduction, number of galls, AMF colonization and nematophagous CFU were recorded three months after inoculation. AMF root infection levels were assessed from randomly selected root material which was cut into 1 cm pieces, cleared in KOH and stained in tryphan blue (Phillips and Hayman 1970); per cent root colonization was determined as per Giovannetti and Mosse (1960). The CFU of nematophagous fungi in the soil was estimated by taking one gram soil and serial dilution plating on PDA media. As two independently conducted experiments produced almost identical results, the data were pooled and subjected to analysis of variance (P = 0.05). Tukey's tests (P = 0.05) were then used to distinguish differences between treatments. All these analyses were performed using SPSS statistical programme (SPSS, 2007).

Results and discussion

Significant increase in the growth of sugarcane was observed with application of AMF alone. Both AMF *G. mosseae* and *G. fasciculatum* caused significant increase in sugarcane fresh shoot weight (40 and 36% respectively) over uninoculated control plants without nematode treatment (Table 1). Treatment with nematophagous fungi *P. lilacinus*, *P. chlamydosporia* and *A. oligospora* had no significant effect on fresh shoot weight in the absence of nematode. The combination of AMF with nematophagous fungi recorded increased fresh shoot weight than the treatment with nematophagous fungi alone. Among the combined treatments without nematode, *G. fasciculatum* + *P. lilacinus* recorded maximum fresh shoot weight (41%) followed by *G. fasciculatum* + *A. oligospora* (38%). Inoculation of nematode *M. javanica* alone

Table 1. Effect of AMF, nematophagous fungi and *Meloidogyne javanica* on shoot weight of sugarcane

Treatments		Shoot fresh wt (g)		Shoot dry wt (g)	
		- Nematode	+ Nematode	- Nematode	+ Nematode
Control		68.4 ef	50.6 g	24.0 fg	16.9 h
AMF	*G. fasciculatum* (Gf)	93.3 a	67.3 ef	33.9 abc	29.0 cdefg
	G. mosseae (Gm)	95.6 a	63.1fg	36.4 ab	25.5 defg
Nematophagous fungi (NF)	*P. lilacinus* (Pl)	68.6 ef	65.3 f	24.6 efg	25.2 defg
	P.chlamydosporia (Pc)	67.6 ef	65.3 f	23.7 g	24.6 efg
	A. oilgospora (Ao)	67.8 ef	68.1 ef	24.9 defg	24.5 efg
G. fasciculatum + NF	Gf + Pl	96.5 a	79.7 bcde	36.6 a	36.4 ab
	Gf + Pc	71.6 cdef	74.0bcdef	28.9 cdefg	26.6 defg
	Gf + Ao	94.3 a	73.1 cdef	27.1 defg	30.4bcde
G. mosseae + NF	Gm + Pl	84.8 abc	74.2 bcdef	30.8 abcd	29.9 cdef
	Gm + Pc	86.9 ab	70.3 def	29.4 cdefg	26.0 defg
	Gm + Ao	83.6 abcd	72.7 cdef	30.5 bcde	30.0 cdef

Values within each column followed by the same letter are not significantly different (Tukey's test $p<0.05$)
Pl= *Paecilomyces lilacinus*; Pc= *Paecilomyces chlamydosporia*; Ao= *Arthrobotrys oligospora*; Gm= *Glomus mosseae*; Gf= *Glomus fasciculatum*

significantly reduced fresh shoot weight (26%) over uninoculated control plants. In general application of either AMF or nematophagous fungi alone or in combination significantly increased fresh shoot weight of nematode inoculated plants than control treatment with nematode alone. Similar trend was also observed with shoot dry weight (Table 1). Among the treatments, *G. fasciculatum* + *P. lilacinus* recorded maximum shoot dry weight (52.3% increase over control) followed by *G. fasciculatum* + *P. lilacinus* + nematode treatment (51.6%) but both were on par with each other and showed that combined application of AMF and nematophagous fungus could compensate the nematode damage and improve the growth of sugarcane plants. In case of root weight (wet weight and dry weight) also, combinations of *G. fasciculatum* + *P. lilacinus* and *G. fasciculatum* + *P. lilacinus* + nematode recorded maximum value than other treatments and the per cent increase of root wet weight was 26 and 24 per cent respectively over the control treatment (Table 2). Among the AMF and nematophagous fungi combination with nematode, *G. fasciculatum* with nematophagous fungi combination favoured increased root growth than *G. mosseae* and nematophagous fungi combinations.

