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Integrated Management of *Meloidogyne incognita* Infecting Eggplant by Certain Organic Amendments, *Bacillus thuringiensis* and Oxamyl with Reference to N P K and Total Chlorophyll Status

[1]A.G. El-Sherif, [1]A.R. Refaei, [2]M.E. El-Nagar and [2]Hagar M.M. Salem,
[1]Nematology Research Unit, Department of Agricultural Zoology,
Faculty of Agriculture Mansoura University, Egypt
[2]Plant Protection Institute, Agriculture Research Center, Giza, Egypt

Abstract: Greenhouse experiment on the impact of horse manure or sesame-oil-cake or *Bacillus thuringiensis* singly and/or integrated with oxamyl on *Meloidogyne incognita* infecting eggplant cv. Black long at 25±5°C, revealed that all tested treatments obviously improved plant growth response and reduced nematode development. Among the tested components, *B. thuringiensis* applied alone or mixed with oxamyl gave the highest percentage of increase in plant growth parameters i.e., plant fresh weight and shoot dry weight with values of 105.9 and 85.8% and 127.8 and 107.5%, respectively, as well as the lowest percent reduction of nematode developmental criteria. Moreover, in concomitant application of horse manure plus oxamyl appeared to be the best treatment in suppressing nematode development and improving plant growth parameters, followed by sesame oil-cake plus oxamyl, then sesame oil-cake and horse manure alone. Meanwhile, length of shoot and number of leaves were positively affected by the tested materials either when applied singly or integrated with oxamyl as compared to nematode alone. Regarding N, P and K status in shoots of eggplant, their concentrations were remarkably reduced by nematode infection. Moreover, single application of any organic amendment or bacterium or oxamyl achieved the highest concentration of N, P and K, whereas the concomitant application of horse manure plus oxamyl showed the highest concentration of phosphorus with value of 0.56 ppm. In addition, the previous treatments obviously decreased the total chlorophyll content of eggplant shoot, whereas nematode infection alone recorded the highest increase percentage for this plant parameter with value of 51.3%

Key words: Integrated management, *Meloidogyne incognita, Bacillus thuringiensis*, chlorophyll, horse manure, sesame-oil-cake, oxamyl, eggplant, organic amendments

INTRODUCTION

Eggplant, *Solanum melongena* L., is one of the major economic solanaceae crops in the Egyptian agriculture with total production in Dakahlia governorate amounted to 4690 tons each year. Root-knot nematodes, *Meloidogyne* sp. (J2) were recently recorded to be associated with eggplant plantations in three soil types surveyed within the cultivated areas of Dakahlia (Salem, 2006). The wide host range of such nematodes and the favorable environmental conditions provoked reasonable control measures to achieve satisfactory results. An effective integrated pest management program is quite suitable for such plant parasitic nematodes.

Biological control of the plant parasitic nematodes with certain natural plant products or animal wastes or microbial agents singly or in combination with nematicides have been recorded by several researches El-Sherif, 1984; Nahar *et al*., 1996; Farahat *et al*.; 1999; Maareg *et al*., 2000; Devi and Hassan, 2002; Nour El-Deen, 2002; Hossain *et al*., 2003; El-Sherif, *et al*., 2004; Hammad, 2005). In India, Anver and Alam (1996) reported that oil-seed-cakes of *Azadirachta indica, Ricinus communis, Brassica campestris* and *Eruca sativa* reduced single and concomitant inoculation with *M. incognita* and *Rotylenchulus reniformis* on okra. Moreover, the application of sterilized horse manure or pigeon-dung singly or mixed with oxamyl or *Serritia marcescens* achieved the highest percentage of *M. incognita* population reduction on peach plant cv. Meet-Ghamer under greenhouse conditions (Nour-El-Deen, 2002). El-Sherif *et al*. (2004) found that great suppression in number of root-knot nematodes,

Corresponding Author: El-Sherif, A.G., Nematology Research Unit, Department of Agricultural Zoology, Faculty of Agriculture, Mansoura University, Egypt

Table 1: Source of sesame oil-cake and horse manure as soil organic amendments

English name	Scientific name	Family	Parts	Product tested
Sesame	*Sesamum indicum* L.	Pedaliacede	Seeds	Oil-cake
Horse manure	*Sesamum indicum* L.	Sun-dried manure	Seeds	Oil-cake

M. incognita galls per sunflower root system was recorded with sesame oil-cake mixed with oxamyl and improved plant growth parameters. In a recent study, Hammad Sandy (2005) evaluated the bio control activity of supernatant and pellet of *Bacillus thuringiensis* isolate 3 against *M. incognita* on eggplant and indicated that treatment with Bt, isolate supernatant significantly reduced number of galls (67%) and eggmasses (74%) in comparison with the control. Moreover, *B. thuringiensis* (protecto) surpassed horse manure or marigold dried leaf powder treatments in suppressing citrus nematode, *Tylenchulus semipenetrans* population on lemon plant (El-Sherif *et al*., 2006).

