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ORIGINAL ARTICLE

Comparative efficacy of different approaches to managing *Meloidogyne incognita* on green bean

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Abstract A greenhouse study was conducted to compare the relative efficacy of different approaches to managing *Meloidogyne incognita* on green bean. These approaches included chemical (fumigant, non-fumigant, seed dressing, and seed dip), biological (the egg-parasitic fungus, *Paecilomyces lilacinus* and the mycorrhizal fungus *Glomus* sp.), physical (soil solarization), and cultural (chicken litter and urea) methods. Accordingly, nine different control materials and application methods plus nematode-infected and non-infected controls were compared. Two important parameters were considered: plant response (plant growth and root galling) and nematode reproduction (production of eggs and the reproduction factor Rf). The results showed that the use of chicken litter as an organic fertilizer severely affected the growth and survival of the plants. Therefore, this treatment was removed from the evaluation test. All of the other eight treatments were found to be effective against nematode reproduction, but with different levels of efficacy. The eight treatments decreased (38.9–99.8%) root galling, increased plant growth and suppressed nematode reproduction. Based on three important criteria, namely, gall index (GI), egg mass index (EMI), and nematode reproduction factor (RF), the tested materials and methods were categorized into three groups according to their relative control efficacy under the applied test conditions. The three groups were as follows: (1) the relatively high effective group (GI = 1.0–1.4, Rf = 0.07–0.01), which included the fumigant dazomet, the non-fumigant fenamiphos, soil solarization, and seed dip with fenamiphos; (2) the relatively moderate effective group (GI = 3.4–4.0, Rf = 0.24–0.60), which included seed dressing with fenamiphos and urea; and (3) the relatively less effective group (GI = 5.0, Rf = 32.2–37.2), which included *P. lilacinus* and *Glomus* sp.

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1. Introduction

Green bean (*Phaseolus vulgaris* L.) is an important vegetable crop worldwide. The crop is usually attacked by many plant pathogens, including plant-parasitic nematodes (Hall, 1991). However, root-knot nematodes (*Meloidogyne* spp.) are the

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most frequent damaging plant-parasite nematodes in greenhouses and in vegetable production in general (Koenning et al., 1999).

Meloidogyne spp. cause crop losses of approximately 10% in vegetable crops (Koenning et al., 1999). However, some studies have reported higher percentages (up to 30%) in some local regions, depending on the host cultivar, population density and *Meloidogyne* species involved (Sikora and Fernandez, 2005; Ornat and Sorribas, 2008).

In Saudi Arabia, green bean is grown in open fields and greenhouses mainly for its green pods. The crop is frequently attacked by *Meloidogyne javanica* (Treub) chitwood and *Meloidogyne incognita* (Kofoid & White) chitwood. Although no accurate estimates of crop losses of green bean in the country have been determined, root-knot nematodes generally cause high damage (40–100%) in some local vegetable farms (Al-Hazmi et al., 1983). In a recent study, *M. incognita* was found to be very important and damaging pest on green bean plants (Al-Nadhari, 2014).

Controlling *Meloidogyne* spp. is sometimes difficult because of their extensive host range, short life cycle, high reproductive rate and endoparasitic nature (Manzanilla-lopez et al., 2004). *Meloidogyne* spp. are also difficult to control with a single control method (Barker et al., 1985).

After many years of use, methyl bromide has been completely phased out by January 1st, 2015. Therefore, we must evaluate the application of other available alternatives to methyl bromide to protect our vegetable production, especially in greenhouses.

Different approaches have been used to manage root-knot nematodes in vegetable crops, including the use of fumigant and non-fumigant nematicides, resistant cultivars and biological and physical control measures (Zuckerman and Esnard, 1994; Collange et al., 2011), although, varied in their efficacy due to several factors. Collange et al. (2011) presented an excellent and extensive review of root-knot nematode management in vegetable crop production, including the role of sanitation, soil management, organic and inorganic fertilizers, biological control and heat-based methods.

The aim of this present study was to compare the relative efficacy of different approaches (chemical, biological, physical, and cultural practices) as alternatives to methyl bromide for managing *M. incognita* on green bean under greenhouse conditions in Saudi Arabia.

2. Materials and methods

2.1. Treatments and design

Eight different approaches of *M. incognita* management (Table 1) were comparatively evaluated in a greenhouse pot experiment. *M. incognita*-infected and non-infected control treatments were also included. Thus, 11 treatments with five replicates were arranged in a complete randomized design (CRD) on a greenhouse bench (25 ± 2 °C).