Application of AMF and nematophagous fungi significantly reduced galling and nematode population over the nematode control (Table 3). Application of single netmatophagous fungus to sugarcane plants showed a reduction of root knot galls and it ranged from 36 per cent (*A. oligospora*) to 57 per cent (*P. lilacinus*). The gall reduction was more when nematophagous fungi were combined with AMF and reductions in galling ranged from 38 to 67 per cent.

Glomus fasciculatum and nematophagous fungi combination reduced the galling in the range of 54-67 % than *G. mosseae* and nematophagous combinations (39 to 55%). Among the AMF and nematophagous combinations, maximum reduction of galls (67%) was recorded with *G. fasciculatum*

Table 2. Effect of AMF, nematophagous fungi and *Meloidogyne javanica* on root weight of sugarcane

Treatments		Root fresh wt (g)		Root dry wt (g)	
		- Nematode	+ Nematode	- Nematode	+ Nematode
Control		24.5 def	16.3 i	4.7 cdef	2.7 h
AMF	*G. fasciculatum* (Gf)	29.4 ab	21.4 gh	5.8ab	5.1 bcdef
	G. mosseae (Gm)	28.8 abc	18.9 hi	5.8 ab	3.0 gh
Nematophagous fungi (NF)	*P. lilacinus* (Pl)	24.3 defg	26.6bcde	4.8cdef	5.4abc
	P. chlamydosporia (Pc)	23.9 efg	21.6 fgh	4.7 cdef	5.2 bcdef
	A. oilgospora (Ao)	25.5 de	22.2 fg	4.7 def	3.5 g
G. fasciculatum + NF	Gf + Pl	31.0 a	26.8 bcd	6.0 a	5.7 ab
	Gf + Pc	26.5 cde	25.5 de	5.5 ab	5.5 ab
	Gf + Ao	26.7 bcde	26.3 cde	5.1 bcdef	5.7 ab
G. mosseae + NF	Gm + Pl	28.9 abc	29.1 abc	5.4 abc	4.5 f
	Gm + Pc	26.4 cde	24.5 def	5.7 ab	4.6 ef
	Gm + Ao	29.8 a	26.5 cde	5.3 abcde	4.6 ef

Values within each column followed by the same letter are not significantly different (Tukey's test $p<0.05$)
Pl= *Paecilomyces lilacinus*; Pc= *Paecilomyces chlamydosporia*; Ao= *Arthrobotrys oligospora*; Gm= *Glomus mosseae*; Gf= *Glomus fasciculatum*

Table 3. Effect of AMF, nematophagous fungi on root galling and *Meloidogyne javanica* population in sugarcane

Treatments		No. of root knot galls/ 5g root	Nematode population/200g soil
Control		44.0 a	346.7 a
AMF	*G. fasciculatum* (Gf)	23.3 bcde	223.3 bc
	G. mosseae (Gm)	27.6 bc	203.3 bc
Nematophagous fungi (NF)	*P. lilacinus* (Pl)	20.0 def	205.0 bc
	P. chlamydosporia (pc)	20.0 def	205.0 bc
	A. oilgospora (Ao)	28.0 b	243.3 b
G. fasciculatum + NF	Gf + Pl	14.6 f	180.0 c
	Gf + Pc	20.3 cdef	193.3 bc
	Gf + Ao	17.3 ef	190.0 bc
G. mosseae + NF	Gm + Pl	20.0 def	190.0 bc
	Gm + Pc	27.0 bcd	203.3 bc
	Gm + Ao	22.0 bcdef	196.7 bc

Values within each column followed by the same letter are not significantly different (Tukey's test $p<0.05$)
Pl= *Paecilomyces lilacinus*; Pc= *Paecilomyces chlamydosporia*; Ao= *Arthrobotrys oligospora*; Gm= *Glomus mosseae*; Gf= *Glomus fasciculatum*

Table 4. Mycorrhizal and nematophagous fungi development in sugarcane with or without *Meloidogyne javanica*

Treatments		AMF colonization (%)		Nematophagous fungi CFUg^{-1} soil	
		- Nematode	+ Nematode	- Nematode	+ Nematode
Control		0.0	0.0	0.0	0.0
AMF	*G.fasciculatum* (Gf)	55.0 bcd	50.0 d	0.0	0.0
	G. mosseae (Gm)	54.0 bcd	52.7 cd	0.0	0.0
Nematophagous fungi (NF)	*P. lilacinus* (Pl)	0.0	0.0	4.0×10^5	5.0×10^5
	P. chlamydosporia (Pc)	0.0	0.0	3.0×10^4	4.6×10^4
	A. oligospora (Ao)	0.0	0.0	3.3×10^3	4.3×10^3
G.fasciculatum + NF	Gf + Pl	66.7 a	64.0 ab	4.3×10^5	5.6×10^5
	Gf + Pc	56.7 abcd	56.3 abcd	3.3×10^4	5.3×10^4
	Gf + Ao	61.7 abc	54.0 bcd	3.6×10^3	4.6×10^3
G. mosseae + NF	Gm + Pl	56.3 abcd	56.7 abcd	4.0×10^5	5.0×10^5
	Gm + Pc	54.7 bcd	55.7 abcd	3.0×10^4	4.3×10^4
	Gm + Ao	58.3 abcd	53.3 bcd	3.3×10^3	4.0×10^3

