EFFICACY OF *BACILLUS THURINGIENSIS* INTEGRATED WITH OTHER NON-CHEMICAL MATERIALS TO CONTROL *MELOIDOGYNE INCOGNITA* IN TOMATO

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Summary. The effects of *Bacillus thuringiensis* (Bt, Dipel 2X, at 12 mg/kg soil), grape marc (GM), chicken litter (CL), both at 10 g/kg soil, and the neem-based product Achook® (AC), at 500 mg/kg soil, alone or as combinations of Bt with each of the other three products, on *Meloidogyne incognita* infecting tomato were assessed in a glass-house pot experiment. Controls consisted of non-inoculated pots, untreated pots inoculated with *M. incognita*, and pots treated with oxamyl at 10 mg a.i./kg soil. All treatments significantly improved plant growth and suppressed the nematode compared to untreated inoculated plants. Among the organic materials, CL and GM were the most effective with root gall reductions of 75.5% and 72.2%, respectively, similar to oxamyl (79.0%). The efficacy of *B. thuringiensis* against *M. incognita* was significantly increased by addition of the organic amendments to the soil. The best combination with which to reduce root galling (by 86.2%) and second-stage juveniles in the soil (by 80.5%) was Dipel 2X + CL, followed by Dipel 2X + GM and Dipel 2X + AC. These combined treatments also improved plant growth parameters. Therefore, *B. thuringiensis* (Dipel 2X) applied to soils in combination with organic amendment materials may be considered as a promising alternative to chemicals for controlling *M. incognita*.

Key words: Bio-control agents, integrated control, root-knot nematodes, soil amendments.

Root-knot nematodes (*Meloidogyne* spp.) are among the most common and important plant parasitic nematodes in tropical and subtropical regions of the world (Luc *et al*., 2005). *Meloidogyne incognita* (Kofoid *et* White) Chitw. is one of the most harmful root-knot nematode species, and is widespread and economically important on a range of field and vegetable crops on lighter soil types in Egypt (Ibrahim *et al*., 2000). The use of nematicides is one of the major means for controlling root-knot nematodes (Whitehead, 1998). However, high cost and a reduced availability of nematicides, as a result of increasing concern for the environment and for public health, have augmented interest in alternative methods for the management of root-knot nematodes (Noling and Becker, 1994).

Organic soil amendments and biocontrol agents have been used successfully as effective alternative eco-friendly methods for controlling root-knot nematodes (Rodriguez-Kábana *et al*., 1987; Stirling 1991; D'Addabbo, 1995; Siddiqui and Mahmood, 1999; Akhtar and Malik, 2000; Barker, 2003).

Most studies have demonstrated that organic amendments have low efficacies, which make them unacceptable as control agents as they need to be applied in large amounts for effective nematode management (Muller and Gooch, 1982). However, integrating them with microorganisms, such as *Bacillus thuringiensis* Berliner, could be effective for nematode control (Radwan *et al*., 2004).

Little is known about the combined effectiveness of *B. thuringiensis* and organic amendments against root-knot nematodes (Chen *et al*., 2000; Radwan *et al*., 2004). Therefore, the objective of this study was to evaluate the efficacy of *B. thuringiensis* combined with organic amendment in the management of *M. incognita* on tomato, in a pot experiment under glass-house conditions.

MATERIALS AND METHODS

The root-knot nematode *M. incognita* was isolated from infected roots of eggplant (*Solanum melongena* L.) obtained from Rosetta, Behera Governorate, Egypt. Eggs and second-stage juveniles (J_2) were extracted from infected roots by the sodium hypochlorite method (Hussey and Barker, 1973).

The commercial products used were *B. thuringiensis* subsp. *kurstaki* (Dipel 2X, 6.4% WP) obtained from Rhone Poulenc Company, the nematicide oxamyl (10% G) supplied by E. I. du Pont de Nemours & Company Inc., used for comparison, and Achook® (0.15% E.C), a neem-based biopesticide, provided by Bahar Agrochem & Feeds, India. The organic amendments were chicken litter, collected at the University of Alexandria, Agricultural Experimental Farm, Poultry Division, and grape marc obtained from Ganaklis Vineyard Company, Desert Rood, West Alexandria.

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The experiment was conducted in clay pots of 15 cm diameter filled with 1 kg of steam sterilized sandy clay loam soil (51% sand, 15% silt and 34% clay; pH 7.8; organic matter 0.73%). All pots were arranged in a completely randomized block design on the bench in a greenhouse that averaged 27-32 °C and each treatment was replicated three times.

The experiment included the following ten treatments: 1) non-inoculated control; 2) untreated control inoculated with *M. incognita*; 3) oxamyl; 4) Dipel 2X (Bt); 5) grape marc (GM); 6) chicken litter (CL); 7) Achook® (AC); 8) Bt + GM; 9) Bt + CL; and 10) Bt + AC (Table I). Treatments 2 to 10 were all inoculated with *M. incognita*.

