

RECIPROCAL INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGUS AND ROOT KNOT NEMATODE AND INTERACTION EFFECTS ON BLACKGRAM

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Summary. Experiments were conducted under glasshouse conditions to study the reciprocal influence of the arbuscular mycorrhizal fungus (AMF) *Glomus fasciculatum* and the root-knot nematode *Meloidogyne incognita* and their interaction effects on the growth of blackgram. Prior inoculation of AMF increased significantly shoot and root growth and pod yield of blackgram, especially when applied 20 days before nematode inoculation, and suppressed root gall index and the nematode population in the soil, with earlier application of AMF resulting in greater suppression of the nematode. Inoculation of the nematode prior to AMF affected negatively root mycorrhizal colonization and spores in the soil with the suppressing effects being more pronounced when nematodes were inoculated 20 days prior to AMF. AMF treatments increased phosphorus content of shoots and roots of blackgram.

Keywords: *Glomus fasciculatum*, interaction, *Meloidogyne incognita*, *Vigna mungo*.

Plant-parasitic nematodes, including endoparasitic nematodes and arbuscular mycorrhizal fungi (AMF) often occur together in the rhizosphere and colonize the same area of roots of host plants and, therefore, interact with each other. The interaction between AMF and nematodes has been studied by several workers (Hussey and Roncadori, 1982; Elsen *et al.*, 2003; de la Peña *et al.*, 2006) and it has resulted in nematode reduction (Sankaranarayanan and Rajeswari Sundarababu, 1994; John and Bai, 2004; Kantharaju *et al.*, 2005; Siddiqui and Akhtar, 2007), no effect (Hasan and Jain, 1987) or even an increase in numbers of nematodes (Atilano *et al.*, 1981). Numerous studies have reported that AMF can increase host tolerance or resistance in many plant/nematode systems. Recently, Elsen *et al.* (2008) demonstrated that AMF have the ability to induce systemic resistance against plant-parasitic nematodes in the roots. However, most of the studies did not address whether the improved host response was a result of improved host nutrition, antagonism or competition between the nematode and mycorrhizal fungus. Thus, there is a need to study the interaction effects between these two groups of organisms in terms of their effects on plant growth or yield and in terms of their reciprocal influence.

Early evidence of possible interactions between AMF and nematodes was obtained from surveys in which spores and mycelia of AMF were found to be more abundant in fields free of *Meloidogyne incognita* (Kofoid *et al.* White) Chitw. than in fields where this nematode

was present (Schenck and Kinloch, 1974). The sequence in which plants are inoculated with nematodes and AMF was found to affect the interaction between the two organisms. Pre-inoculation of cowpea with either *Glomus fasciculatum* (Thaxter) Gerd. *et* Trappe or *G. epigaeus* Daniels *et* Trappe, to allow these slow growing symbionts to become established in the roots 15 days before introduction of *M. incognita* or *Heterodera cajani* Koshy, resulted into fewer juveniles penetrating and developing to maturity in roots of mycorrhizal plants than in roots of non-mycorrhizal plants (Jain and Sethi, 1988). Pre-inoculation of plants with AMF reduced *M. incognita* infestation in tomato (Diedhiou *et al.*, 2003) and cucumber (Zhang *et al.*, 2008). The effects of nematodes on mycorrhizal development have been variable. Atilano *et al.* (1981) found that spore production of *G. fasciculatum* was reduced by *M. incognita*. The other variables of mycorrhizal formation that may be affected by nematodes include percent mycorrhizal colonization, development of extra-matrical mycelia and vesicle formation in roots (DeSouza, 1979; Germani *et al.*, 1981). However, in most studies the presence of nematodes did not significantly alter the arbuscular mycorrhizal colonization (Kellam and Schenck, 1980; Saleh and Sikora, 1984).

Meloidogyne incognita is considered to be one of the more serious pests of blackgram *Vigna mungo* (L.) Hepper. Previous studies suggested that plant-parasitic nematodes, including *Meloidogyne* spp., adversely affect nodulation, N₂ fixation and yield in legumes (Hussaini and Seshadri, 1975). Among the various kinds of organisms engaged in biological control of nematodes, mycorrhizal fungi, especially AMF, are now attracting greater attention as potential biocontrol agents. Therefore, the

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objective of this research was to study the interactions between the AMF *G. fasciculatum* and *M. incognita* and their effects on growth and yield of blackgram.

