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Glomus mosseae (Gerd and Trappe) and Neemex Reduce Invasion and Development of *Meloidogyne incognita*

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ARK, SAK and WA designed the study, executed experimental work and analyzed the data. NJ and STS supervised the work. TM helped in preparation of the manuscript.

Key words
Bio-control, *Glomus mosseae*, Neemex, Root knot nematode.

ABSTRACT

Among plant parasitic nematodes, root knot nematodes are the major problem for vegetables including eggplant. Chemical control of nematodes is hazardous to health and causes environmental pollution by contaminating underground water. The bio-protectant potential of mycorrhizal fungus (*Glomus mosseae*) and neemex® (Azadirachtin) against invasion and development of *Meloidogyne incognita* was tested in eggplant roots in greenhouse pot trials. Neemex (5 g, 10 g and 15 g) and *G. mosseae* (100 g, 150 g and 200 g) were applied as protective treatment. The roots of eggplant were inoculated with 1000 second stage juveniles of *M. incognita*. Eggplants inoculated with nematodes only served as control. Each treatment was replicated tenfold. Data were recorded after one week interval up to five weeks to record different developmental stages of *M. incognita*. After each harvest, neemex in combination with *G. mosseae* proved the most effective as the development of nematode was adversely affected. Developing juveniles and adults were less in number in the combined treatment.

INTRODUCTION

Root knot nematode infestation is causing severe losses to crop production worldwide with decrease in tolerance to other abiotic factors (Oka *et al*., 2000). The heavy infestation of nematodes may cause the complete failure of the crop. Infected roots are unable to utilize water, minerals and fertilizers. *Meloidogyne* species represent the foremost nematode dilemma in developing countries. In Pakistan, root knot nematode, *M. incognita*, causes damage to eggplant and other vegetables (Kayani *et al*., 2013, 2017; Mukhtar *et al*., 2013a; Hussain *et al*., 2016) and was found economically most important (Mukhtar *et al*., 2013b; Tariq-Khan *et al*., 2016). Associations of root knot nematodes with other soil borne pathogens aggravate crop losses (Iqbal and Mukhtar, 2014; Iqbal *et al*., 2014; Shahbaz *et al*., 2015). Many chemical nematicides are being gradually prohibited for protecting vegetable crops. Hence, the improvement of other management practices and durable methods are immediately required to minimize the use of nematicides (Martin, 2003; Mukhtar *et al*., 2013c, 2014, 2017a, b; Hussain *et al*., 2014).

Biological control of nematodes using rhizosphere micro-organisms was considered in several reviews to be a potential management tactic and an effective alternative to nematicides (Kerry, 2000; Mukhtar *et al*., 2013d; Hussain *et al*., 2017a, b; Rahoo *et al*., 2017). Mycorrhizal fungi perform a significant role as bio-protectant against pathogens (Naher *et al*., 2013). Mycorrhizal fungi and root-knot nematodes share a striking feature, which is their ability to form associations with the roots of the majority of plant species, whereas other biotrophs generally show a restricted host range (Trudgill and Block, 2001). Mycorrhizal fungus and neem product have the ability to suppress root knot nematodes in different crops including vegetables (Hasan and Khan, 2004); however, no information is available on the combined application of neemex and mycorrhizal fungus for root knot nematode management and their impact on invasion and development in eggplant. Therefore, the present study was designed to exploit the potential of neemex and mycorrhizal fungus (*G. mosseae*) in reducing the invasion and development of *M. incognita* in eggplant.

MATERIALS AND METHODS

Seeds of eggplant (*Solanum melongena* L.) were grown under greenhouse conditions (temperature ranges

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from 22–28°C and 80% relative humidity) and were transferred to earthen pots at 3 to 4 leaf stage. The mycorrhizal fungus, originally isolated from the field area of University of Agriculture, Faisalabad, and identified on the basis of morphological characters, was kept as a greenhouse stock culture on maize, and was applied as mycorrhizal inoculum at sowing of the test plants (Elsen *et al*., 2008). The inoculum consisted of rhizosphere soil from 4 months old maize pot cultures containing spores, hyphae and heavily colonized root pieces. Mycorrhizal colonization was determined by staining the roots with an ink-vinegar solution (Vierheilig *et al*., 1998). Each treatment was replicated ten times. *M. incognita* was originally isolated from susceptible tomato cultivar and afterwards maintained as a greenhouse stock culture on eggplant. When used for inoculation, egg masses were extracted from the eggplant roots and freshly hatched second-stage juveniles were collected by using modified Baermann dishes (Hooper *et al*., 2005).