Each organic amendment (GM or CL) was incorporated into the soil at the rate of 10 g/kg, two weeks before transplanting one-month-old seedlings of tomato (*Lycopersicon esculentum* Mill.) cv. Super strain B. Bt, AC and oxamyl were applied to the soil at the rate of 0.012, 0.5 and 0.01 g/kg, respectively, sequentially after nematode inoculation. Each plant was inoculated with 5,000 nematode eggs + J_2/pot by pouring the nematode suspension into holes made 2-4 cm below the soil surface around the base of the plants. After 50 days, plants were removed from the pots and the roots were washed free of soil. Top and root length and fresh weight, number of galls/root system and number of J_2/250 g soil were determined for each of the plants. The second stage juveniles (J_2) were extracted from the soil by the decanting and sieving technique (Goodey, 1963) and counted.

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):

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

This factor was used to determine the type of interaction that occurred between Bt and the non-chemical treatments. 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.

Data were subjected to analysis of variance and means were compared for significance by LSD at the probability of 0.05 (Cohort Software Inc., 1985).

RESULTS AND DISCUSSION

Tomato root galling was significantly reduced by all the different treatments compared to the untreated inoculated control (Table I). The greatest reduction was by Bt + CL followed by Bt + GM and Bt + AC (Table I). Similarly to root galling, the reduction in the numbers of J_2 obtained from soil of the different treatments ranged from 63.0 to 80.5%. The greatest reductions were by Bt + CL, Bt + GM and Bt + AC (80.5, 79.7 and 79.3%, respectively) (Table I).

The combinations of the organic amendments with Bt resulted in greater reductions of the *M. incognita* than when they were applied alone. The best combination to reduce root galling (by 86.2%) and J_2 (by 80.5%) was that of Bt + CL, followed by Bt + GM and Bt + AC.

Table I. Effects of *Bacillus thuringiensis* and non-chemical materials, alone and in combination, on the numbers of galls per plant and second-stage juveniles (J_2) per 250 g of soil of *Meloidogyne incognita* in a pot experiment with tomato plants.*

Treatment	Galls/plant (N°)	Decrease in galls over control (%)	J_2/250 g soil	Decrease in J_2 over control (%)
Non-inoculated control				
Untreated inoculated control	133.2		739.0	
Oxamyl	28.0	79.0	161.0	78.2
Dipel 2X (Bt)	38.7	70.8	250.0	66.2
Grape marc (GM)	37.0	72.2	273.3	63.0
Chicken litter (CL)	32.7	75.5	165.0	77.7
Achook® (AC)	45.0	66.2	260.0	64.8
Bt + GM	22.0	83.5	150.0	79.7
Bt + CL	18.3	86.2	144.3	80.5
Bt + AC	24.7	81.5	152.7	79.3
L.S.D. P = 0.05	9.72		54.95	

Grape marc, chicken litter and Achook® were incorporated into the soil at 10, 10 and 0.5 g/kg, respectively.
Dipel 2X and oxamyl were applied to the soil at 0.012 and 0.01g/kg, respectively.
*Data are means of three replicates.

Bacillus thuringiensis synthesizes delta endotoxins, which may inhibit the parasitism of plant parasitic nematodes. The commercial product of the bacterium (Dipel 2X) suppressed the nematode as it reduced the numbers of galls on tomato roots and J_2 in the soil, confirming previous findings. Dipel 2X and SAN 415 *B. thuringiensis* strains suppressed populations of *M. javanica* (Treub) Chitw. on tomato and of *Tylenchulus semipentrans* Cobb on citrus under greenhouse conditions (Osman *et al*., 1988). A strain of *B. thuringiensis* reduced damage by root-knot nematodes and *Rotylenchulus reniformis* Lindford *et* Oliveira and the commercial products of *B. thuringiensis* Turex and Dipel 2X reduced J_2 in the soil and galls on the roots of tomatoes grown in *M. incognita* infested soil (Zuckerman *et al*., 1993; Radwan *et al*., 2004; Radwan, 2007).

The results obtained with *M. incognita* on tomato by amending soil with CL were similar to those of Mian and Rodríguez-Kábana (1982), Chindo and Khan (1990), Kaplan and Noe (1993), Riegel and Noe (2000), D'Addabbo *et al*. (2003) and Lopez-Perez *et al*. (2005). GM soil amendment confirmed the suppressive effect on *Meloidogyne* spp. previously reported by D'Addabbo and Sasanelli (1998), Oka and Yermijahu (2002) and Nico *et al*. (2004). Moreover, AC was effective against *M. incognita* and this is in agreement with Akhtar and Mahmood (1994), who reported that the addition of Achook® to the soil significantly reduced populations of plant parasitic nematodes and root galling on tomato and increased numbers of free-living nematodes.

