INTEGRATED MANAGEMENT OF *MELOIDOGYNE INCOGNITA* INFECTING TOMATO USING BIO-AGENTS MIXED WITH EITHER OXAMYL OR ORGANIC AMENDMENTS

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Summary. The effects of three commercial bio-products containing the bio-agents *Bacillus megaterium* (Bioarc®), *Trichoderma album* (Biozeid®) or *Ascophyllum nodosum* (Algaefol®), dried seed powder of *Matricaria chamomilla* and chitosan, each at 5 g or ml/kg soil, and the nematicide oxamyl at 0.01 g a.i/kg soil, alone or in combination, on *Meloidogyne incognita* in tomato were assessed in a glasshouse pot experiment. All treatments significantly improved plant growth and suppressed the nematode compared to untreated inoculated plants. Among the single treatments, the bio-products were the most effective with root gall reductions of 94.6% (Biozeid®), 89.1% (Bioarc®) and 81.7% (Algaefol®), similar to those obtained with the nematicide oxamyl (88.3%). The corresponding values for the suppression of the second-stage juveniles (J_2) were 92.4%, 92.4% and 96.0%, again similar to oxamyl (91.2%). Chitosan and *M. chamomilla* were the least effective in suppressing the nematode. The efficacy of each of the bio-products against *M. incognita* was increased by the addition of oxamyl to the soil. The best combinations to reduce root galling (95.5%) and J_2 in the soil (97.5%) were Biozeid® + oxamyl and Bioarc® + oxamyl, respectively. The combination of Algaefol® + oxamyl significantly reduced tomato galling (88.4%) and *M. incognita* J_2 in the soil (95.8%). The addition of the organic amendments chitosan and *M. chamomilla* to each of the bio-products increased the control of the nematode except for Biozeid® + *M. chamomilla*, Biozeid® + chitosan and Algaefol® + chitosan, which exhibited antagonistic interaction effects. Bioarc® + oxamyl and Algaefol® + *M. chamomilla* produced the greatest shoot length and weight. None of the combined treatments affected root length and weight of tomato, except Biozeid® + oxamyl which decreased and Algaefol® + chitosan which increased root weight.

Key words: Biological control, root-knot nematodes, soil amendments, *Solanum lycopersicum*.

Plant-parasitic nematodes cause diseases in nearly all crop plants of economic importance, with estimated losses of US$125 billion per year worldwide (Chitwood, 2003). Among these, the root-knot nematodes, *Meloidogyne* spp., are one of the most economically important nematode pest groups in both tropical and sub-tropical crop production regions (Sikora and Fernandez, 2005).

Chemical control is widely used for the management of root-knot nematodes. However, synthetic nematicides are now being reappraised with respect to environmental hazard, high costs, limited availability in many developing countries and their diminished effectiveness following repeated applications (Dong and Zhang, 2006). This has encouraged scientists to search for sources of effective and eco-friendly methods for nematode management alternative to synthetic nematicides (Noling and Becker, 1994).

Biological agents and organic soil amendments have been used successfully as effective alternative methods for controlling root-knot nematodes (Stirling, 1991; D'Addabbo, 1995; Akhtar and Malik, 2000; Oka, 2010). However, biological control alone is often inadequate and/or insufficient to maintain nematode populations below their economic threshold under normal agricultural conditions. Therefore, it must be integrated with other management means (Akhtar, 1997; Wu *et al.,* 1998; Hildalgo-Diaz and Kerry, 2008). Enhancement or application of bio-control agents within an integrated pest management protocol must be promoted and investigated in more detail.

An increase in nematicidal efficacy of microorganisms appears possible when such bio-control agents are integrated with either organic amendments or nematicides into an integrated control package (Al-Rehiayani *et al.,* 1999; Radwan, 1999, 2007; Radwan *et al.*, 2004; Ashraf and Khan, 2010).

