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An integrated control trial of *Meloidogyne incognita* using *Bacillus thuringiensis* associated with nematicides

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ABSTRACT

The experimental product of the bacterial endo-toxin, *Bacillus thuringiensis* and the granular nematicides; carbofuran, oxamyl and terbufos were evaluated alone and in combination against the root-knot nematode, *Meloidogyne incognita* infecting tomato plants in a pot trial. In addition, their effects on tomato growth was also investigated. Results revealed that the *B. thuringiensis* toxin or nematicide alone reduced the number of juveniles in soil and number of tomato galling and enhanced plant growth. However, the integration of these treatments was better in reducing nematode and improving plant growth than that of their single application. *B.thuringiensis* / nematicide mixture exhibited an additive effect against *M. incognita*. *B.thuringinesis* / carbofuran was the best mixture, enhancing maximum growth and nematode control. It is suggested that the low nematicidal properties of carbofuran, can be increased by using in combination with the bacteria toxin. This mixture has potential for use in the integrated nematode management.

INTRODUCTION

The root-knot nematode, *Meloidogyne* spp., has been recognized as one of the serious pests causing remarkable losses of vegetable production especially tomato crop in localities with light sandy soil (Ibrahim ,1985) .

Chemical nematicides are still the most effective measures for controlling plant parasitic nematodes. However, their use in high concentrations, posses a severe problem of mammalian toxicity and environmental hazards (Eto, 1977; Fukuto, 1983 and Marshall, 1985). In recent years, considerable attention has been directed toward effective alternative methods of nematode control to avoid such hazards. A bacterial toxin insecticide, *Bacillus thuringiensis* is one of the biocontrol agents used today as an excellent alternative to chemical pesticides. This bacterial toxin seems to be compatible and less sensitive to low concentrations of most carbamates and organophosphate pesticides (Benz,1971 and Jaques and Morris,1971). Few articles have suggested using strains of the microbial pathogen, *Bacillus thuringiensis* to control plant parasitic nematodes (Osman *et al.*,1988; Mena *et al.*,1996 and Sharma and Gomes,1996) and to affect population growth of free-living nematodes (Meadows *et al.*,1990; Leyns *et al.*,1995 and Borgonie *et al.*,1996).

The potential interactions between the biocide, *Bacillus thuringiensis* and insecticides against different insects was studied by several investigators (Salama *et al.*, 1984; Abdallah,1986; and Kelada and Shaker, 1988), while no work so far has been undertaken to evaluate its efficiency in combination with nematicides for the control of plant parasitic nematodes. Therefore, the present pot trial was conducted to explore the possibility of using *B. thuringiensis*, certain nematicides (at low dosage rate) and their mixtures for the management of *M. incognita* infecting tomato plants.

MATERIALS AND METHODS

Nematode inoculate :

Root- knot nematode, *Meloidogyne incognita* was isolated from infected roots of eggplants (*Solanum melongena*). Root galls were homogenized in a blender and passed through 200,400 mesh sieves to obtain free eggs directly before carrying out the experiment.

Bacterial insecticide tested :

Experimental preparation of the bacterial toxin insecticide, *Bacillus thuringiensis* Berl., was used as SAN 415 and was obtained from Sandoz company. It is a dry powder containing 32000 I.U./ mg .

Nematicides Used:

Three different nematicides were tested : (a) A carbamate insecticide-nematicide, carbofuran (Furadan 10%G), 2,3-dihydro-2,2-dimethylbenzofuran-7-yl methyl carbamate. (b) An oxime carbamate nematicide, oxamyl (Vydate 10%G), Methyl N,N-dimethyl-N-{[methylcarbamoy]}oxy}-1-thiooxamidate. (c) An organophosphate insecticide/ nematicide, terbufos (Counter 10%G), S-(tert.butylthio) methyl O,O-diethyl phosphorodithioate.