The co-toxicity factors for the treatments of Bt with GM, CL or AC showed additive interaction effects on the reduction of tomato root galling. Bt + CL was the most effective followed by Bt + GM and Bt + AC (Table II). These results conform with those of Radwan *et al*. (2004), who found that the combination of the commercial product of *B. thuringiensis* (Turex) with poultry manure or sawdust exhibited an additive effect against *M. incognita* on tomato.

The length and weight of plant shoots and roots were also influenced by the treatments (Table III). The application of either Bt or other single treatments, except oxamyl, significantly increased length and fresh weight of the shoots compared to the untreated inoculated control. A significant increase in tomato root length was also recorded in pots receiving AC, GM, CL or Bt. Similar re-

Table II. Types of interaction between *B. thuringiensis* and non-chemical materials against *M. incognita*.

Treatment	% effectiveness for galls		Co-toxicity factor	Type of interaction
	Observed	Expected		
Bt + GM	83.48	91.93	- 9.2	Additive
Bt + CL	86.23	92.88	- 7.2	Additive
Bt + AC	81.48	90.20	- 9.7	Additive

Bt = *B. thuringiensis*; GM = Grape marc; CL = chicken litter; and AC = Achook®

Table III. Effects of *B. thuringiensis* and non-chemical materials, alone and in combination, on the growth of tomato plants infected with *M. incognita* in a pot experiment.*

Treatment	Fresh shoot		Fresh root	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)
Non-inoculated control	24.0	3.7	11.4	2.9
Untreated inoculated control	18.9	3.2	10.1	1.9
Oxamyl	22.2	3.5	10.2	2.2
Dipel 2X (Bt)	24.1	4.1	12.0	2.3
Grape marc (GM)	26.8	5.2	11.7	2.6
Chicken litter (CL)	25.9	4.9	11.0	2.3
Achook® (AC)	27.6	5.0	12.2	3.3
Bt + GM	30.9	5.6	13.8	4.6
Bt + CL	34.2	6.0	15.0	3.7
Bt + AC	28.6	5.6	13.5	3.1
L.S.D. P = 0.05	4.48	0.73	1.12	0.77

Grape marc, chicken litter and Achook® were incorporated into the soil at 10, 10 and 0.5 g/kg, respectively.
Dipel 2X and oxamyl were applied to the soil at 0.012 and 0.01g/kg, respectively.
*Data are means of three replicates.

sults on root weight were obtained in pots amended with AC but not with CL, Bt or GM. All combined treatments improved tomato growth compared to single treatments, with greatest growth given by Bt + CL or Bt + GM.

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. These materials provide the nutrients needed for initial growth of the bio-control agents in soil, and may be used as carriers to facilitate distribution. Breakdown of organic materials may release toxic and nematicidal substances that contribute to nematode control (Rodríguez-Kábana *et al*., 1987). The integration of *B. thuringiensis* with organic materials produced a greater reduction 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 confirm results previously obtained by Radwan *et al*. (2004). In contrast, Chen *et al*. (2000) reported that organic amendments did not enhance the efficacy of bio-control agents *B. thuringiensis*, *Paecilomyces marquandii* (Masse) S. Hughes and *Streptomyces costaricanus* Esnard, Potter *et* Zuckerman against *M. hapla* Chitw. infecting lettuce.

Under the conditions of this experiment, application of *B. thuringiensis*, either alone or combined with the tested organic amendment materials, provided an effective means for reducing nematode levels and could be an alternative control option for the management of root-knot nematodes. Further work will be needed to determine their effectiveness under field conditions.

LITERATURE CITED

Akhtar M. and Mahmood I., 1994. Nematode populations and short-term tomato growth in response to neem-based products and other soil amendments. *Nematropica, 24*: 169-173.

Akhtar M. and Malik A., 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. *Bioresource Technology, 74*: 35-47.

Barker K., 2003. Perspectives on plant and soil nematology. *Annual Review of Phytopathology, 41*: 1-25.

Chen J., Abawi G.S. and Zuckerman B.M., 2000. Efficacy of *Bacillus thuringiensis*, *Paecilomyces marquandii* and *Streptomyces costaricanus* with and without organic amendments against *Meloidogyne hapla* infecting lettuce. *Journal of Nematology, 32*: 70-77.

Chindo P.S. and Khan F.A., 1990. Control of root-knot nematodes, *Meloidogyne* spp., on tomato, *Lycopersicon esculentum* Mill., with poultry manure. *Tropical Pest Management, 36*: 332-335.

Cohort Software Inc., 1985. *Costat Users manual. Version 3*. Cohort. Tucson, Arizona, USA.

