EFFICACY OF SELECTED BIO-CONTROL AGENTS ON *MELOIDOGYNE INCOGNITA* ON EGGPLANT

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Summary. The biocontrol effects of *Serratia marcescens* (1×10^9 bacterium cells/ml water), ground ascaris (*Ascaris lumbricoides*) cuticle (10 g/pot), two entomopathogenic nematode species (*Heterorhabditis bacteriophora* strain EGG and *Steinernema carpocapsae* strain All, each at 125 infective juveniles/cm^2), and garlic extract (600 g ground garlic cloves/l water), used as soil treatments, were assessed on *Meloidogyne incognita* attacking eggplant (cv. Roomi Balady) in the glass-house. These treatments were compared with spray application of the nematicide oxamyl (15 ml solution/pot from a stock of 3 litres of 24% liquid oxamyl + 600 litres water). Fifty-three days after *M. incognita* inoculation, all of the bio-control agents increased various measures of plant growth. The root weights of plants treated with ascaris cuticle were almost doubled, but shoot weights were greatest in plants treated with oxamyl, followed by those of plants that received *S. marcescens, S. carpocapsae* and ascaris cuticle. Since much *Bacillus subtilis* was found in ascaris cuticle-treated pots (138 bacterial cells/g of treated soil) with respect to the control (28 bacterial cells/g), it is likely that ascaris cuticle could be used for culturing this bacterium, which apparently controlled *M. incognita.* The treatments delayed the development of *M. incognita* and second stage juveniles occurred only in control pots. Numbers of third and fourth stage juveniles, females and egg masses of the nematode, root galls and gall index were reduced by all treatments, with the greatest reduction (95-98%) given by ascaris cuticle.

Key words: *Ascaris lumbricoides*, *Heterorhabditis bacteriophora*, oxamyl, *Serratia marcescens*, *Steinernema carpocapsae*.

Over-reliance on the use of synthetic pesticides in crop protection has resulted in disturbances to the environment, pest resurgence, pest resistance to pesticides, and lethal and sub-lethal effects on non-target organisms, including humans (Prakash and Rao, 1997). These side effects have raised public concern about the routine use and safety of chemical nematicides. At the same time, increases in the populations of plant-parasitic nematode and a tendency to use ever greater quantities of pesticides are causing ever greater environmental problems, that is, provision of sufficient clean food whilst at the same time protecting water supplies and wild life habitats.

Although crop damage caused by plant parasitic nematodes accounts for annual losses of about 12% of food and fiber production in the world (Barker *et al.*, 1994), yield loss of vegetables, including eggplant, due to nematodes exceeds this figure (Netscher and Sikora, 1990). In Egypt, plant parasitic nematodes, especially root-knot nematodes, are important pests and cause considerable loss to many economic crops. Recently, the use of environmentally friendly bio-nematicides has been proposed (Abd-Elgawad and Aboul-Eid, 2005). Osman *et al.* (2005) found a clear correlation between application of an increasing amount of crushed garlic and percentage reduction of *Meloidogyne incognita* (Kofoid *et* White) Chitw. galls, egg masses and females on cowpea, along with increases in *Rhizobium* nodulation and plant growth. They also found that Nemaless (a water suspension of *Serratia marcescens* Bizio containing 1×10^9 bacterium cells/ml water, produced by the Egyptian Ministry of Agriculture and Land Reclamation) reduced nematode galls, egg masses, females and other developmental stages in the roots and juveniles in soil. Other authors (Grewal *et al.*, 1999; Abd-Elgawad and Aboul-Eid, 2002; Perez and Lewis, 2002) have reported different levels of suppression of plant-parasitic nematode populations by soil application of entomopathogenic nematodes (EPN). For example, Bird and Bird (1986) demonstrated that entomopathogenic nematodes crowded along the roots of tomato plants, forcing plant-parasitic nematodes away; thus reducing their root penetration and consequent damage to plants. Fallon *et al.* (2002) tested two Hawaiian isolates of *Steinernema feltiae* (Filipjev) Wouts, Mracek, Gerdin *et* Bedding MG-14 and *Heterorhabditis indica* Poinar, Karunakar *et* David MG-13, a French isolate of *S. feltiae* SN, and a Texan isolate of *S. riobrave* Cabanillas, Poinar *et* Raulston for their efficacy against the root-knot nematode, *Meloidogyne javanica* (Treub) Chitw., in the laboratory and greenhouse. They demonstrated that a single application of 10^4 *S. feltiae* MG-14 or SN infective juveniles per 100 cm^3 of sterile soil, together with 500 (MG-14) or 1,500 (SN) second-stage juveniles of *M. javanica*, reduced root penetration of the nematode three days after its inoculation. Entomopathogenic nematode infective juveniles applied to assess the effects on *M. javanica* egg production did not cause a significant reduction compared to that of the water control treatment. There was no dose