2.2. Test plants

Clean plastic pots (14 cm diam.) were filled with 1500 g/pot of a mixture of equal parts sand and sandy loam soil. The potting mixture was previously steam-sterilized with an autoclave. Pots were then seeded with three green bean seeds (cv. Contender). A week after emergence, the seedlings were thinned to one seedling/pot.

2.3. Nematode inoculum and inoculation

As inoculum, an egg suspension of *M. incognita* (race 2), was prepared (Hussey and Barker, 1973) from a pure greenhouse culture on tomato. Inoculation always took place when seedlings were 3-week-old. Each seedling was inoculated with 10,000 eggs/pot (6.7 eggs/g soil).

2.4. Treatments with nematicides

The soil in each pot to be treated with the fumigant nematicide dazomet was mixed thoroughly in a plastic bag with the recommended dose (50 g/m^2 = 0.76 g/pot). Treated soils were returned to their pots, irrigated to field capacity, and covered with plastic sheets. A week later, the covers were removed, and the soils were aerated for two weeks. Soils were then returned to pots and seeded with bean seeds. Seedlings were thinned and inoculated with *M. incognita* as mentioned before. A similar procedure was followed with the nematicide fenamiphos (9.6 kg/ha = 0.15 g/pot) and the nematode inoculation but without plastic to cover the pots.

For seed dressing (coating), bean seeds were moistened with water and then mixed thoroughly in a plastic bag (seed

Table 1 Control approaches and methods used in the study.

Control approaches	Control method	Tested material	Rate used/remarks
Chemical	Fumigant	Dazomet	50 g/m^2
	Non-fumigant	Fenamiphos	Soil treatment @ 9.6 kg a.i./ha
			Seed dressing @ 2.0% a.i. (w:w)
			Seed-dip @ 2.0% a.i. (w:v)
Biological	Parasitic fungus	*Paecilomyces lilacinus*	0.7% of culture on grains
	Mycorrhiza	*Glomus* sp.	1×10^3 spore/kg soil
Physical	Soil solarization		For 8 weeks (June–July)
Cultural	Organic fertilizer	Chicken litter	2.0% (w:w dry base)
	Inorganic fertilizer	Urea (46-0-0)	600 kg/ha
Check			*M. incognita* (6.7 egg/g soil)
			Non-infected and non-treated seedlings

dressing) with fenamiphos @ 2.0% a.i. (w:w). The soil in each designated pot was mixed in a plastic bag with the nematode inoculum, returned to its pot, and seeded with the nematicide-treated seeds. A similar procedure was followed for seed dip and nematode inoculation. However, seeds of the seed-dip treatment were immersed in a solution of fenamiphos @ 2.0% a.i. (w:v) for six hours. Seeds were then air-dried and used for direct seeding in the designated pots. After seedling emergence, seedlings were thinned and inoculated with *M. incognita* as mentioned before.

2.5. Paecilomyces lilacinus inoculum and inoculation

The egg-parasitic fungus *P. lilacinus* (Thom.) Samson was originally isolated on potato dextrose agar (PDA) from a greenhouse culture of *M. incognita*-infected tomato plants. For inoculum, several discs of the fungus culture on PDA were transferred to flasks (250 cm^3) containing autoclaved wheat grains, which were then incubated at 25 °C for 3 weeks (Jatala, 1986). At inoculation, the fungal culture was mixed thoroughly in a plastic bag with the pot soil of the designated treatment @ 0.7% (10.5 g/pot). The infested soil was then returned to the pots and kept moist in the greenhouse for fungal colonization for two weeks. Green bean seeds were sown in the pots, thinned and inoculated with *M. incognita* as mentioned before.

2.6. Soil solarization

Soil in the pots of this treatment was first mixed with the nematode egg inoculum (10,000 eggs/pot). Infested soils in the pots were irrigated to the field capacity. The pots were then covered with a double polyethylene film (25–30 μm) and were kept in direct sun for eight weeks (during June and July). The soil was then aerated for one week, then returned to pots and planted with the green bean seeds. Seven days after emergence, seedlings were thinned to one seedling/pot.

2.7. Treatments with fertilizers

Chicken litter and urea (46-0-0) were used as organic and inorganic fertilizers, respectively. The chicken litter was left on a board to be air-dried for a week, then ground and sieved. The powder-like litter was thoroughly mixed in a plastic bag with the pot soil of the designated treatment @ 2.0% (w:w) (20 g/kg soil) and returned to pots. Treated soils in pots were kept moist in the greenhouse for two weeks, then planted with green bean seeds (Ibrahim and Ibrahim, 2000). Seedlings were thinned and inoculated with *M. incognita* as mentioned before. Urea was used @ 600 kg/ha (0.939 g/pot) on two equal applications (doses); a week after emergence and a month after the first application.