In this context, the efficacy of the bio-products Bioarc®, Biozeid® and Algaefol®, alone and in combination with either oxamyl or organic amendments, in the management of *M. incognita* (Kofoid *et* White) Chitw. in tomato, was evaluated in a pot experiment under glasshouse conditions.

MATERIALS AND METHODS

The root-knot nematode *M. incognita* was isolated from infected roots of eggplant (*Solanum melongena* L.) obtained from El-Nubaria region, Behera Governorate, Egypt. Eggs were extracted from infected roots using the sodium hypochlorite method (Hussey and Barker, 1973).

The commercial products Bioarc®, containing *Bacillus megaterium* De Bary (25 × 10^6 cfu/g) and Biozeid®, containing *Trichoderma album* Nagy AbuZeid (25 × 10^6

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spores/g), were obtained from the Plant Disease Research Institute, Agricultural Research Centre (ARC), Giza, Egypt; and the concentrated alkaline liquid extract of Algaefol®, containing *Ascophyllum nodosum* Le Jolis, was provided by Chema Industries, Alexandria, Egypt. The organic amendments were: seeds of the medicinal plant *Matricaria chamomilla* L., purchased from a local market, and chitosan obtained from Chema Industries, Alexandria, Egypt. The nematicide oxamyl (10G) was supplied by E.I. du Pont de Nemours & Company Inc. Each of these products was used alone and each of the bio-products was used in combination with either amendments or the nematicide (Tables I-III). Controls were non-inoculated pots and inoculated but non-treated pots.

The experiment was conducted in clay pots of 15 cm diameter filled with 2 kg of steam sterilized sandy clay loam soil (68% sand, 6% silt and 26% clay, pH 7.8, 0.7% organic matter). All pots were arranged in a completely randomized design on a bench in a greenhouse at 27-32 °C. Each treatment was replicated three times.

Bio-products or organic amendments were incorporated into the soil at the rate of 5 g or ml/kg two weeks before transplanting one-month-old seedlings of tomato (*Solanum lycopersicum* L.) cv. Super strain B. All pots were irrigated daily to ensure the establishment of the organisms in the soil or to ensure proper decomposition of the amendments. Oxamyl was applied to the soil at the rate of 0.01 g a.i./kg, two days after transplanting. Each pot was inoculated with 5000 eggs and J_2 of the nematode. Fifty days after inoculation, the plants were removed from the pots and the roots were washed free of soil. Plant top and root length and fresh weight, number of galls/root system and number of J_2/250 g soil were determined for each plant. The J_2 were extracted from the soil by the decanting and sieving technique (Goodey, 1963) and counted under a stereo microscope.

Interactions within the treatment combinations were calculated using Limpel's formula (Richer, 1987)

$$E = X + Y - XY/100$$

where: E = expected additive effect of the components A and B; X = effect due to component A, and Y = effect due to component B.

The co-toxicity factor was calculated according to Mansour *et al.* (1966):

$$\text{Co-toxicity factor} = \frac{\text{Observed effect (\%) - Expected effect (\%)}}{\text{Expected effect (\%)}} \times 100$$

This factor was used to determine the type of interaction that occurred between each bioagent and either oxamyl or the organic materials. A co-toxicity factor of 20 or more is considered potentiation, a negative factor of 20 or more means antagonism, and intermediate values between -20 and +20 indicate an additive effect.

All data were subjected to statistical analysis of variance according to the SAS software programme (SAS Institute, 1998). Data of the numbers of nematode root galls and J_2 were transformed to $\sqrt{x+1}$ before being subjected to the analysis. Treatment means were compared by least significant difference (LSD) at the 5% level of probability.