Therefore, the aim of the present investigation was to study the efficacy of horse manure, sesame oil-cake, *Bacillus thuringiensis* (protecto) as a bio-agent either singly and/or mixed with oxamyl against *Meloidogyne incognita* infecting eggplant and to determine N, P and K status and total chlorophyll contents of eggplant shoot grown under greenhouse conditions (25±5°C).

MATERIALS AND METHODS

Source of nematodes inoculum: Second stage juveniles (J2) of the root-knot nematodes, *Meloidogyne incognita* (Kofoid & White) Chitwood, were obtained from a pure culture of *M. incognita* by sieving and modified Baermann technique (Goodey, 1957) that was previously initiated by a single eggmass and propagated on coleus plants, *Coleus blumei* in the greenhouse of Nematology Research Unit, Agricultural Zoology Department, Faculty of Agriculture, Mansoura University, Egypt.

Source of the bacterium tested as a biological agent and the pesticide, oxamyl: Protecto is the trade name of *Bacillus thuringiensis* which is recorded under No. 541 at the Ministry of Egyptian Agriculture with an active ingredient 9.4% inert ingredient carrier 90.6% and the recommended dose per feddan (300 g). This product is produced by Research Institute of Plant Protections, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt; whereas, the pesticide tested has the trade name, Vydate 24% EC with the chemical name oxamyl Methyl-N' N'-dimethyle-N (methyl) carbamycocy)-1- hioxamidate.

Integrated management of *Meloidogyne incognita* infecting eggplant, *Solanum melongena* L.: An experiment was conducted to study the impact of sesame oil-cake as well as horse manure as organic amendments and the bioproduct Bt (protecto) singly or in combination with oxamyl as nematicide on eggplant cv. Black long infected with *M. incognita* under greenhouse conditions (25±5°C) (Table 1). In order to carry-out this experiment, twenty-seven plastic pots 10-cm-d. filled with steam-sterilized sand loam soil (V : V) (1 : 1) were planted with one eggplant seedling/pot at 60 days-old after dipping its roots in vitavax solution (Fungicide) for 10 sec. Plants received water as required Oxamyl or *B. thuringiensis* was added at the recommended dose that was 0.3 mL or 0.01 g/pot, respectively, whereas that of sesame oil-cake or horse manure was amounted to 5 g/pot, respectively. Meanwhile, each integrated treatment contained oxamyl at the half recommended dose (0.15 mL) plus half dose of any component under study. All components were added to soil of eggplant seedling at the time of introducing the juveniles of *M. incognita*. Twenty four seedlings were inoculated with 1000 J2 each. Each treatment was replicated three times. Treatments were as follows:

1- N+horse manure (5 g), 2- N+sesame cake (5 g), 3- N+Bt (0.01 g), 4- N+oxamyl (0.3 mL), 5-N+(2.5 g) horse+(0.15 mL) oxamyl, 6-N+(2.5 g) sesame+(0.15 mL) oxamyl, 7- N+(0.05 g) Bt+(0.15 mL) oxamyl, 8- N alone and 9- Plants free of N or any treatment.

After 45 days of inoculation with nematode juveniles, plants were harvested. Data on both shoots and roots regarding the length, Fresh weight and dry mass for shoot only was recorded. Infected plant roots were examined for number of galls, females and eggmasses after staining by lactic acid-fuchsin and recorded (Byrd *et al*., 1983).

Regarding N, P and K determination, 0.2 g shoot dry weight was subjected to chemical analysis as follows: total nitrogen content was determined according to the improved kjeldahl method (AOAC, 1980) modified by distilling the ammonia into saturated boric acid solution and titration with 0.1 NaCl standard. Total phosphorus was colorimetrically determined using the chlorostannous reduced Molybdophosphoric Blue colour method, while total potassium was flam photometry estimated as described by Jakson (1967).

Chlorophyll content was spectrophotometrically measured in leaves of the harvested plant using Fadeel's 1962 method, chlorophyll concentrations were calculated according to Wellburn and Lichtenthaler (1984). The content of chlorophyll was then expressed as μg/g. F.wt.