Pot experiment:

A pot experiment was carried out using tomato, *lycopersicon esculantum* cv. *castlerocke* as host plant for *Meloidogyne incognita*. Clay pots (12 cm in diameter) were containing 1 kg of sandy clay loam soil. The pots were then arranged in a complete randomized block design. There were eight treatments (each repilcated three times) as follows: *M. incognita* alone; *M. incognita* + *B. thuringiensis*; *M. incognita* + carbofuran; M.incognita + oxamyl; *M. incognita* + terbufos; *M. incognita* + *B. thuringiensis* + carbofuran; *M. incognita* + *B. thuringiensis* + oxamyl; and *M. incognita* + *B. thuringiensis* + terbufos. Each nematicide or bacterial pathogen was applied to the soil at the rate of 5 Kg/feddan (0.05 gm/Kg soil). Immediately following the addition of nematode inocula (5000 eggs/pot), bacterial insecticide and each nematicide were added to soil as side-treatment. The efficiency of the eight treatments was judged, eight weeks after treatment, by determining the number of *2nd* juveniles per 250 gm soil, number of galls per root system and recording the growth indices length as well as fresh weight of root and shoot systems.

Analysis of interactiom data for mixtures :

Interaction data for mixtures were estimated by using limpel's formala reported by Richer (1987) as follow:

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

where: E = The expected additive effect of the mixture.
X = The effect due to component A alone.
Y = The effect due to component B alone.

The expected effect was compared with the actual effect obtained experimentally for mixture to determine the additive or synergistic and antagonistic effect according to the equatien given by Mansour *et al.*, (1966) as follows:

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

This factor was used to classify results into three categories. A positive factor 20 or more is considered potentiation, a negative factor 20 or more means antagonism and intermediate values between -20 and +20 indicate only additive effect. All data were subjected to analysis of variance and means were compared for significance by LSD method at the probability of 0.05 (Steel and Torrie,1980).

RESULTS AND DISCUSSION

The effect of the *Bacillus thuringiensis* and the three nematicides namely: carbofuran, oxamyl and terbufos either alone or in combination on *Meloidogyne incognita* infecting tomato plants and their effect on tomato growth was evaluated in a pot trial and the obtained results are given in Tables 1,2 and 3.

Effect on number of juveniles and galls:

Data presented in Table (1), showed significant effect for all individual treatments in reducing the number of juveniles in soil and the number of galls per root system compared with the untreated check. The *B. thuringiensis* alone suppressed the number of juveniles in soil and galls in tomato roots as compared with control by 72% and 73.37%, respectively. Terbufos was apparently the most effective among the tested nematicides application in reducing the number of juveniles in soil (84.58%), followed by oxamyl (79.19%), and carbofuran (69.24%). With respect to the galls, also terbufos gave the highest effectiveness (84.56%), followed by oxamyl (78.30%) and carbofuran (70.69%) when compared with control. The biopesticide *B.thuringensis* was ranked in between carbofuran and oxamyl in suppresing the nematode both in soil and in tomato roots (Table 1). Abu-Elamayem *et al.*, (1985) showed that terbufos was the most toxic nematicide followed by isazafos, oxamyl, aldicarb, phenamiphos and carbofuran against *M.incognita* juveniles in aqueous solutions. Terbufos proved to be the best nematicide for contorlling the root-knot nematode, *M.incognita* infecting tomato plants followed by oxamyl, whereas carbofuran and isazafos were the least effective compounds in both clay loam and sandy clay loam soils (Radwan, 1988). Also, the application of oxamyl and carbofuran on carrots reduced the number of juveniles in soil and inhibited gall formation of the root-knot nematode, *M.hapla* (Berbec and Dolna,1990).