RESULTS AND DISCUSSION

All treatments significantly reduced root galling and J_2 in the soil compared to the untreated inoculated control (Table I). The greatest reduction of the individual treatments was given by Biozeid® followed by Bioarc® and oxamyl. However, Algaefol®, *M. chamomilla* and chitosan did not significantly differ from Bioarc® or oxamyl. Of the combined treatments, the best combination to reduce root galling was Biozeid® + oxamyl (95.5%), followed by Bioarc® + oxamyl (94.7%) and Bioarc® + *M. chamomilla* (90.5%) (Table I).

The percent reduction of J_2 in the soil of the individual treatments ranged from 88.1 to 96. Algaefol® caused the greatest reduction (96%), followed by Biozeid® (92.4%) and Bioarc® (92.4%). On the other hand, *M. chamomilla* (88.1%) and chitosan (89.5%) gave the least reduction of J_2 (Table I). Of the combined treatments, the greatest reduction of J_2 in the soil was obtained with Bioarc® + oxamyl (97.5%), followed by Bioarc® + chitosan (97.1%), Algaefol® + oxamyl (95.8%) and Algaefol® + chitosan (93.8%) (Table I).

Bacillus megaterium, a biological control agent, has been poorly studied. The local commercial product of this bacterium, Bioarc®, reduced the numbers of galls on tomato roots and J_2 of the nematode in the soil, confirming previous findings. Al-Rehiayani *et al.* (1999) reported that *B. megaterium* reduced the population densities of *M. chitwoodi* by up to 50% on potato plants. Secondary metabolites produced by strains of *B. megaterium* caused a significant reduction of the reproduction of *M. exigua* on coffee (Oliveira *et al.*, 2007, 2009). Also, *B. megaterium* inhibited hatching and reduced the development of *M. graminicola* infecting rice (Padgham and Sikora, 2007). Bioarc® suppressed the development of *M. javanica* in sunflower (Hammad and Zaid, 2007). Moreover, Huang *et al.* (2009) found that a culture of *B. megaterium* exhibited nematicidal activity against *M. incognita* through the production of nematicidal volatiles.

The local commercial product of the fungus *T. album*, Biozeid®, gave encouraging results in the control of *M. incognita* as it reduced the numbers of galls on tomato roots and J_2 in the soil. Our results complement those of Hammad and Zaid (2007). These authors demonstrated that, when Biozeid® was used as a soil amendment, it effectively reduced the incidences of *M. javanica* infecting sunflower. Also, our results confirmed previous findings on the use of isolates of *Trichoderma* spp. for the management of root-knot nematodes in tomatoes (Spiegel and Chet, 1998; Meyer *et al.*, 2000;

Table I. Effects of three bio-products containing the bio-agents *Bacillus megaterium* (Bioarc®), *Trichoderma album* (Biozeid®) and *Ascophyllum nodosum* (Algaefol®), two organic amendments (*Matricaria chamomilla* and chitosan) and oxamyl, alone and in combination, on the numbers of galls per plant and second-stage juveniles (J_2) of *Meloidogyne incognita* in the soil, in a pot experiment with tomato plants.

Treatment	Dosage (g/kg)	Galls / root system	Transformed data (√x+1)	J_2/250 g soil	Transformed data (√x+1)
Non-inoculated control					
Untreated inoculated control		1108	33.1 ± 6.5	3824	61.7 ± 8.3
Matricaria chamomilla (ground seeds)	5	211	14.3 ± 3.6	457	21.3 ± 2.5
Chitosan	5	185	13.6 ± 1.5	402	20.1 ± 1.5
Bioarc®	5	121	10.9 ± 2.1	290	16.9 ± 2.8
Biozeid®	5	60	7.6 ± 2.0	289	14.0 ± 5.8
Algaefol®	5 ml	202	14.1 ± 2.7	152	11.9 ± 4.1
Oxamyl	0.01	129	11.3 ± 2.2	338	17.1 ± 5.3
Bioarc® + *M. chamomilla*	5 + 5	105	10.3 ± 0.2	418	20.5 ± 3.1
Bioarc® + chitosan	5 + 5	192	13.9 ± 0.9	109	10.5 ± 1.0
Bioarc® + oxamyl	5 + 0.01	58	7.5 ± 2.0	94	9.7 ± 0.4
Biozeid® + *M. chamomilla*	5 + 5	322	17.7 ± 3.7	824	28.6 ± 3.2
Biozeid® + chitosan	5 + 5	230	15.2 ± 1.8	306	16.5 ± 2.4
Biozeid® + oxamyl	5 + 0.01	50	7.1 ± 0.3	274	15.6 ± 4.7
Algaefol® +*M. chamomilla*	5 ml + 5	242	15.4 ± 3.3	339	16.8 ± 2.3
Algaefol® + chitosan	5 ml + 5	277	16.5 ± 2.9	235	13.5 ± 1.8
Algaefol® + oxamyl	5 ml+0.01	129	11.0 ± 3.0	162	12.5 ± 3.2
$LSD_{0.05}$			4.1		9.1