Eggplants were planted under greenhouse. The pots contained soil, sand and manure in a 2:1:1 ratio, a substrate shown previously to be compatible with a high nematode infection potential (Vos *et al*., 2012). For the mycorrhizal treatment, different dosages like 100, 150 and 200 g of rhizosphere soil colonized by mycorrhizal fungus were added. Three doses of neemex, 5, 10 and 15 g were added alone and in combination. Plants from the non-mycorrhizal control treatment received 200 g of rhizosphere soil from non-colonized maize plants. After 6 weeks, eight mycorrhizal plants were uprooted to determine mycorrhizal colonization by staining the roots with ink-vinegar (Vierheilig *et al*., 1998). After clearing, staining and de-staining, 20 root pieces of 1 cm were mounted on permanent slides and observed with a light microscope. The mycorrhizal colonization percentage was observed according to Chaurasia and Khare (2005). After confirmation of mycorrhizal colonization, plants were inoculated with 1000 freshly hatched *M. incognita* second-stage juveniles. Invasion and life stages of the nematodes that invaded the control or treated roots were visualized by acid fuchsin staining of the whole root system of all the plants (Byrd *et al*., 1983) followed by observation with a light microscope. The number of second-stage, third-stage, fourth-stage juveniles and females were counted in acid fuchsin stained roots at 1, 2, 3, 4 and 5 weeks after inoculation.

Statistical analysis

The data were analyzed statistically by using the Fischer analysis of variance technique using MINITAB/STAT statistical analysis software (Minitab, 2010), and treatment means were compared using least significant difference test at 5% probability level (Steel *et al*., 1997).

RESULTS

The results showed that distinct dose levels of mycorrhizal fungus and neemex differ significantly from control treatments. At the first harvest after 1 week, minimum number of J2 invaded in combined application of MF and neemex at level 3 in which 200 g soil colonized by mycorrhizal fungus was used along with 5 g neemex (Table I). Vermiform and swollen roots, developmental stages of *M. incognita* were recorded in all treatments but significantly lower as compared to control. When mycorrhizal fungus was applied alone with 200 g dose level, vermiform and swollen roots were recorded 27 and 14 respectively as compared to control (240 and 80, respectively). Neemex alone also showed significant difference over control as vermiform and swollen roots were 33 and 20, respectively.

Table I. Effect of mycorrhizal fungus and neemex on invasion and development of *M. incognita* after one week.

Treatment	Levels (g)	Developmental stages	
		Vermiform	Swollen
M. incognita	0	240.0 a	79.1 a
	0	240.4 a	80.0 a
	0	240.1 a	80.0 a
MF + *M. incognita*	100	37.0 bc	23.0 bc
	150	31.0 e	19.0 d
	200	27.0 f	14.0 e
Neemex + *M. incognita*	5	40.0 b	25.0 b
	10	36.0 cd	22.0 bcd
	15	33.0 de	20.0 cd
MF + Neemex + *M. incognita*	100 + 15	31.0 e	19.0 d
	150 + 10	24.2 fg	15.0 e
	200 + 5	23.0 g	12.0 e
LSD at $P<0.05$		3.6037	3.8308

Means sharing similar letters are statistically non-significant at $P<0.05$; MF, Mycorrhizal fungus (*Glomus mosseae*).

After 2 weeks, a minimum number of vermiform, swollen and sausage shaped was observed as 3, 30.4 and 41.7 units, respectively, at level 3 in which mycorrhizal fungus and neemex was applied together as compared to control (Table II). Mycorrhizal fungus and neemex alone also showed significant difference over control. Subsequent to 3 weeks; sausage shaped, immature females, adult females and egg masses were observed significantly higher in control (Table III). Combination of mycorrhizal fungus and neemex at level 3 showed minimum number of sausage (6), immature females (4.4), adult females (19.9) and egg masses (2.8) followed by level 2 and 1 (Table III). Later than 4 weeks; vermiform (168.8), swollen (114), immature females (28.9), adult females (283.3) and egg masses (158.1) were recorded significantly higher in control (Table IV) while minimum developmental stages were recorded in MF and neemex combined treatment (level 3) as vermiform stage, swollen stage, immature females, adult females and egg masses were 8.1, 8.3, 1.4, 14.7 and 13.4 units again respectively followed by level 2 and 1. Mycorrhizal fungus and neemex alone also showed significant difference as compared to control. After 5 weeks, significantly lower number of developmental stages of *M. incognita* were observed in combined application of MF and neemex (level 3) as vermiform (16.2), swollen (6.9), sausage (4.7), immature females (3.6), adult females (38.4) and egg masses (27.8) followed by level 2 and 1 as compared to control in which vermiform, swollen, sausage, immature females, adult females and egg masses were 219.4, 182.7, 112.6, 51.8, 347.3 and 184.3, respectively (Table V). Minimum number of developmental stages were recorded in MF (level 1, 2, 3) and neemex alone treatments (level 1, 2, 3) as compared to control.