response effect by *Steinernema* spp. on *M. javanica* root penetration or egg production. *Steinernema* spp. did not affect the growth or development of *M. javanica*-infected plants, but *H. indica* MG-13-treated plants had lower biomass than untreated plants infected with *M. javanica.* Infective juveniles of *S. riobrave* TX*, S. feltiae* SN and MG-14 but not those of *H. indica* MG-13 were found inside root cortical tissues of M. *javanica*-infected plants. They concluded that entomopathogenic nematode antagonism to *M. javanica* on soybean or tomato was insufficient in their study to provide a consistent level of nematode suppression at the afore mentioned doses of infective juveniles applied. On the other hand, Grewal *et al.* (1997) found that *S. riobrave* applied at 6×10^9 infective juveniles/acre in turf grass provided up to 95-100% control of root-knot, *Meloidogyne* sp., sting, *Belonolaimus longicaudatus* Rau, and ring, *Criconemella* sp., nematodes in Georgia. *Steinernema riobrave* was as effective as the chemical nematicide Fenamiphos (Nemacur 10G) at 4 weeks after treatment and more effective at 8 weeks after treatment.

Integrated pest management (IPM) places emphasis on pro-active measures to redesign the agricultural ecosystem to the disadvantage of a pest and to the advantage of its biocontrol agent(s). Building on these principles, approaches that adopt such measures have been tested. For example, we noticed that ascaris cuticle can be used for culturing bacteria which may reduce population densities of plant-parasitic nematode. Therefore, the present experiment attempts to test this assumption as a pre-requisite for consideration of ascaris cuticle as a non-traditional nematicide.

For comparison, a variety of nematode control agents have been included in the experiments. For instance, Nemaless and Nemastop (Nemastop is a suspension containing 600 g ground garlic cloves/litre of water produced by the Egyptian private sector), which have recently been produced in Egypt as bio-nematicides, have been included in our tests. The present study compared the efficacy of some non-traditional bio-nematicides, including two species of entomopathogenic nematodes (EPN) (*Heterorhabditis bacteriophora* Poinar and *Steinernema carpocapsae* (Weiser) Wouts, Mracek, Gerdin *et* Bedding), the bacterium *S. marcescens*, garlic (*Allium sativum* L.) extract and the cuticle of ascaris, with that of the chemical oxamyl for the control of the root-knot nematode *M. incognita* on eggplant in pots.

MATERIALS AND METHODS

Plant material and M. incognita inoculum. In a glasshouse, 35-day-old eggplant (*Solanum melongena* L. cv. Roomi Balady) seedlings, of similar age and size, were singly transplanted into seventy 15-cm-diameter pots, each containing 2 kg of steam sterilized loamy soil. There were ten pots per treatment. Ten days after transplanting, each pot was inoculated with a suspension containing 1000 ± 4 of at most seven-day-old second stage juveniles (J_2) of *M. incognita*, poured into three holes in the soil around the base of the plant stem. The nematode inoculum was obtained from tomato (*Lycopersicon esculentum* L. cv. Super Strain B) roots as described by Taylor and Sasser (1978). After dissolving the gelatinous matrix of the nematode egg masses using sodium hypochlorite solution, centrifuging and washing with tap water, the nematode suspension was left in aerated water for seven days at 27 ± 2 °C and then examined under stereomicroscope to confirm that all viable eggs had hatched just before application.

Treatments. Treatments consisted of: 1) two additions of 2 ml of Nemaless (a commercial suspension of *Serratia marcescens* having 1×10^9 bacterium cells/ml water) per pot, one immediately and the other one week after *M. incognita* inoculation; 2) 10 g of cuticle of the ascaris worm (*Ascaris lumbricoides* L.) per pot; the ground cuticle was mixed thoroughly with the soil of each pot ten days before *M. incognita* inoculation; 3) *Steinernema carpocapsae* strain All, at the rate of 125 infective juveniles (IJs)/cm^2 (i.e.16,600 IJs/pot), was applied immediately after *M. incognita* inoculation; 4) spraying oxamyl on plant shoots (3 litres of 24% liquid oxamyl in 600 litres water; 15 ml of the solution/pot) immediately and again one week after *M. incognita* inoculation; 5) 5 ml of Nemastop (a suspension of garlic extract, 600 g ground garlic cloves/litre of water) per pot immediately after nematode inoculation; 6) *Heterorhabditis bacteriophora* strain EGG at the rate of 16,600 IJs/pot was applied immediately after *M. incognita* inoculation; 7) untreated controls. EPN were routinely cultured on *Galleria mellonella* L. (Woodring and Kaya, 1988). The pots were arranged in a randomized complete block design, maintained at 23 ± 5 °C and watered as needed.