MATERIAL AND METHODS

Nematode culture. The inoculum required for raising a pure culture of *M. incognita* was obtained from tomato plants maintained in a glass-house at the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore. Perineal patterns of the females were prepared for confirmation of the species as *M. incognita*. Roots with conspicuous galls were selected, washed gently but thoroughly in water and examined for the presence of egg masses under a stereo microscope. Galls which showed the protruding bodies of the mature females covered with gelatinous matrix were dissected and the egg masses were kept individually in embryo cups half-filled with water. The egg masses collected were utilized for raising pure cultures. Tomato cv. Co.5 seedlings were raised in 2-litre capacity earthen pots containing autoclaved pot mixture (red soil:sand:FYM, 2:2:1 v/v). Three seedlings were maintained in a pot and the juveniles that emerged from the egg masses were collected, mixed and inoculated at 2000 juveniles/pot into small holes made in the soil at the base of the tomato plants. The pots were maintained in a glass-house and regularly irrigated with tap water passed through a 325 (44 µm pore size) mesh sieve. The plants were up-rooted 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 water in which they were incubated for 10 days in the laboratory at room temperature (27 °C ± 2). The juveniles that emerged were collected in beakers, aerated daily and 2- or 3-day-old cultures were used in the study.

AMF inoculum. The inoculum of *G. fasciculatum* used in the experiments was maintained in the culture

collection of the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore. The starter cultures consisted of living AMF spores, root tissues of the previous host and soil and was inoculated in earthen pots (20 cm diameter) containing red soil, sand and FYM (2:2:1 v/v). Pearl millet cv. WCC 75 seeds were sown and thinned to eight plants/pots after germination. Sixty days after planting, the number of spores and mycorrhizal colonization was assessed. About 20 g of AMF inoculum, containing spores (25 ± 3 spores/g soil), hyphae and mycorrhizal root fragments along with soil, was used per pot in both experiments.

Effect of AMF on nematode. The pot culture experiment was conducted to assess the effect of AMF *G. fasciculatum* on the root knot nematode *M. incognita* inoculated at different timings under glass-house conditions (temperature range 30 ± 2 °C and day light length of 12 h and 30 min.). For this, clay pots of 20 cm diameter were filled with 2 kg of 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). The characteristics of the soil were: sandy loam, pH 7.8, electrical conductivity 0.5 dS/m, available N 74 kg/acre, available P 4 kg/acre and available K 259 kg/acre. Two seeds/pot of the blackgram cv. Co.5 were sown and plants were thinned to one per pot after seed germination. There were seven treatments *viz.*, 1) AMF alone, 2) AMF followed by nematode inoculation 5 days later; 3) AMF followed by nematode inoculation 10 days later; 4) AMF followed by nematode inoculation 15 days later; 5) AMF followed by nematode inoculation 20 days later; 6) Nematode alone and 7) uninoculated control. Each treatment was replicated four-fold and the experiment set out in a completely randomized design. Clay pots were first inoculated with 20 g/pot of AMF, then sown with two seeds/pot of the blackgram cv. Co.5 and thinned to one seedling/pot after seed germination. Nematode inoculation timing was according to treatments. Second stage juveniles of *M. incognita* were inoculated at the rate of 2,000/pot into small holes made in the rhizosphere in the soil around

Table I. Effects of *Glomus fasciculatum* inoculated prior to *M. incognita* at different timings on growth components of blackgram¹.

Treatment	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Pod yield (g)
AMF alone	34.2	33.2	33.7	7.0	6.6
AMF + Nematode 5 days later	23.4	17.5	36.6	4.2	4.2
AMF + Nematode 10 days later	26.8	20.2	26.0	4.4	4.7
AMF + Nematode 15 days later	30.5	25.4	30.8	4.8	5.1
AMF + Nematode 20 days later	32.1	26.6	31.6	5.7	5.8
Nematode alone	16.8	9.7	11.8	3.1	1.8
Uninoculated control	21.3	15.4	23.8	2.9	3.2
LSD (P = 0.05)	2.7	1.3	2.3	1.1	1.2

¹Means were compared by Fisher's protected LSD test.