Table II. Effect of mycorrhizal fungus and neemex on invasion and development of *M. incognita* after two weeks.

Treatment	Levels (g)	Developmental stages		
		Vermiform	Swollen	Sausage
M. incognita	0	35.0 a	214.0 a	117.3 a
	0	36.0 a	215.5 a	118.4 a
	0	36.0 a	215.0 a	118.3 a
MF + *M. incognita*	100	11.3 b	046.1 b	63.2 b
	150	8.1 cd	41.0 cd	54.3 cd
	200	6.1 de	35.0 ef	48.8 de
Neemex + *M. incognita*	5	13.0 b	48.1 b	61.0 b
	10	10.9 bc	44.1 bc	58.3 bc
	15	9.1 cd	39.0 de	53.5 cd
MF + Neemex + *M. incognita*	100 + 15	6.5 de	37.0 def	49.9 de
	150 + 10	5.0 ef	34.0 fg	46.9 ef
	200 + 5	3.0 f	30.4 g	41.7 f
LSD at P<0.05		3.6037	3.2304	4.3641

Means sharing similar letters are statistically non-significant at P<0.05; MF, Mycorrhizal fungi (*Glomus mosseae*).

Table III. Effect of mycorrhizal fungus and neemex on invasion and development of *M. incognita* after three weeks.

Treatment	Levels (g)	Developmental stages			
		Sausage	Immature females	Adult females	Egg masses
M. incognita	0	37.0 a	33.4 a	201.0 a	118.9 a
	0	36.6 a	32.2 a	200.5 a	119.8 a
	0	37.6 a	33.0 a	201.6 a	119.1 a
MF + *M. incognita*	100	19.6 bc	16.0 bc	33.9 bcde	14.3 bcde
	150	19.1 bc	16.3 bc	47.9 b	22.2 b
	200	12.0 e	9.7 de	26.2 cde	7.0 def
Neemex + *M. incognita*	5	22.2 b	18.8 b	40.0 bc	18.6 bc
	10	19.1 bc	15.5 bc	36.3 bcd	15.3 bcd
	15	16.2 cd	12.8 cd	33.1 cde	12.9 cde
MF + Neemex + *M. incognita*	100+15	13.2 de	9.0 de	29.8 cde	9.0 def
	150+10	10.2 e	7.0 ef	24.2 de	5.9 ef
	200+5	6.0 f	4.4 f	19.9 e	2.8 f
LSD at P<0.05		3.6037	4.1730	4.0840	14.117

Means sharing similar letters are statistically non-significant at P<0.05; MF, Mycorrhizal fungus (*Glomus mosseae*).

Table IV. Effect of mycorrhizal fungus and neemex on invasion and development of *M. incognita* after four weeks.

Treatment	Levels (g)	Developmental stages				
		Vermiform	Swollen	Immature females	Adult females	Egg masses
M. incognita	0	186.8 a	114.0 a	28.9 a	283.3 a	158.1 a
	0	186.4 a	114.2 a	29.1 a	283.6 a	157.9 a
	0	186.2 a	114.1 a	29.0 a	283.4 a	158.0 a
MF + *M. incognita*	100	23.0 cd	19.3 cde	8.7 de	35.2 cd	28.3 bcd
	150	37.6 b	28.0 b	10.8 cd	55.8 b	39.5 b
	200	16.5 cde	14.2 def	3.3 f	26.2 cd	21.1 de
Neemex + *M. incognita*	5	27.1 bc	25.2 bc	16.2 b	43.6 bc	37.5 b
	10	24.8 bcd	22.0 bcd	13.5 bc	39.3 bcd	34.2 bc
	15	23.9 cd	20.2 bcde	10.7 cd	37.5 bcd	30.3 bcd
MF + Neemex + *M. incognita*	100 + 15	16.7 cde	16.6 def	7.7 de	23.2 de	24.5 cde
	150 + 10	12.4 de	12.6 ef	4.8 ef	19.2 de	19.5 de
	200 + 5	8.1 e	8.3 f	1.4 f	14.7 e	13.4 e
LSD at P<0.05		13.444	8.4063	4.3443	20.345	11.506

Means sharing similar letters are statistically non-significant at P<0.05; MF, Mycorrhizal fungus (*Glomus mosseae*).