Assessment of plant and nematode parameters. Fifty-three days after *M. incognita* inoculation, the tops of the eggplants were cut off and the roots gently washed free of soil. Fresh weights of the roots and shoots, as well as their lengths, were recorded. Roots were stained in hot acid fuchsin-lactophenol, cleared with lactophenol and nematode galls and egg masses were counted (Taylor and Sasser, 1978). The centrifugal-flotation technique (Jenkins, 1964) was used to extract nematode juveniles from soil (200 g soil/pot was processed and used to calculate the nematode population per pot). A microbiological analysis was made on 10 g soil samples from the ascaris-treated pots as well as the non-treated control to determine the bacterial flora, using a soil extract-yeast agar medium procedure, according to Mahmoud *et al.* (1964). Before sampling, the soil of each pot was mixed thoroughly.

Statistical analysis. Data were subjected to analysis of variance and averages of shoot and root weights and lengths as well as numbers of each nematode developmental stage were compared using Duncan's New Multiple Range Test.

Table I. Numbers of each developmental stage of *Meloidogyne incognita*, nematode galls and gall index (GI) per root system of eggplant (cv. Roomi Balady), as influenced by different treatments, 53 days after inoculation of the nematode in the glass-house.

Treatment	J_2 in soil	J_3	J_4	No. of females	No. of galls	No. of egg masses	GI %
Control	410	169 *Aa	88 Aa	262 Aa	279 Aa	243 Aa	100
Nemaless	___	33 Bb	21 Bb	66 Bb	74 Bb	55 Bb	24.7
Ascaris	___	6 Ef	4 De	5 Ff	11 Ef	3 Ed	2.7
S. carpocapsae	___	11 CDEde	13 Ccd	23 CDcd	29 Dd	18 Cc	9
Vydate	___	8 DEef	15 Cc	13 Ee	24 De	16 CDc	7.7
Nemastop	___	14 CDcd	5 De	18 DEde	27 Dde	9 DEd	6.9
H. bacteriophora	___	16 Cc	11 Cd	27 Cc	38 Cc	22 Cc	11.5

Each value is the mean of ten replicates. J_2,J_3 and J_4 = Second, third and fourth stage juveniles of *M. incognita.*
* Averages in each column sharing a common letter are not significantly different according to Duncan's New Multiple Range Test. Small letters for $P \leq 0.05$ and capital letters for $P \leq 0.01$.
GI% = total number of galls and eggmasses on the root system/their corresponding number in the control.

RESULTS AND DISCUSSION

Nematode development and reproduction were greatly reduced by all treatments (Table I). The data suggest that the treatments delayed nematode development or did not allow the nematode to complete its life cycle by the end of the experiment. As a result, newly hatched J_2 were extracted only from non-treated soil (Table I). The greatest reduction in numbers of *M. incognita* juveniles was caused by ascaris ground cuticle, followed by garlic extract, oxamyl, *S. carpocapsae*, *H. bacteriophora* and *S. marcescens.* Ascaris cuticle treatment caused a significantly greater ($P \leq 0.01$) decrease in numbers of *M. incognita* females than any other treatment, followed by oxamyl (Table I). Similar trends were observed with nematode galls, galling index and number of nematode egg masses (Table I).

Eggplant biomass was markedly increased by most of the treatments (Table II). Increases ranged from 12.3% in the shoot weight of *H. bacteriophora*-treated pots to 96.3% in the root weight of ascaris cuticle-treated pots. The largest increase in shoot and root weight and length was attained by oxamyl and ascaris cuticle treated plants, respectively.

The idea of amending the soil with a substance similar to that present on the outer layer of a pathogen was first suggested by Mitchell and Alexander (1961). For example, chitin is present in egg shells of root-knot nematodes (Spiegel and Cohn, 1985) and nematode cuticle and the use of chitinous products as soil amendments was reported to control plant parasitic nematodes (Mian *et al.*, 1982; Spiegel *et al.*, 1986). Such a rationale (Mitchell and Alexander, 1961) for the control of plant parasitic nematodes would probably be best satisfied by using collagen as a soil amendment to increase the collagenolytic and proteolytic microflora (Galper *et al.*, 1990). Collagen is a long rod-like molecule consisting of three polypeptide chains wound about each other. The first step in the degradation of collagen is the initial attack by collagenase, which gives rise to two peptides that can be further digested by proteases (Harper, 1980). These two types of enzymes have potential to harm external structures of nematodes in the eggshell and the cuticle, or in cuticular structures such as cysts or stylets due to their protein-collagen nature, thus leading to nematode control. Our study showed that soil treated with ground ascaris cuticle contained much *Bacillus subtilis* (138 bacterial cells/g of treated soil compared to 28 bacterial cells/g in the control). Therefore, ascaris cuticle, containing up to 90% collagen (D'Auria *et al.*, 2000), might have served as a food source for this bacterium, which in turn may have controlled plant-parasitic nematodes. We speculate that the initial population of *B. subtilis* may have come from a trace carried to the soil by contaminated air and then thrived on the ground ascaris cuticle. The ability of

Table II. Weight and length of shoots and roots of eggplant (cv. Roomi Balady) infested by *M. incognita* as influenced by the treatments, 53 days after inoculation of the nematode in the glass-house.