D'Addabbo T., 1995. The nematicidal effect of organic amendments: a review of the literature, 1982-1994. *Nematologia Mediterranea, 23*: 299-305.

D'Addabbo T. and Sasanelli N., 1998. The suppression of *Meloidogyne incognita* on tomato by grape pomace soil amendments. *Nematologia Mediterranea, 26*: 145-149.

D'Addabbo T., Sasanelli N., Lamberti F., Greco P. and Carella A., 2003. Olive pomace and chicken manure amendments for control of *Meloidogyne incognita* over two crop cycles. *Nematropica,* 33: 1-7.

Goodey J.B., 1963. *Laboratory methods for work with plant and soil nematodes*. Ministry of Agriculture, Fisheries and Food, Technical Bulletin 2, HMSO, London, UK, 44 pp.

Hussey R.S. and Barker K.R., 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter, 57*: 1025-1028.

Ibrahim I.K.A., Handoo Z.A. and El-Sherbiny A.A., 2000. A survey of phytoparasitic nematodes on cultivated and non-cultivated plants in north western Egypt. *Journal of Nematology, 32*: 478-485.

Kaplan M. and Noe J.P., 1993. Effects of chicken-excrement amendments on *Meloidogyne arenaria*. *Journal of Nematology*, *25*: 71-77.

Lopez-Perez J.A., Roubtsova T. and Ploeg A., 2005. Effect of three plant residues and chicken manure used as biofumigants at three temperatures on *Meloidogyne incognita* infestation of tomato in greenhouse experiments. *Journal of Nematology, 37*: 489-494.

Luc M., Sikora R.A. and Bridge J., 2005. *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. 2nd Edition. CABI Publishing, Wallingford, UK, 871 pp.

Mansour N.A., El-Dafrawi M.E., Tappozada A. and Zeid M.I., 1966. Toxicological studies on the Egyptian cotton leaf worm, *Proedenia litura*. VI. Potentiation and antagonism of organophosphorus and carbamate insecticides. *Journal of Economic Entomology, 59*: 307-311.

Mian I.H. and Rodríguez-Kábana R., 1982. Soil amendments with oil cakes and chicken litter for control of *Meloidogyne arenaria. Nematropica, 12*: 205-220.

Muller R. and Gooch P.S., 1982. Organic amendments in nematode control. An examination of the literature. *Nematropica, 12*: 319-326.

Nico A.I., Jimenez-Diaz R.M. and Castillo P., 2004. Control of root-knot nematodes by composted agro-industrial wastes in potting mixtures. *Crop Protection, 23*: 581-587.

Noling J.W. and Becker J.O., 1994. The challenge of research and extension to define and implement alternatives to methyl bromide. *Journal of Nematology, 26*: 573-586.

Oka Y. and Yermiyahu U., 2002. Suppressive effects of composts against the root-knot nematode *Meloidogyne javanica* on tomato. *Nematology, 4*: 891-898.

Osman G.Y., Salem F.M. and Ghattas A., 1988. Bio-efficacy of two bacterial insecticide strains of *Bacillus thuringiensis* as a biological control agent in comparison with a nematicide Nemacur on certain plant parasitic nematodes. *Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz, 61*: 35-37.

Radwan M.A., 2007. Bioactivity of some commercial products of *Bacillus thuringiensis* on *Meloidogyne incognita* infecting tomato. *Indian Journal of Nematology, 37*: in press.

Radwan M.A., Abu-Elamayem M.M., Kassem Sh.M.I. and El-Maadawy E.K., 2004. Management of *Meloidogyne incognita*, root-knot nematode by integration of *Bacillus thuringiensis* with either organic amendments or carbofuran. *Pakistan Journal of Nematology, 22*: 135-142.

Richer D.L., 1987. Synergism - a patent view. *Pesticide Science, 19*: 309-315.

Riegel C. and Noe J.P., 2000. Chicken litter soil amendment effects on soilborne microbes and *Meloidogyne incognita* on cotton. *Plant Disease, 84*: 1275-1281.

Rodríguez-Kábana R., Morgan-Jones G. and Chet I., 1987. Biological control of nematodes: soil amendments and microbial antagonists. *Plant and Soil, 100*: 237-247.

Siddiqui Z.A. and Mahmood I., 1999. Role of bacteria in the management of plant parasitic nematodes: a review. *Bioresource Technology, 69*: 167-179.

Stirling G.R., 1991. *Biological control of plant parasitic nematodes. Progress, Problems and Prospects*. CAB International, Wallingford, UK, 282 pp.

Whitehead A.G., 1998. *Plant nematode control*. CAB International, Wallingford, UK, 384 pp.

Zuckerman B.M., Dicklow M.B. and Acosta N., 1993. A strain of *Bacillus thuringiensis* for the control of plant parasitic nematodes. *Biocontrol Science and Technology, 3*: 41-46.

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