Table V. Effect of mycorrhizal fungus and neemex on invasion and development of *M. incognita* after five weeks.

Treatment	Developmental stages						
	Levels (g)	Vermiform	Swollen	Sausage	Immature females	Adult females	Egg masses
M. incognita	0	219.4 a	182.7 a	112.6 a	51.8 a	347.3 a	184.3 a
	0	219.3 a	182.6 a	112.5 a	52.1 a	347.4 a	184.5 a
	0	219.5 a	182.5 a	112.7 a	52.0 a	347.5 a	184.4 a
MF + *M. incognita*	100	30.7 cde	21.0 cd	18.0 bcd	13.5 bcd	63.0 cd	48.5 bcde
	150	47.5 b	35.7 b	23.2 b	15.9 bc	59.1 d	57.5 b
	200	21.1 de	18.3 cde	9.0 ef	7.9 efg	53.1 e	41.1 def
Neemex + *M. incognita*	5	39.5 bc	29.8 bc	22.7 bc	18.6 b	68.1 b	53.8 bc
	10	37.7 bc	23.9 bcd	18.5 bc	15.5 bc	64.6 bc	49.6 bcd
	15	32.7 bcd	21.6 cd	18.2 bc	13.0 cde	62.3 cd	44.3 cdef
MF + Neemex + *M. incognita*	100 + 15	25.3 cde	16.3 de	14.5 cde	9.9 def	50.4 e	37.7 efg
	150 + 10	21.2 de	12.3 de	9.5 def	6.5 fg	44.5 f	33.5 fg
	200 + 5	16.2 e	6.9 e	4.7 f	3.6 g	38.4 g	27.8 g
LSD at P<0.05		15.625	13.090	8.5393	5.2329	4.1416	11.366

Means sharing similar letters are statistically non-significant at P<0.05; MF, Mycorrhizal fungus (*Glomus mosseae*).

DISCUSSION

Data of the developmental stages of *M. incognita* was recorded up to 5 weeks after inoculation. Depending on environmental conditions, normally *M. incognita* completes its life cycle in 24 to 35 days (Ploeg and Maris, 1999). As J2 penetrated the roots, developmental life stage of RKN comprised three extra moults prior to adult stage and it takes about 2 weeks, earlier than J2 moult converted into third stage, whereas fourth stage juveniles normally appear quickly and this stage prevail for almost 1 week (Moens *et al*., 2009). Invasion and development rate of life stages of *M. incognita* was decreased in the roots of eggplant treated with mycorrhizal fungus. Developmental stages of *M. incognita* appear progressively lower in the roots treated with mycorrhizal fungus and neemex than control. The present findings revealed that minimum J2s were penetrated in the roots, inoculated with mycorrhizal fungus and neemex alone or in combination, and in addition, development of life stages were reduced compared to the control. Reports about mycorrhizae and neemex in combined form reduced the infection and reproduction of nematodes (Khan *et al*., 2015; Hol and Cook, 2005; Akhtar and Siddiqui, 2008). Symbiotic association of mycorrhizal fungus effectively reduced the infection of nematodes as reported for other pathogens (Slezack *et al*., 2000). Current research was focused on the phases of infection that lead the invasion and development of nematodes. It showed that lesser invasion of J2 following development was partly liable for the lengthy generation time and reduced the reproduction of nematodes in plants colonized with mycorrhizal fungus. The decline in the invasion of *M. incognita* might be due to the allelopathic effect of mycorrhizal fungus that affects nematode motility and food finding ability (probing) in the rhizosphere. For root invasion, *M. incognita* directs towards appropriate host and site of infection (Curtis *et al*., 2009). The application of root exudates from mycorrhized tomato plants appreciably reduced the invasion of *M. incognita* (Vos *et al*., 2012).

CONCLUSION

This study demonstrated a continuously suppressing effect of MF and neemex alone or in combined form on penetration and further development of *M. incognita*. J2 penetration was constantly lower in the roots colonized by mycorrhizal fungus and nematode developmental stages were also reduced. Neemex and mycorrhizal fungus can be successfully used against *M. incognita* invasion and development in eggplant.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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