Each transformed figure is the average of three replicates ± SD.

Dababat and Sikora, 2007; Sahebani and Hadavi, 2008; Abd-Elgawad and Kabeil, 2010; Affokpon *et al*., 2011). The explanation for the suppression of root-knot nematodes using *Trichoderma* spp. might be due to the effect of secondary metabolites produced by the fungus in the soil and direct parasitism of nematode eggs through the increase in extra cellular chitinase activity as indicated by egg infection capability and the induction of plant defence mechanism leading to systemic resistance (Sharon *et al*., 2001; Shebani and Hadavi, 2008). Moreover, soil drench of commercially available alkaline extract of the brown alga, Algaefol®, confirmed the suppressive effect on *M. incognita* previously reported by Whapham *et al.* (1994), Wu *et al.* (1997, 1998), Javed *et al.* (2001) and Massa (2010). These authors reported that the addition of *A. nodosum* extracts to the soil decreased the infestation of tomato plants by root-knot nematodes, reducing the number of eggs when compared to untreated controls. The effects of chitosan were similar to those obtained by Zinov'eva *et al.* (1999) and Aboud *et al.* (2002), who reported that chitosan reduced the infection of *M. javanica* and that it may have potential to induce systemic acquired resistance in tomato plants.

The co-toxicity factors for all treatments of the three tested bioagents with *M. chamomilla*, chitosan or oxamyl

Table II. Type of interaction (additive or antagonistic) among three bio-products containing the bio-agents *B. megaterium* (Bioarc®), *T. album* (Biozeid®) and *A. nodosum* (Algaefol®) and two organic amendments (*M. chamomilla* and chitosan) or oxamyl, for their activity against *M. incognita*.

Treatment	% effectiveness for galls		Co-toxicity factor	Type of Interaction
	Expected	Observed		
Bioarc®+ *Matricaria chamomilla*	97.9	90.5	-7.6	Additive
Bioarc®+ chitosan	98.2	82.7	-15.8	Additive
Bioarc®+ oxamyl	98.7	94.7	- 4.0	Additive
Biozeid®+ *M. chamomilla*	99.0	70.9	-28.3	Antagonistic
Biozeid®+ chitosan	99.1	79.2	-20.1	Antagonistic
Biozeid®+ oxamyl	99.4	95.5	-3.9	Additive
Algaefol®+ *M. chamomilla*	96.5	78.1	-19.1	Additive
Algaefol®+ chitosan	96.9	75.0	-22.7	Antagonistic
Algaefol®+ oxamyl	97.9	88.7	-9.7	Additive

showed additive interaction effects on the reduction of root galling of tomato, except for Biozeid® with *M. chamomilla* or chitosan and Algaefol® with chitosan which, instead, exhibited an antagonistic effect (Table II). These results confirm those of Radwan (1999), who found that the combinations of *B. thuringiensis* with carbofuran, oxamyl or terbufos exhibited additive interaction effects against *M. incognita* infecting tomato. Oxamyl increased the efficacy of the obligate bacterial parasite *Pasteuria penetrans* (Thorne) Sayre *et* Starr in trials against *M. javanica* infection of tomato and cucumber crops and the effects on nematode control were additive (Tzortzakakis and Gowen, 1994). In addition, Radwan *et al.* (2004) and Radwan (2007) reported that combinations of treatments of *B. thuringiensis* with poultry manure, sawdust, grape marc, chicken litter or Achook® showed additive interaction effects against *M. incognita* on tomato.

