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Integrated Management of *Meloidogyne incognita* Infecting Soybean by Certain Organic Amendments, *Bacillus thuringiensis*, *Trichoderma harzianum* and Oxamyl with Reference to NPK and Total Chlorophyll Statuus

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Abstract: The integrated control of *M. incognita* infecting soybean cv. Giza 21 using camel manure, dried leaf powder of marigold, *Trichoderma harzianum* 100% filtrate, *Bacillus thringiensis* singly or in combination with oxamyl under greenhouse condition (22±3°C) indicated that the concomitant treatments obviously gave better results than single ones did. Moreover, *B. thringiensis* plus oxamyl at half dose each surpassed all other tested materials in percentage increase of total plant fresh and shoot dry weights (99 and 88 %), respectively, followed by *T. harzianum* filtrate plus oxamyl (95 and 61%) in this respect. On the other hand, pots received *T. harzianum* filtrate plus oxamyl ranked first in suppressing final nematode population (90%), root galling (65%) and eggmass numbers (62.79%), followed by B.t. plus oxamyl, marigold powder +oxamyl and then camel manure + oxamyl, where their reduction percentage values amounted to 88.7, 62.5 and 61%; 88.5, 59.87 and 58.9% and 67, 50.5 and 52.97%, respectively. Of the tested singly applications, *T. harzianum* filtrate showed the best results in improving plant growth and suppressing nematode development criteria, followed by B.t., whereas, marigold powder and camel manure achieved the lowest values in this respect comparing to nematode alone. Regarding the N, P and K concentrations in leaves of soybean plants inoculated with *M. incognita* treated with certain organic matters or fungal filtrate or bacterium alone or mixed with oxamyl, they were obviously enhanced by all tested materials whereas the opposite results was recorded for total chlorophyll content comparing to nematode alone.

Key words: *Bacillus thringiensis,* camel manure, marigold powder, *Meloidogyne incognita*, oxamyl, soybean, *Trichoderma harzianum*, integrated control, NPK and chlorophyll

INTRODUCTION

Soybean, *Glycine max* L. is considered to be the most important economic oil and cash crops in Egypt. Root-knot nematodes, *Meloidogyne* spp. were recorded as pathogens of many vegetable as well as field crops in tropical and sub-tropical areas including the Arab Republic of Egypt. They are widely distributed in the cultivated areas of Egypt causing remarkable crop losses, particularly with soybean yield. Moreover, losses caused by the root-knot nematodes, *Meloidogyne* spp. can be reduced effectively by applying nematicides to the soil. However, chemical nematicides are being reappraised because of health, environmental hazard and high costs. Organic amendments integrated with other control measure i.e., bioagents *Bacillus thuringiensis* (El-Sherif *et al*., 2007) or fungi culture filtrates, *Trichoderma harzianum* (Ansari *et al*., 2002; Nasr *et al*., 2005; El-Sherif and Ismail, 2007) or plant product (Refaei *et al*., 2008) or animal manure (Aboubaker *et al*., 2004; El-Sherif *et al*., 2008) could play an important role in the management of the target nematodes and keep nematode low at the safe level. El-Sherif and Ismail (2007) reported that the culture filtrates of *Trichoderma harzianum* or *T. viride* at 40, 80 and 100% concentrations obviously caused remarkable increase in potato plant growth characters and significantly reduced root galling, females and egg masses on root system as well as number of *M. incognita* (J2) per cc soil. In the same year, El-Sherif *et al.* (2007) studied the impact of horse manure or sesame oil cake or *Bacillus thuringiensis* singly and/or integrated with oxamyl on *M. incognita* infecting eggplant cv. Black Long at 25±5°C and found that all tested treatments obviously improved plant growth response and reduced nematode development. The effect of poultry manure singly or integrated with *Hirsutella rhossiliensis* and/or oxamyl on soybean plant infected with *Rotylenchulus reniformis*

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indicated that poultry manure + oxamyl treatment gave the highest value of percentage increase in the fresh weight of whole plant (57.73 %) as well as the higher reduction in nematode population density (71.24%) over other treatments (El-Sherif *et al.*, 2008). Refaei *et al.* (2008) found that drenching application of the three dried plant leaf extracts i.e., marigold, periwinkle and eucalyptus ranked as the best method followed by root-tip treatments in controlling citrus nematode, *Tylenchulus semipenetrans* on sour-orange and improving plant growth. The objective of the present research conducted to study the integrated management of *M. incognita* infecting soybean by certain organic amendments, fungal filtrate of *Trichoderma harzianum* 100%, *Bacillus thuringiensis* singly or mixed with oxamyl with reference to NPK and total chlorophyll status under greenhouse conditions.

MATERIALS AND METHODS

Nematode stock cultures, propagations and preparing nematode inoculum: Single eggmass related to *Meloidogyne incognita* previously identified female (Taylor *et al*., 1955) was used to inoculate coleus plant, *Coleus blumei* grown in 25 cm-d, plastic pots filled with sterilized loamy sand soil. Pots were kept on a clean bench in the greenhouse receiving water and fertilizers as needed. Two months later, plants were uprooted and roots were washed and examined for nematode infection. Infected roots were used to inoculate other coleus plants. Sub-culturing and maintenance were continuously carried out to obtain sufficient inoculum as second stage juveniles from soil that extracted by sieving and modified Baermann-technique (Goodey, 1957) and for further greenhouse experiments at the Nematology Research Unit, Agriculture Zoology Department, Faculty of Agriculture, Mansoura University, Egypt where this study was done.