2.8. Glomus sp. inoculum and inoculation

The mycorrhizal fungus *Glomus* sp. was cultured on corn plants (*Zea mays* L.) in a sandy soil for two months in the greenhouse. Chlamydospores were then harvested using the wet-sieving method (Gerdemann and Nicolson, 1963). The soil in each pot of the designated treatment was mixed thoroughly in a plastic bag with the spore suspension @ one spore/g soil (1500 spores/pot). Infested soils were returned to the pots and kept moist in the greenhouse for three weeks. Pots were then planted with green bean seeds, and the emerged seedlings were thinned and inoculated with *M. incognita* as mentioned before.

2.9. Control treatments

Two control treatments were included in this study: non-infected, non-treated seedlings and seedlings inoculated only with *M. incognita* (10,000 eggs/pot).

2.10. Test termination and data recording and analysis

Treated seedlings were irrigated and fertilized with Hogland's solution (Hoagland and Arnon, 1950) as needed until the end of the test. Sixty days after nematode inoculation, the test was terminated. Fresh plant weights and the number of galls, egg masses and eggs per plant were recorded. The gall and egg mass indices (on a 0–5 scale both) (Sasser et al., 1984), and nematode reproduction factor (Oostenenbrink, 1966) were also determined. Data were subjected to the analysis of variance (ANOVA), and the means were separated by Fisher's protected $LSD_{0.05}$ (SAS, 2013).

3. Results

Chicken litter severely affected the seedlings' growth and survival, causing numerous deaths. Therefore, this treatment was removed from the test. All of the other tested approaches decreased ($P \leqslant 0.05$) the number of galls and gall indices (Table 2). With some exceptions, the tested approaches increased (up to 68%) the total fresh weight of the plants (Table 2). All approaches also suppressed nematode reproduction to different levels (Table 3).

Three important criteria were used to compare the efficacy of the tested approaches: the gall index (GI), egg mass index (EMI) and nematode reproduction factor (Rf). Based on these criteria, the tested approaches applied under our test conditions were categorized into three groups according to their relative control efficacy (Table 4): (1) the relatively high effective group (GI = 1.0–1.4, Rf = 0.07–0.01), which included the fumigant dazomet, non-fumigant fenamiphos (soil treatment), soil solarization, and seed-dip with fenamiphos, (2) the relatively moderate effective group (GI = 3.4–4.0, Rf = 0.24–0.60), which included the seed dressing with fenamiphos and mineral fertilizer urea, and (3) the relatively less effective group (GI = 5.0, Rf = 32.2–37.2), which included the parasitic fungus *P. lilacinus* and the mycorrhizal fungus *Glomus* sp.

4. Discussion

Our results confirm previous reports on the efficacy of the two used nematicides (Melton et al., 1995; Giannakou et al., 2002), and the efficacy of *P. lilacinus* (Jatala, 1986; Goswami and Mital, 2004; Krishnamoorthi and Kumar, 2008), soil solarization (Ioannon, 2002; Kaskavalci, 2007) *Glomus* spp. (Verma and Nandal, 2006) and urea (Al-Hazmi and Dawabah, 2014). However, the tested approaches showed differences in

Table 2 Effects of different control methods on the plant growth and root galling of green bean infected with *Meloidogyne incognita*, 60 days after inoculation (greenhouse 25 ± °C).

Treatment	Plant fresh weight (g)	% change from *M. incognita* control	No. of galls/root system	Gall index (GI) (0–5)
Non-treated and non-infected control	11.9 cd	+17.8		
M. incognita (N)	10.1 de		509.0 a	5.0 a
N+ dazomet	9.5 de	−4.8	1.0 e	1.0 e
N+ fenamiphos	14.4 abc	+43.6	2.0 e	1.2 de
N+ fenamiphos (seed dressing)	16.7 a	+68.2	56.0 d	4.0 b
N+ fenamiphos (seed dip)	15.0 ab	+51.4	2.0 e	1.4 d
N+ *P. lilacinus*	15.2 ab	+52.9	212.0 c	5.0 a
N+ soil solarization	13.9 bc	+43.3	1.0 e	1.0 e
N+ *Glomus* sp.	8.6 e	−12.2	311.0 b	5.0 a
N+ urea (46%)	4.9 f	−47.9	44.0 d	3.40 c

Values are means of five replicates.
Means within the same column that are followed by the same letter(s) are not significantly different according to Fisher's Protected LSD ($P \leqslant 0.05$).
Gall index (GI): 0 = none, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = more than 100 galls per root system.

Table 3 Effects of different control methods on the reproduction of *Meloidogyne incognita* on green bean, 60 days after inoculation (greenhouse 25 ± °C).