Table 2: Impact of certain organic amendments and the bioproduct B.t. (Protecto) *Bacillus thuringiensis* alone or integrated with oxamyl on growth of eggplant cv. Black long infected with *Meloidogyne incognita* under greenhouse conditions (25±5°C).

	Plant growth response								
	Length (cm)		Fresh weight (g)		No. of shoot	Fresh weight of	Increase	Shoot dry	Increase
Treatments	Shoot	Root	Shoot	Root	leaves	whole plant	(%)	weight (g)	(%)
Horse manure+N	23.33cd	26.00bc	8.50ab	8.07d	5.00a	16.57ab	75.2	1.88a	77.4
Sesame cake+N	22.00d	24.00bcd	8.40ab	7.90d	5.00a	16.30ab	77.2	1.61ab	51.9
B.t+N	24.16bcd	28.56abc	7.52ab	11.41a	5.66a	18.94ab	105.9	1.97a	85.8
½ Horse manure +/2 Oxamyl+N	27.00ab	23.00cd	10.06a	10.16abc	6.00a	20.22a	119.8	2.07a	95.3
½ Sesame cake +1/2 Oxamyl+N	26.50abc	28.00abc	9.76a	9.06cd	6.00a	18.82ab	104.7	1.68ab	58.5
½ B.t.+1/2 Oxamyl+N	25.00abcd	33.00a	10.20a	10.76ab	5.00a	20.96a	127.8	2.20a	107.5
Oxamyl+N	23.33cd	23.66cd	7.08ab	7.05d	4.33ab	14.13bc	53.6	1.41ab	33.0
N alone	15.25e	19.25d	5.24b	3.96e	3.00b	9.20c	-	1.06 b	-
CK (Untreated)	27.66a	29.66ab	10.03a	9.59bc	5.66a	19.62ab	113.3	2.07a	95.3

N = 1000 J2 of *M. incognita*. Each value is a mean of three replicates. Means in each column followed by the same letter(s) did not differ at $p<0.05$ according to Duncan's multiple-range test. B.t = *Bacillus thuringiensis*

Data were statistically subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984), followed by Duncan's multiple-range test to compare means (Duncan, 1955).

RESULTS AND DISCUSSION

Data in Table 2 revealed the effect of horse manure, sesame oil cake and the bioproduct (Protecto) Bt singly or in combination with oxamyl on the growth of eggplant cv. Black long under greenhouse conditions at 25±5°C. Results indicated that all tested materials obviously caused remarkable increase in eggplant growth with various degrees. It is evident that pots received *B. thuringiensis* (Protecto) either alone or added with oxamyl at the half dose of both components significantly higher than all other treatments tested in improving plant growth parameters, since the percentages of increase of total plant fresh weight as well as shoot dry weight averaged 105.9, 85.8; 127.8 and 107.5%, respectively. However, significant increase in the previous growth criteria was also achieved by the application of horse manure, followed by sesame oil cake with value of 75.2, 77.4, 77.2 and 51.9%, respectively. Likewise, similar results were obtained when horse manure or sesame oil cake integrated with oxamyl regarding the percent increase of total plant fresh weight as well as shoot dry weight over control with values of 119.8, 95.3% and 104.7 58.5%, respectively. On the other hand, oxamyl alone gave the lowest percentage of increase in the previous growth parameters of eggplant with values of 53.6 and 33.0%, respectively (Table 2). Moreover, plants free of nematodes that received none of the tested components showed remarkable percentages of increase in total plant fresh weight as well as shoot dry weight with values of 113.3 and 95.3%, respectively when compared to that of nematode alone.

As a whole it can be indicated that oxamyl integrated with (Protecto) *B. thuringiensis* was ranked the first in increasing plant fresh weight of eggplant infected with *M. incognita* followed by oxamyl plus horse manure then (Protecto) *B. thuringiensis* alone with percentages of increase averaged 127.8, 119.8 and 105.9%, respectively. It is worth to note that length of shoot as well as number of leaves were significantly affected by tested components either singly or integrated with oxamyl when compared to nematode alone (Table 2).

Data in Table 3 revealed the impact of horse manure, sesame oil cake and (Protecto) *B. thuringiensis* singly or in concomitantly with oxamyl on root galling, female and eggmasses number of *M. incognita* infecting eggplant cv. Black long under greenhouse conditions.