The *B. thuringiensis* toxin reduced juveniles and galls significantly by 72% and 73.37%, respectively.The data indicated that *B. thuringiensis* was as effective as carbofuran or oxamyl (Table 1). These results support those reported by Osman *et al.* (1988). They showed that Dipel and SAN 415 (both containing *B. thuringiensis Berliner*), each was used at the rate of 5 kg/feddan, suppressed population of *M.javanica* and *Tylenchulus semipentrans* and reduced egg hatchability. SAN 415 was more effective in this respect than Dipel, and was about as effective as the standard nematicide,fenamiphos. *B. thuringiensis var. Kurstaki* controlled *M.incognita* and *Radopholus similis* on *Cucurbita pepo* (Mena *et al.*,1996) Also, Microbial toxin of *B.thuringiensis* strains affect the oviposition and juvenile emergence from cysts of *Heterodera glycines* (Sharma and Gomes,1996).

The results shown in Table (1) evidently indicated that the addition of *B. thuringiensis* toxin to nematicidal treatments increased the efficiency

Table (1): Effect of *Bacillus thuringiensis*, certain nematicides, and their mixtures on *Meloidogyne incognita* infecting tomato plants.

Treatment*	Mean No. of 2nd juveniles/ 250 gm soil	Effective-ness of juveniles (%)	Mean No. of galls/ root system	Effective -ness on galls (%)
M.incognita alone (Conlrol)	445.33		149.00	
M.incognita + *B.thuringiensis*	124.67	72.00	39.67	73.37
M.incognita + Crbofuran	137.00	69.24	43.67	70.69
M.incognita + Oxamyl	92.67	79.19	32.33	78.30
M.incognita + Terbufos	68.67	84.58	23.00	84.56
M.incognita + *B. thuringiensis* + Carbofuran	43.00	90.34	15.67	89.48
M.incognita + *B. thuringiensis* + Oxamyl	86.00	80.69	26.67	82.10
M.incognita + *B. thuringiensis* + Terbufos	62.67	85.93	22.00	85.23
$L.S.D_{0.05}$	43.20		23.39	

* Each bacterial or nematicidal treatment was applied to the soil at the rate of 5 kg/feddan.

of the nematicide in reducing the number of juveniles in soil and the number of root galling. This effect was pronounced with carbofuran with the percent effectiveness of 90.34% and 89.48%, respectively.

The nematicidal properties of carbofuran, which are less than those of oxamyl and terbufos, can be increased by using it together with *B.thuringiensis*. On the other hand, *B. thuringiensis* in combination with oxamyl or terbufos resulted in a greater reduction in nematode both in soil and in the roots by 80.69% and 82.10% or 85.93% and 85.23%; respectively, than in the case of bacterium only.

Table (2): Combined efficacy of *Bacillus thuringiensis* mixed with certain nematicides against *Meloidogyne incognita*.

B. thuringiensis - nematicide combination	Percent effectiveness for galls Expected	Observed	Co-toxicity Factor	Type of Interaction
B. thuringiensis + Carbofuran	92.19	89.48	- 2.93	Additive
B. thuringiensis + Oxamyl	94.22	82.10	-12.86	Additive
B. thuringiensis + Terbufos	95.89	85.23	-11.12	Additive

Table (2) shows the expected and observed percent effectiveness of nematode galling, co-toxicity factor and type of the interaction which resulted from *B.thuringiensis*/ nematicide mixtures. The results in Table (2) showed an additive effects as reflected by the reduction of tomato galling obtained when the *B. thuringiensis* and the nematicide were applied together against *M.incognita*. However, *B. thuringiensis* + carbofuran was the most effective binary mixture followed by *B. thuringiensis* + terbufos and *B. thuringiensis* + oxamyl in this respect.

Effect on the growth of tomato plants:

The effect of *B.thuringiensis*, certain nematicides and their combinations on tomato growth indices, including root and shoot length as well as fresh root and shoot weight were recorded and presented in Table(3).