Table II. Effect of *G. fasciculatum* on *M. incognita* inoculated at different timings on nematode reproduction and mycorrhizal colonization on blackgram¹.

Treatment	Gall index ²	Nematode population/ 200 g soil	Spore population/ 50 g soil	Mycorrhizal colonization (%)	Total P content (%)	
					Shoot	Root
AMF alone	1.0	0.0	173.2	65.4	0.56	0.17
AMF + Nematode 5 days later	3.6	422.6	145.0	39.8	0.45	0.33
AMF + Nematode 10 days later	3.2	363.0	139.0	42.0	0.47	0.36
AMF + Nematode 15 days later	2.4	266.0	137.0	50.2	0.32	0.41
AMF + Nematode 20 days later	2.0	188.4	155.4	54.3	0.53	0.42
Nematode alone	5.0	616.7	0.0	0.0	0.18	0.12
Uninoculated control	1.0	0	0.0	0.0	0.26	0.14
LSD (P = 0.05)	0.5	0.5	4.8	2.3	0.02	0.02

¹ Means were compared by Fisher's protected LSD test. Analyses were carried out using $\sqrt{(n + 0.5)}$ transformed data for nematodes and spores in soil; untransformed means are given in the table.

² Root galling was rated on a 1-5 scale: 1 - no gall; 2 - 1-25% galls; 3 - 26-50% galls; 4 - 51-75% galls and 5 - >75% galls.

the plant stems. The plants were fertilized weekly with Hoagland's nutrient solution (Hoagland and Arnon, 1950) lacking P. The experiment was repeated once.

Effect of nematode on AMF. In another experiment, the effect of *M. incognita* on multiplication of AMF *G. fasciculatum* inoculated at different timings was evaluated in pots in the same conditions as indicated above. There were seven treatments *viz.*, 1) Nematode alone, 2) nematode followed by AMF inoculation 5 days later; 3) nematode followed by AMF inoculation 10 days later; 4) nematode followed by AMF inoculation 15 days later; 5) nematode followed by AMF inoculation 20 days later; 6) AMF alone and 7) uninoculated control. One week after germination the plants were inoculated with second stage juveniles followed by AMF at the different timings. Inoculation of nematode and AMF, pot size, soil, blackgram seeds, experimental design and crop maintenance were as in the previous experiment. The experiment was repeated once.

Assessment of variables and data analysis. Data on plant growth and yield components, nematode reproduction, gall index (Heald *et al.*, 1989), AMF spore population, mycorrhizal colonization and total phosphorus content of plants were recorded 70 days after sowing. AMF root infection levels were assessed from randomly selected root material after cutting the entire root systems into 1-cm-long pieces. Roots were cleared in KOH and stained in trypan blue (Phillips and Hayman, 1970). Per cent root colonization was determined as observed by Giovannetti and Mosse (1980). The total P content in the plant material was estimated by vanadomolybdate in the nitric acid system (Jackson, 1973).

Data on plant growth variables, nematode reproduction and mycorrhizal colonization were analyzed by analysis of variance (ANOVA) when the conditions for ANOVA (*i.e.*, normal distribution and homogeneity of variances) were met. Experimental results were similar

in both experiments so the data were pooled for analysis. Data on nematode count and mycorrhizal spore population were transformed to square root ($n + 0.5$) before analysis. Means were compared using Fisher's protected least significant difference test (LSD) at $P = 0.05$.

RESULTS

Effect of AMF on nematode. Inoculation of *M. incognita* alone significantly reduced plant growth relative to uninoculated control plants. Significant increases in the growth of blackgram plants were observed with prior application of *G. fasciculatum* when compared to uninoculated plants and those inoculated with nematodes alone (Table I). Treatment with *G. fasciculatum* significantly increased growth of nematode-inoculated plants compared to uninoculated plants. Among the dual inoculation treatments, greatest shoot growth, root growth and pod yield of blackgram was observed when nematodes were inoculated 20 days after the application of *G. fasciculatum*, and this was significantly different from other dual inoculated plants. Inoculation of nematodes 5 and 10 days after AMF application resulted in reduced growth of blackgram.