Data indicated that concomitant treatments obviously gave better results than single treatments (Table 3). Among single application, it was evident that pots received sesame oil seed cake accomplished the highest percentage of reduction in number of galls, females and eggmasses, followed by horse manure with values of 60.9, 69.0, 74.6; 42.0, 40.8 and 45.4%, respectively, whereas, the bioproduct, *B. thuringiensis* (Protecto) showed the lowest values that amounted to 32.2, 36.6 and 37.7%, respectively. However in concomitant treatments, horse manure plus oxamyl achieved the highest percentage of nematode reduction, followed by sesame oil cake plus oxamyl with values of 81.26, 85.2, 80.8; 75.4, 77.8 and 79.2% for number of galls, females and eggmasses on eggplant roots, respectively. Moreover, the least value of the same previous parameters was also achieved by *B. thuringiensis* plus oxamyl that amounted to 34.6, 42.2 and 44.6%, respectively. It is worth to note that oxamyl as a nematicide at the recommended dose (0.3 mL pot) gave the highest percentage of nematode parameters reduction values over all tested components as well as

Table 3: Effect of certain organic amenders and *Bacillus thuringiensis* alone or mixed with oxamyl on *Meloidogyne incognita* infecting eggplant cv. Black long under green house conditions (25+5°C)

Treatments	No. of galls	Reduction (%)	RGI	No. of females	Reduction (%)	No. of eggmasses	Reduction	EGI
Horse manur+N	65.33b	42.00	4.0	56.00b	40.8	47.30b	45.4	3.6
Sesame cake+N	44.00c	60.90	4.0	29.33c	69.0	22.00c	74.6	3.0
B.t.+N	76.33b	32.20	4.0	60.00b	36.6	54.00b	37.7	4.0
½ Horse manure +1/2 Oxamyl+N	20.66d	81.26	3.0	14.00c	85.2	16.66c	80.8	2.0
½ Sesame cake +1/2 Oxamyl+N	27.66cd	75.40	3.6	21.00c	77.8	18.00c	79.2	2.6
½ B.t.+1/2 Oxamyl+N	73.66b	34.60	4.0	54.66b	42.2	48.00b	44.6	4.0
Oxamyl+N	25.00cd	77.80	3.0	17.66c	81.3	17.00c	80.4	3.0
N alone	112.66a	-	5.0	94.66a	-	86.66a	-	4.0

N = 1000 J2 of *M. incognita*. Each value is a mean of three replicates. Means in each column followed by the same letter(s) did not differ at p<0.05 according to Duncan's multiple-range test. *Root gall index (RGI) or eggmass index (EGI): 0 = no galling or eggmasses, 1 = 1-2 galls or eggmasses; 2 = 3-10 galls or eggmasses; 3=11-30 galls or eggmasses; 4=31-100 galls or eggmasses and 5 = more than 100 galls or eggmasses. (Talyor and Sasser, 1978) **RGI or EGI = the average of three replicates

Table 4: Nitrogen, phosphorus and potassium concentrations as well as chlorophyll content in dry shoot of eggplant cv. Black long influenced by *Meloidogyne incognita* treated with horse manure, sesame oil-cake, *Bacillus thuringiensis* singly or incombination with oxamyl under greenhouse conditions (25+5°C)

Treatments	Nitrogen (N) µg mg	Phosphorus (P) ppm	Potassium (K) ppm	Chlorophyll content		
				Chlorophyll A	Chlorophyll B	Total Chlorohyll
Horse manure+N	30.93b	0.463b	27.86b	637.30b	369.03ef	1006.36c
Sesame cake+N	33.50b	0.340c	26.20bc	664.53b	410.46de	1075.00b
B.t.+N	26.93c	0.226d	33.80a	563.50c	512.10b	1075.60b
½ Horse manure +1/2 Oxamyl+N	13.63f	0.56a	20.90e	539.76c	439.96cd	979.73c
½ Sesame cake +1/2 Oamyl+N	23.30de	0.260d	23.40d	545.70c	342.30f	888.06d
½ B.t. +1/2 Oxamyl+N	24.90cd	0.39c	24.9cd	426.76d	487.13be	913.9d
Oxamyl+N	37.00a	0.543a	35.53a	430.00d	437.33cd	867.33d
N alone	21.43e	0.246d	15.13f	902.53a	748.23a	1650.76a
Untreated (CK)	32.90b	0.486b	34.00a	646.16b	445.23cd	1091.40b

N = 1000 J2 of *M. incognita*. Each value is a mean of three replicates. Means in each column followed by the same letter(s) did not differ at p<0.05 according to Duncan's multiple-range test. B.t. = *Bacillus thuringiensis*

when integrated with only horse manure (2.5 g/pot) at its half dose (0.15 ml/pot). However, these values were 77.8, 81.3, 80.4, 81.26, 85.3 and 80.8%, respectively. These previous results indicated that among the two tested organic amendments, sesame oil-cake applied singly or integrated with oxamyl showed a reasonable results in controlling *M. incognita* development on eggplant as well as improving its growth parameters.