Values within each column followed by the same letter are not significantly different (Tukey's test $p<0.05$)
Pl= *Paecilomyces lilacinus*; Pc= *Paecilomyces chlamydosporia*; Ao= *Arthrobotrys oligospora*; Gm= *Glomus mosseae*; Gf= *Glomus fasciculatum*

+ *P. lilacinus*. Almost the same trends were observed for nematode population also (Table 3).

Application of nematophagous fungi favoured the mycorrhizal colonization in sugarcane roots. Increased mycorrhizal colonization in combined treatments with nematophagous fungi ranged from 55 to 67 per cent (Table 4). In presence of nematode, *G. fasciculatum* + *P. lilacinus* combinations recorded the maximum mycorrhizal colonization (64%) followed by the combination of *G. mosseae and P. lilacinus*. Application of AMF favoured the nematophagous fungal development and spore production as evidenced from the CFU value.

In the present study, AM fungi and nematophagous fungi were tested either alone or in combinations to know which bioagent combinations are most suitable for the bio-management of root-knot nematode *M. javanica* in sugarcane. Application of AMF had reduced the nematode population compared to nematode alone treatment. The interaction between these fungi and nematodes have been studied and reduction in nematode population densities demonstrated by several workers (Bagyaraj et al. 1979; Sankaranarayanan and Rajeswari Sundarababu 2009, 2010). Such a reduction of nematode population and gall index resulted in increased growth of the plants. This reduction in the severity of disease caused by *Meloidogyne* spp. in mycorrhizal plants might be due to the altered biochemical constituents in the host plant (Sikora and Schonbeck 1975) or improved plant nutrition especially phosphorus or alteration of compounds of root exudates or alteration of the physiological components of AMF root due to increased lignin levels in the exodermis of mycorrhizal plants. Presence of increased quantities of sugars, amino acids like phenylalanine and serine, and phosphorus may individually or collectively play a role in suppressing the development of *M. incognita* in mycorrhizal plants (Krishnaprasad 1971). It is speculated that the same kind of mechanism might

have reduced the nematode population in sugarcane examined in the present study. The three nematophagous fungi used in this present study recordd drastic reduction of *M. javanica* population. It is a well-known fact that *A. oligospora*, *P. lilacinus* and *P. chlamydosporia* infect the eggs and females of root-knot nematodes and destroy the embryo within five days (Jatala 1986) and are known to be successful biocontrol agents under different conditions (Siddiqui and Mahmood 1996). These microorganisms are thought to coexist and not compete for substrates and space on the host root. AMF with nematophagous fungi had an additive effect in reducing galling and nematode population. In the present study, application of *G. fasciculatum* and *P. lilacinus* was the most successful combination treatment for reducing nematode multiplication on sugarcane roots, possibly due to their different mechanisms of action. Arbuscular mycorrhizal fungi reduce nematode damage by competition for nutrient sources and space, as well as by prevention of juvenile penetration (Hallmann and Sikora 1996). *Paecilomyces lilacinus* is an egg-parasite, which suppresses the nematode inoculum in the soil (Kiewnick and Sikora 2004). Maximum growth of crop plants and control of root knot nematodes *Meloidogyne* spp. were achieved by several workers by integration of AMF with nematode bioagents *P. lilacinus* (Bhat and Irshad Mahmood 2000) *P. chlamydosporia* (Rao et al. 2003) as compared to individual application of any bioagent (Siddiqui and Akhtar 2009; Guetsky et al. 2002; Siddiqui and Akhtar 2008). Different modes of action by these organisms probably resulted in synergistic effects in increasing plant growth (Guetsky et al. 2002). The present study clearly indicated the beneficial role of nematophagous fungi in enhancing the mycorrhizal development and resulting in maximum colonization. AMF also enhanced the CFU of nematophagous fungi in the soil. To conclude, in the combined application of *G. fasciculatum* and *P. lilacinus* for root knot nematode control, the two fungi appear to be compatible with each other indicating their potential for integration in root knot nematode management in sugarcane crop system.

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