Root gall index and nematode population were greatest in plants treated with the nematode alone (Table II). Prior establishment of the AMF had adverse effects on *M. incognita*, significantly suppressing the formation of the galls on the roots and the nematode population in the soil, irrespective of the time of inoculation. However, the degree of such suppressive effects depended upon the time of AMF inoculation, with earlier application resulting in greater suppression of the nematode. The AMF suppressed root galling to the range of 28 to 60% and nematode population in the soil to the range of 31 to 69% of the values for plants treated with the nematode alone. Among the dual inoculated plants, the greatest

gall reduction and nematode population in the soil were when nematodes were inoculated 20 days after the AMF.

The plants treated with AMF alone had the greatest spore and mycorrhizal colonization. The combined treatments had adverse effects on mycorrhizal spore population and mycorrhizal colonization in roots. Root knot nematodes suppressed the spore population to the extent of 10 to 20% and mycorrhizal colonization to the extent of 16 to 40% of the values for plants treated with AMF alone. The least spores were found in the AMF + nematodes 15 days later treatment and the least mycorrhizal colonization was in the AMF + nematodes 5 days later treatment.

All AMF treatments resulted in significantly higher shoot and root phosphorus contents of blackgram plants compared to plants inoculated with nematodes alone and uninoculated plants (Table II). Among the dual inoculated treatments, AMF + nematodes 20 days later had the greatest shoot and root P content. The AMF + nematodes 5 days later treatment had the lowest phosphorus content, which was decreased by 19 and 29% in shoots and roots, respectively, relative to the AMF alone treatment.

Effects of nematode on AMF. The effects of application of AMF at different time intervals after nematode inoculation on the various growth measures of blackgram are presented in Table III. Inoculation of *M. incognita* caused significant reductions in shoot length, shoot weight and root length relative to the uninoculated control plants. Application of *G. fasciculatum* caused significant increases in shoot length, shoot weight, root length, root weight and pod yield relative to nematode inoculated plants. Among the dual inoculated treatments, the greatest shoot and root lengths were found when AMF was applied 5 days after the nematode inoculation and these values were significantly greater than in other dual treatments. The two treatments of AMF applied 10 days and 15 days after inoculation of nematodes were similar with respect to shoot and root length. Plants inoculated with AMF alone had the greatest root

weights, followed by uninoculated control plants. Among the dual inoculated plants, AMF inoculation 20 days after nematodes gave the greatest root weight. Highest pod yield was obtained in plants inoculated with AMF alone followed by AMF application 5 days after nematode inoculation.

Plants inoculated with the nematode alone had the greatest density of root knot galls and the greatest nematode population in the soil. Nematode soil population density and intensity of the disease increased with delaying the application time of AMF. Plants inoculated with AMF 5 days after inoculation of the nematode had the lowest gall indices (Table IV) but the values were not significantly different to those of plants inoculated with AMF 10 and 15 days after inoculation with nematodes. Post-application of AMF to blackgram plants reduced the nematode population in soil by 10 to 30%, with the greatest reduction occurring when AMF was inoculated 5 days after nematodes, although this was on par with AMF inoculated 10 days after inoculation of nematodes.

Pre-inoculation of nematodes affected mycorrhizal development adversely and this effect was more pronounced when nematodes were inoculated 20 days prior to AMF. The adverse effect of the nematode on AMF was 35 to 64% on mycorrhizal spore population and 38 to 72% on mycorrhizal colonization compared to plants receiving AMF alone. Greatest spore population and colonization were in plants inoculated with AMF 5 days after nematode inoculation.

The amount of phosphorus in shoots and roots significantly increased in all the mycorrhizal inoculated plants compared to the nematode alone treatment. Shoot phosphorus content was increased from 0.19% in the nematode alone treatment to 0.41% when the plants were inoculated with AMF 5 days after nematode inoculation. There was a progressive decrease from 0.41% to 0.28% of shoot phosphorus in plants inoculated with AMF 5 days and 20 days after nematode inoculation. A similar trend was seen with respect to root phosphorus content.

Table III. Effects of inoculation of *M. incognita* prior to *Glomus fasciculatum* inoculated at different timings on the growth of blackgram¹.