Data in Table 4 showed the influence of the two organic amendments i.e., horse manure, sesame oil cake and *B. thuringiensis* alone or in combination with oxamyl on Nitrogen (N), Phosphorous (P) and Potassium (K) concentrations in eggplant infected with *M. incognita*. It was evident that N, P and K concentrations were obviously reduced by nematode infection. Most tested treatments showed remarkable increase in N, P and K concentrations exceeding those of nematode alone. Oxamyl application ranked first in increasing N, P and K concentrations over all materials tested and those of untreated uninoculated plants. The highest increment in nitrogen and phosphorus concentrations was achieved by sesame oil cake or horse manure when singly applied with values of 33.5 µg mg and 0.34 ppm or 30.9 µg mg and 0.46 ppm, respectively. On the other hand, *B. thuringiensis* was significantly superior over the two organic amenders in potassium concentration with value of 33.8 µg mg (Table 4). However, among the concomitant applications, *B. thuringiensis* plus oxamyl showed the highest increment in nitrogen, phosphorus and potassium concentration, followed by sesame plus oxamyl whereas horse manure plus oxamyl resulted the least concentrations of N, P and K. These values were amounted to 24.9, 0.39, 24.9, 23.3, 0.26, 23.4, 13.6, 0.56 and 20.9, respectively (Table 4).

The impact of nematode infection to eggplant seedlings in pots receiving either horse manure or sesame oil-cake or *B. thuringiensis* singly or in combination with oxamyl on the total chlorophyll content, indicated that all tested components significantly decreased the total chlorophyll content of eggplant shoot comparing to nematode alone. Moreover, nematode infection without any materials added revealed the highest percentage of

increase with value of 51.3% over the untreated uninoculated plant. Meanwhile, *B. thuringiensis* or sesame oil-cake showed the lowest reduction percentages of chlorophyll content followed by horse manure with values of 1.4, 1.5 and 7.78%, respectively (Table 4).

Moreover, the concomitant treatments also achieved the highest reduction percentages of chlorophyll content as compared to the untreated uninoculated plants with values of 10.2, 16.3 and 18.6% for horse manure plus oxamyl, *B. thuringiensis* plus oxamyl and sesame oil-cake plus oxamyl, respectively. In addition, oxamyl treatment alone surpassed all tested components in resulting the highest reduction percentage of this plant criterion with value of 20.53% (Table 4) comparing with the untreated uninoculated plants.

Regarding the impact of the tested two organic amenders i.e., sesame oil-cake or horse manure or *B. thuringiensis* applied singly or mixed with oxamyl on *M. incognita* infecting eggplant cv. Black long; B.t. (Protecto) either alone or in combination with oxamyl gave the highest percentage of increase in plant growth parameters as well as the lowest percent reduction of nematode criteria. Meanwhile, sesame oil-cake alone ranked the first in suppressing nematode development parameters and the second when applied integrated with oxamyl for the same nematode parameters, whereas it ranked the third in promoting plant growth parameters. Moreover, in concomitant application of horse manure plus oxamyl appeared to be the best treatment in suppressing nematode development and in improving plant growth parameters followed by sesame oil-cake plus oxamyl then sesame oil-cake and horse manure alone as compared to nematode alone.

The present results support the findings of Nour El-Deen (2002) in respect to horse manure; El-Sherif *et al.* (2004) and Youssef and El-Nagdi (2004) in regard to sesame oil-cake and Hammad (2005) in respect to *B. thuringiensis*. This result also agree with the finding of Ignoffo and Dropkin (1977) who reported that the toxin preparation "thuringiensin" of *B. thuringiensis* was capable of protecting tomato plants from infection by root-knot nematodes. Improving eggplant growth and suppression of *M. incognita* following the application of horse manure could be attributed to the presence of high concentration of nitrogen (2.131%) in the present results which are in harmony with those that previously mentioned according to Eno *et al.* (1955).

Obviously, the present investigation showed the potential of the bacterium *B. thuringiensis* as a biofertilizer as well as its thermostable toxin on root-knot nematodes infecting eggplant seedlings and improving plant growth. Organic amendements, of such plant product and animal manure tested play an important role in improving soil structure, promoting plant growth and may activate different organisms against the target nematode. The safety of such materials and its low cost is one of its advantages. However, additional researches are needed using plant or animal organic amenders or the indigenous bacterium, *B. thuringiensis* in microplot and field experiments before using effectively in Integrated Pest Management (IPM).

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