Length and weight of plant shoots and roots were also influenced by the treatments (Table III). The application of either Biozeid® or Algaefol® significantly increased the length of shoots compared to the untreated inoculated control. On the other hand, a significant decrease in tomato root length was recorded in pots receiving Biozeid®. No significant effects on shoot and root weights were observed with any of the single treatments as compared to the untreated inoculated control. All the combined treatments significantly increased the shoot length as compared with the control treatment, except for the mixtures of Biozeid® + *M. chamomilla*, Biozeid® + chitosan and Bioarc® + *M. chamomilla*. No significant differences in root length were noticed between any of the combined treatments and the control. Bioarc® + oxamyl and Algaefol® + *M. chamomilla* gave the greatest shoot weight relative to the control. None of the combined treatments affected root weight significantly as compared with the control, except the combination Biozeid® + oxamyl, which significantly decreased it, and Algaefol® + chitosan, which significantly increased it when compared with the control.

Application of organic matter to the soil is known to have beneficial effects on soil nutrients, soil physical properties, soil biological activity and crop performance. The nutrient content of the amendments and the large quantities of these materials added to the soil result in increased soil fertility and hence plant growth. This helps the plant to tolerate nematode attack (Rodríguez-Kábana *et al.*, 1987; Stirling, 1991). The enhancement of plant growth by organic amendments in the present study could be due to the combination of the suppressive effect on nematodes with a direct fertilizing effect on the plants.

Biological control agents are often applied to soils in combination with organic materials that contribute to enhanced biological activities against the target pathogen. The organic materials provide the nutrients needed for initial growth of the bio-control agents in soil, and may be used as carriers to facilitate distribution. The breakdown of the organic materials may release toxic and nematicidal substances that contribute to nematode control (Rodríguez-Kábana *et al.*, 1987). The integration of the tested bio-agents with either organic materials or nematicides produced a greater re-

Table III. Effects of three bio-products containing the bio-agents *B. megaterium* (Bioarc®), *T. album* (Biozeid®) and *A. nodosum* (Algaefol®), two organic amendments (*M. chamomilla* and chitosan) and oxamyl, alone and in combination, on the growth of tomato plants infected with *M. incognita* in a pot experiment.