Treatment	Shoot		Root	
	Length	Weight	Length	Weight
Control	18 Bc*	65 Cc	19 Bc	27 Cc
Nemaless	30 Aab	90 Aa	28 Aa	35 Bb
Ascaris	24 ABbc	77 Bb	25 ABab	53 Aa
S. carpocapsae	28 ABb	80 ABb	25 ABab	33 BCb
Vydate	32 Aa	90 Aa	22 ABbc	32 BCbc
Nemastop	26 ABbc	74 BCb	27 Aa	36 Bb
H. bacteriophora	25 ABbc	73 BCbc	25 ABab	33 BCb

Each value is the mean of ten replicates. Lengths are expressed as cm and weights as gram fresh weight
* Averages in each column sharing a common letter are not significantly different according to Duncan's New Multiple Range Test. Small letters for $P \leq 0.05$ and capital letters for $P \leq 0.01$.

Bacillus species to suppress nematodes has been attributed to reduced egg hatching by modification of root exudates, which also interferes with the host finding processes of the nematodes and may even lead to production of metabolites that are toxic to the nematodes (Sikora and Hoffman-Hergarten, 1992; Mankau 1995; Hallmann *et al.*, 1998).

Numerous species of EPN have recently been introduced for control of soil insects in Egypt (Abd-Elgawad, 2001). Recent reports (Grewal *et al.*, 1999; Abd-Elgawad and Aboul-Eid, 2002; Perez and Lewis, 2002) state that EPN can reduce phytonematode populations as well. Our results have confirmed such findings. However, optimal application strategies are needed to maximize field effectiveness of EPN, e.g. delivery of the dauer stage juveniles near the plant roots for effective phytonematode control. Four mechanisms of plant-parasitic nematode suppression caused by entomopathogenic nematodes have been reported: 1) entomopathogenic nematodes crowded along the roots of plants forcing plant-parasitic nematodes away (Bird and Bird, 1986); 2) massive doses of entomopathogenic nematodes leading to a build-up of nematode antagonists, resulting in nematode suppressive soils (Ishibashi and Kondo, 1986; Ishibashi and Choi, 1991); 3) allelochemicals, almost exclusively ammonium (Grewal *et al.,*1999), produced by the entomopathogenic nematodes or their symbiotic bacteria, either repelling or intoxicating plant-parasitic nematodes (Hu and Webster, 1995); and 4) root-penetrating EPN releasing small quantities of nematode antagonistic metabolites upon their death, and the death of the bacterial symbiont, that are dispersed through neighbouring root tissue, thus protecting the root from further penetration by plant-parasitic nematodes or antagonizing plant-parasitic nematodes present in the root. Such a localized effect of mechanism 4 would confer only limited protection to the plant (Fallon *et al.*, 2002). This may explain the variation in the control efficacy of EPN treatments in different experiments. Suppression of *M. incognita* by EPN may vary with density of initial nematode infestation, crop, and soil type (Perez and Lewis, 2002). Further experiments that explain these factors are needed.

Generally, regulation of nematode behavior with environmentally compatible organic amendments (e.g. ground cuticle) or natural compounds, such as garlic extract, should have potential use in developing extremely safe and economically valuable management strategies. But many ecological factors, including the type and composition of the soil and its chemical and biological constituents, may inhibit deleterious effects of these substances on phytonematodes.

Although all treatments affected *M. incognita* populations and increased eggplant growth parameters (Tables I and II), the level of control achieved by the applied agents was quite different. Abd-Elgawad and Aboul-Eid (2001) found similar results concerning some of the bioagents tested here. Their modes of action are quite different. Oxamyl is a systemic nematicide/insecticide that penetrates the leaf cuticle and is translocated from the leaves to the root system through the phloem (Hsu and Kleier, 1996). The suppressive effect of EPN on plant-parasitic nematodes may be due to one or more of the four above-mentioned mechanisms. *Serratia marcescens* produces volatile metabolites, especially when the nitrogen source in the growth medium is organic and in the form of amino groups, toxic to *M. incognita* juveniles (Zavaleta-Mejia and Van Gundy, 1989). Also, the garlic extract proved to have a direct nematicidal activity on the nematodes. The microhabitat is likely to have a significant effect on the effectiveness of EPN, *S. marcescens* and garlic extract treatments.

Further investigations are necessary to confirm the effectiveness of the bio-agents we tested on root-knot nematodes under field conditions.

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