Treatment	No. of egg masses/root system	No. of eggs/root system	Egg mass index (EMI) (0–5)	Reproduction factor (Rf)
Non-infected and non-treated control				
M. incognita (N)	705.0 a	850, 838 a	5.0	85.08 a
N+ Dazomet	1.0 e	99.0 d	1.0	0.01 c
N+ Fenamiphos	1.0 e	208.0 d	1.0	0.02 c
N+ Fenamiphos (seed dressing)	19.4 d	234.9 cd	3.0	0.24 c
N+ Fenamiphos (seed dip)	1.6 d	722.0 cd	1.2	0.07 c
N+ *P. lilacinus*	263.8 c	322.540 b	5.0	32.2 b
N+ Soil solarization	1.0 e	143.0 d	1.0	0.01 c
N+ *Glomus* sp.	418.8 b	372.638 b	5.0	37.2 b
N+ Urea (46%)	27.0 d	5.918 c	3.4	0.60 c

Values are means of five replicates.
Means within the same column that are followed by the same letter(s) are not significantly different according to Fisher's Protected LSD ($P \leqslant 0.05$).
Egg mass index (EMI): 0 = none, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = more than 100 egg masses/root system.
Reproduction factor (Rf) = final nematode population (Pf)/initial inoculum (Pi).

Table 4 Relative efficacy of different control methods of *Meloidogyne incognita* on green bean, 60 days after inoculation (greenhouse 25 ± °C).

Control method	Plant damage Gall index (0–5)	Nematode reproduction	
		Egg mass index (0–5)	Reproduction factor (Rf)
Highly effective = Group 1			
Dazomet	1.0	1.0	0.01
Soil solarization	1.0	1.0	0.01
Fenamiphos (soil treatment)	1.2	1.0	0.02
Fenamiphos (seed dip)	1.4	1.2	0.02
Moderately effective = Group II			
Fenamiphos (seed dressing)	3.4	3.0	0.25
Urea (46%)	4.0	3.4	0.6
Less effective = Group III			
P. lilacinus	5.0	5.0	32.2
Glomus sp.	5.0	5.0	37.2

their relative control efficacy under our experimental conditions. Dazomet, fenamiphos and soil solarization were the most potent and effective materials that were used. Considering the response of the host plant, nematode reproduction and application cost and method, it appears that seed dip with fenamiphos is more appropriate.

Under our arid climate conditions in Saudi Arabia, where summers are long with a dry and high air temperature (during June to August), soil solarization would be the best choice for managing root-knot nematodes in the open fields. In this study, soil solarization reduced both root galling and nematode reproduction and, in contrast to dazomet, increased plant growth up to 43.6%. This finding supports those reported by Kaskavalci (2007), who found that root galling caused by *M. incognita* in tomato plants grown in plots treated with solarized soil or solarized soil plus organic amendments was lower than in plots that underwent other treatments.

Seed dressing with fenamiphos and amendments with urea ranked second in their relative efficacy. Amendments with urea provide additional benefits in suppressing the *M. incognita* population, as shown in our study. Previous reports have shown that urea and ammonia-releasing fertilizers are effective in controlling plant-parasitic nematodes (Santana-Gomes et al., 2013; Seifi and Bide, 2013; Al-Hazmi and Dawabah, 2014).

Unfortunately, the treatment of chicken litter severely affected the survival of the test plants and caused early death to most of the treated seedlings. It appears that we may have used a relatively higher concentration (2% w:w) of the litter, which was enough to be phytotoxic (Wahundeniya, 1991).

Although the egg-parasitic fungus *P. lilacinus* increased plant growth, it did not decrease the indices of root gall, egg masses or nematode reproduction. The poor effect of *P. lilacinus* on the nematode reproduction might be due to the fact that our fungal culture was old, and might lose its effectivity. The used isolate of *Glomus* sp. completely failed to improve host growth or suppress the nematode population. This indicates that the used inoculum was somewhat low, or that the strain of this mycorrhizal fungus was not appropriate in relation to the chemical and physical characteristics of the used soil mixture (Motosugi et al., 2002). The use of the non-fumigant fenamiphos or soil solarization (under the arid climate condition) would be the best alternative to methyl bromide for managing root-knot nematodes. Either approach would be enhanced greatly if combined with other control measures in an integrated control system.

5. Conclusion

Under our experimental conditions, the use of the non-fumigant fenamiphos or soil solarization would be the best alternatives to methyl bromide for managing root-knot nematodes. Either approach would be enhanced greatly if combined with other control measures in an integrated control system. However, further studies, under field conditions, are needed to prove the effectivity and applicability of these approaches.

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