Treatment	Dosage (g/kg)	Fresh shoot		Fresh root	
		Length (cm)	Weight (g)	Length (cm)	Weight (g)
Non-inoculated control		63.9 ± 3.5	14.6 ± 0.4	18.3 ± 0.6	5.9 ± 1.9
Untreated inoculated control		53.0 ± 5.3	12.4 ± 3.5	17.3 ± 2.5	5.6 ± 2.0
Matricaria chamomilla (ground seeds)	5	65.8 ± 7.9	13.9 ± 2.8	16.8 ± 2.4	6.1 ± 1.1
Chitosan	5	55.6 ± 0.6	15.9 ± 0.3	12.6 ± 0.6	5.1 ± 1.1
Bioarc®	5	67.4 ± 2.6	19.1 ± 1.5	14.6 ± 2.6	3.1 ± 1.5
Biozeid®	5	73.5 ± 11.5	13.4 ± 3.5	10.3 ± 0.3	3.0 ± 1.5
A. nodosum	5 ml	72.8 ± 7.3	16.8 ± 4.3	15.7 ± 3.2	8.1 ± 1.5
Oxamyl	0.01	51.3 ± 7.6	13.4 ± 1.3	17.0 ± 3.5	5.8 ± 2.7
Bioarc®+ *M. chamomilla*	5 + 5	45.0 ± 5.0	15.5 ± 5.0	12.0 ± 5.0	3.6 ± 0.1
Bioarc®+ chitosan	5 + 5	75.1 ± 11.4	18.5 ± 3.5	16.3 ± 5.3	7.6 ± 1.1
Bioarc®+ oxamyl	5 + 0.01	85.5 ± 7.5	25.4 ± 4.8	17.8 ± 8.3	4.9 ± 2.4
Biozeid®+ *M. chamomilla*	5 + 5	63.5 ± 4.0	19.2 ± 3.6	11.4 ± 1.1	6.4 ± 1.7
Biozeid®+ chitosan	5 + 5	62.4 ± 0.6	16.7 ± 0.6	15.1 ± 2.6	8.6 ± 1.4
Biozeid®+ oxamyl	5 + 0.01	106.3 ± 4.3	19.1 ± 1.6	14.5 ± 4.5	1.5 ± 0.3
Algaefol®+ *M. chamomilla*	5 ml + 5	76.4 ± 4.6	20.9 ± 4.9	14.3 ± 2.3	6.9 ± 4.5
Algaefol®+ chitosan	5 ml + 5	75.8 ± 7.3	17.3 ± 1.5	18.3 ± 1.3	9.1 ± 3.8
Algaefol®+ oxamyl	5 ml + 0.01	86.7 ± 9.1	20.6 ± 7.1	13.8 ± 4.4	4.9 ± 2.8
$LSD_{0.05}$		15.4	8.3	6.2	3.4

Each figure is the average of three replicates ± SD.

duction of root galling and J_2 of *M. incognita* in the soil and improved growth of the infected plants more than the single treatments. These findings agree with the results found previously by Al-Rehiayani *et al.* (1999), in which the application of *B. megaterium* reduced *M. chitwoodi* populations to a greater extent if oil radish or rapeseed green manures had been added to soil. In other studies, the efficacy of *B. thuringiensis* against *M. incognita* was significantly increased by the addition of organic amendments or nematicides to the soil (Radwan, 1999, 2007; Radwan *et al.*, 2004). Moreover, Radwan *et al.* (2007) found that the nematicidal efficacy of green algae, azolla and yeast and/or molasses in buffalo manure extract against *M. incognita* was significantly increased by the addition of oxamyl. In a different experiment, Ashraf and Khan (2010) stated that the efficacy of the biocontrol agents *Paecilomyces lilacinus* (Thom) Samson and *Cladosporium oxysporum* Berkeley *et* Curtis against *M. javanica* attacking eggplant increased in the presence of oil cakes. On the other hand, the combinations of Biozeid® + *M. chamomilla*, Biozeid® + chitosan and Algaefol® + chitosan in our experiment exhibited antagonistic interaction effects, thus supporting the results of Chen *et al.* (2000), who reported that organic amendments did not enhance the efficacy of biocontrol agents *B. thuringiensis* Berliner, *Paecilomyces marquandii* (Masse) S. Hughes and *Streptomyces costaricanus* Esnard, Potter *et* Zuckerman against *M. hapla* Chitw. infecting lettuce. Also, the combined application of neem oil, *Datura stramonium* L. and *Calotropis procera* Aiton leaves with *T. harzianum* Rifai did not increase the reduction of the multiplication of *M. incognita* attacking okra compared to either neem oil or *Calotropis procera* leaves alone (Sharma *et al.*, 2005).

Under the conditions of our experiment, application of bio-products containing the bio-agents *T. album*, *B. megaterium* and *A. nodosum*, either alone or combined with the organic amendments or oxamyl, provided an effective mean for reducing nematode levels so may be a useful alternative control option for the management of root-knot nematodes. However, the results should be further confirmed under microplot and field conditions.

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