Treatment	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Pod yield (g)
Nematode alone	14.3	10.8	9.9	8.0	2.8
Nematode + AMF 5 days later	30.5	20.7	28.9	4.0	5.0
Nematode + AMF 10 days later	22.8	18.6	15.6	3.9	4.5
Nematode + AMF 15 days later	22.3	17.2	13.7	3.3	3.7
Nematode + AMF 20 days later	22.0	12.7	12.8	4.9	3.0
AMF alone	32.2	31.2	33.7	13.0	6.6
Uninoculated control	22.3	26.4	23.8	11.9	3.2
LSD (P = 0.05)	0.5	1.2	2.2	0.04	0.35

¹Means were compared by Fisher's protected LSD test.

Table IV. Effect of *M. incognita* and *G. fasciculatum* inoculated at different timings on nematode reproduction and mycorrhizal colonization on blackgram¹.

Treatment	Gall index ²	Nematode population/ 100 g soil	Spore population/ 50 g soil	Mycorrhizal colonization (%)	Total P content (%)	
					Shoot	Root
Nematode alone	5.0	637.2	0.0	0.0	0.19	0.15
Nematode + AMF 5 days later	3.4	444.4	112.8	40.6	0.41	0.31
Nematode + AMF 10 days later	3.8	470.4	92.0	33.4	0.39	0.26
Nematode + AMF 15 days later	3.8	511.2	80.4	26.0	0.33	0.23
Nematode + AMF 20 days later	4.4	576.6	61.6	18.4	0.28	0.20
AMF alone	1.0	0.0	173.2	65.4	0.56	0.17
Uninoculated control	1.0	0	0.0	0.0	0.26	0.14
LSD (P = 0.05)	0.8	0.63	0.64	5.4	0.04	0.03

¹ Means were compared by Fisher's protected LSD test. Analyses were carried out using $\sqrt{(n + 0.5)}$ transformed data for nematode and spores in soil, untransformed means are tabulated.

² Root galling was rated on a 1-5 scale: 1 - no gall; 2 - 1-25% galls; 3 - 26-50% galls; 4 - 51-75% galls and 5 - >75% galls.

DISCUSSION

Application of AMF 20 days prior to nematode inoculation suppressed the nematodes to a greater degree than the application of AMF 5 days before the nematodes. This might be related to the time interval necessary for the establishment of the mycorrhiza in the root cortex. A period of five days for the establishment of AM fungi is too short to affect the development of nematode. It is well known that mycorrhiza establish in the root cortex in about 15 to 20 days, e.g. in tomato (Sitaramaiah and Sikora, 1982) and cotton (Saleh and Sikora, 1984). The presence of mycorrhiza in the host can reduce attraction to roots and juvenile penetration and retard nematode development after penetration (Sikora, 1978). Mycorrhizal spore population and mycorrhizal colonization were found to be lower when nematodes were inoculated 5 days after AMF. This agrees with observations by O'Bannon and Nemeč (1979) in rough lemon seedlings infected with *Radopholus similis* and *G. etunicatum* in which vesicle formation and mycelial growth were lower in nematode infected roots. Also, reduced spore production in the presence of *M. incognita* may indicate competition for nutrients (Kotcon *et al.*, 1985).

Delaying application of AMF from 5 to 20 days after the nematode inoculation resulted in increased root gall index and nematode population in the soil and suppression of spore population and colonization by AMF. Changes in AMF colonization and spore population in the presence of nematodes have been observed previously (Elliott *et al.*, 1984; Carling *et al.*, 1989; Waceke *et al.*, 2001; Castillo *et al.*, 2006) and were attributed mainly to competition between AM fungi and *Meloidogyne* spp. for feeding sites and carbon substrates from host photosynthesis (Smith, 1998; Hol and Cook, 2005). After *Meloidogyne* spp. invade the vascular cylinder, the root tissue around developing females usually proliferates to form knots or galls, which disrupt vessels and

thus reduce the transport of water and nutrients through the altered roots (Jenkins and Taylor, 1967). This may interfere with the translocation of metabolites required by mycorrhizal fungi. The disease syndrome initiated by root knot nematodes very often includes the invasion of affected root tissue by secondary pathogens, which cause decay of root tissues (Golden and Van Gundy, 1975) including the cortical tissue colonized by AMF. Mycorrhizal development and growth of mycorrhizal and non-mycorrhizal plants were reported to be reduced in the presence of *M. arenaria* in grapes (Atilano *et al.*, 1981).

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