Data presented in Table (3) indicated that the application of either bacterial or nematicidal treatment alone, except oxamyl treatment, signficantly increased the fresh weight of root and shoot system compared to that of plant grown in untreated soil. However, the highest effect was obtained with the bacterial application, while the lowest effect was in case of oxamyl treatment. Terbufos was significantly higher increased shoot length compared to control treatment followed by the bacterial treatment, carbofuran and then oxamyl. As for the effect of single treatments on the length of root, significant increases were observed with the bactrium, terbufos and oxamyl in descending order, while no significant effect was recorded in the case of carbofuran alone.

B.thuringiensis alone or in combination with carbofuran resulted in a significant increase in the length, fresh weight of root and shoot of tomato plants compared with the untreated check. On the other hand, no significant differences were noticed between the mixture of *B. thuringiensis* with oxamyl or terbufos and the control in the effect of root and shoot weight as well as shoot length. However, these two mixtures caused significant increases in root length of tomato plants when compared with the control (Table 3).

It could be concluded that *B. thuringiensis* toxin proved to be effective against root-knot nematode, *M.incognita*. Also, it is concluded that the addition of the bacterial toxin, *B. thuringiensis* to the nematicide (at low dosage rate) increased the nematicidal action and remarkably increased tomato growth. Bacterium/carbofuran combination was the best mixture in this respect. So, it can be used as a potential mixture in the integrated management of *M.incognita*

Table (3): Effect of *Bacillus thuringiensis*, nematicides alone and in combinations on growth of tomato plants infected by *Meloidogyne incognita*.

Treatment*	Root		Shoot	
	Length (cm)	Fresh Weight(g)	Length (cm)	Fresh Weight(g)
M. incognita alone (control)	11.70	1.52	25.30	2.30
M.incognita + *B. thuringiensis*	18.30	1.95	30.70	3.40
M.incognita + Carbouran	12.20	1.73	28.40	2.95
M.incognita + Oxamyl	14.00	1.40	26.65	2.45
M.incognita + Terbufos	16.30	1.88	31.50	3.25
M.incognita + *B. thuringiensis* + Carbofuran	18.00	1.95	33.00	3.82
M.incognita + *B. thuringiensis* + Oxamyl	13.00	1.66	29.55	2.80
M.incognita + *B. thuringiensis* + Terbufos	16.80	1.61	27.00	2.60
L.S.D $_{0.05}$	1.28	0.20	4.45	0.51

Data are average of three replicates, each of two plants.

* Each bacterial or nematicidal treatment was applied to the soil at the rate of 5kg /feddan.

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محاولة للمكافحة المتكاملة لنيماتودا تعقد الجذور *Meloidogyne incognita* بإستخدام بكتريا *Bacillus thuringiensis* مع المبيدات النيماتودية

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تم تقييم مستحضر بكتريا *Bacillus thuringiensis* والمبيدات النيماتودية المحببة ، الكاربوفيوران ، الأوكساميل ، التربيوفوس مفردة وفى خلائط ضد نيماتودا تعقد الجذور *Meloidogyne incognita* وكذلك تأثير هذه المعاملات على نمو نبات الطماطم.

وقد أوضحت النتائج أن البكتريا أو المبيدات النيماتودية بمفردها قد أدت إلى خفض فى عدد اليرقات وعدد العقد الجذرية وزيادة فى نمو نبات الطماطم – وأوضحت الدراسة أيضا أن تكامل البكتريا مع المبيد النيماتودى قد أدى إلى خفض فى التعداد النيماتودى وزيادة فى نمو النبات أكثر منه فى حالة التطبيق بالمعاملات المفردة ، وقد أظهرت كل الخلائط تأثير اضافة ضد النيماتودا وكانت البكتيريا مع الكاربوفيوران أحسن الخلائط فى زيادة نمو نبات الطماطم وخفض التعداد النيماتودى لذلك أقترح من الدراسة إمكانية إستخدام هذا الخليط فى المكافحة المتكاملة للنيماتودا