Pesticide: Oxamyl: (Vydate 24% E.C.) Methyle-N-N-dimethyl-) N(methyl) carbamycocyl)-1-1hioxamidate.

Table 1 shows source of plant product as soil organic amendment.

Table 2 shows source of animal waste as soil organic amendment.

Table 1: Source of plant product as soil organic amendment

Product tested	Parts	Family	Scientific name	English name
Dried powder	Leaves	Asteraceae	*Tagetes erecta*	Marigold

Table 2: Source of animal waste as soil organic amendment

Product tested	English name
Sun dried manure	Camel manure

Source of the bacterium tested as biological agent: Protecto is the trade name of *Bacillus thuringiensis* that is reported 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 Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt.

Source and preparation of the fungal filtrates of *Trichoderma harzianum*: Cultures of the fungi i.e., *Trichoderma harzianum* on liquid media, Potato Dextrose Broth (PDB) was brought by author from laboratory of Plant Pathology Department, Faculty of Agriculture, Mansoura University after incubating for two weeks. Fungal filtrates without spores were separately obtained to flask (250 mL each) through filter papers (whatmann) which was at 100% concentration. This solution of fungus filtrate was added to the plant at the rate of 25 mL/plastic bag in this investigation.

Integrated management of *Meloidogyne incognita* infecting soybean cv. Giza 21 under greenhouse conditions: An experiment was conducted to study the influence of camel manure as well as dried leaf powder of marigold (*Tagetes erecta*) as soil organic amendements, fungal filtrate of *Trichoderma harzianum*, the bioproduct, B.t. (protecto) singly or in combination with oxamyl as nematicide on soybean infected with *M. incognita* under greenhouse conditions at 22±3°C. In order to carry out this investigation, twenty seven out of thirty three black plastic bags containing 1800 g steam-sterilized sandy loam soil (1:1, v:v) separately received the tested dose of Vydate (oxamyl) or *B. thuringiensis* or fungus filtrate at 100%, or camel manure or dried leaf powders of marigold at the rate of 0.6 mL or 0.02 g or 25 mL or 10 g or 5 g/seedling/bag one week before seed sowing as pre-planting applications, respectively, watered and left one week before planting. Then all plastic bags (33) were planted with three seeds of soybean each and then thinned to one seedling/bag, seven days after germination. Thirty three soybean seedlings at 15 day-old were inoculated with 2000 second stage juveniles of *M. incognita* per seedling. The other three soybean seedling left free of nematode and any treatment as control. Meanwhile, each integrated treatment contained oxamyl at the half recommended dose (0.3 mL) plus half dose of any component under investigation. Each treatment was replicated tree times. Treatments were as follows:

- 1-Camel manure (10 g), 2- Marigold (5 g)

- 3-Fungus filtrate, *T. harzianum* at 100% (25 mL), 4-Bacterium, B.t. (0.02 g)
- 5-Oxamyl (0.6 mL), 6- Camel manure (5g) + oxamyl (0.3 mL),
- 7-Marigold (2.5 g) +oxamyl (0.3 mL), 8- *T. harzianum* at 100% (12.5 mL) +oxamyl (0.3 mL)
- 9-B.t. (0.01g) + oxamyl (0.3 mL), 10-N alone and
- 11-Plant free of N or any treatment

After 45 days from nematode juveniles inoculation, plants were harvested. Data dealing with lengths and fresh weights of shoot and root as well as shoot dry weights were determined and recorded. Infected plant roots were examined for number of galls, developmental stages, females and eggmasses after staining by lactic acid-fuchsin (Byrd *et al*., 1983) and recorded. Number of *M. incognita* juveniles in 250 g soil was also determined by extracting through sieve and modified Baermann-pan technique (Goodey, 1957) and recorded. Regarding N, P and K determination, 0.2 g of dry weight of shoot as well as chlorophyll content 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 chlorostannous reduced Molybdophosphoric blue color method, while total potassium was flame photometry estimated as described by Jackson (1967). Chlorophyll content was spectrophotometrically measured in leaves of the harvested plants using Fadeel's (1962) method. Chlorophyll concentrations were calculated according to Wellburn and Lichtenthaler (1984). The content of chlorophyll was then expressed in $\mu g\ g^{-1}$ Fresh Wt. of the leaves. Data were statistically subjected to Analysis of Variance (ANOVA) (Gomez and Gomez, 1984) followed by Duncan's (1955) multiple-range test to compare means.

RESULTS AND DISCUSSION

Data in Table 3 documented the impact of camel manure, fungal culture filtrate of *T. harzianum* (100%), dried leaf powder of marigold, *B. thuringiensis* (protecto) alone or mixed with oxamyl on the growth of soybean cv. Giza 21 under greenhouse condition 22±3°C. Results revealed that all tested components obviously caused remarkable increments in soybean plant growth to certain extent. It is clear that plants received *B. thuringiensis* plus oxamyl at half dose each surpassed all other tested materials in improving plant growth parameters, since the percentages increase of total plant fresh weight and shoot dry weight averaged 99 and 88%, respectively, when compared with nematode alone (Table 3). Moreover, all tested concomitants treatments significantly improved the same plant growth parameters, where fungus filtrate of *T. harzianum* plus oxamyl ranked second to that of B.t. plus oxamyl for the values of percentage increase of total plant fresh weight and shoot dry weight which averaged 95 and 61%. Likewise, similar results were obtained by marigold dried leaf powder plus oxamyl (72.5 and 56.8%), followed by camel manure plus oxamyl (74.9 and 37%), respectively as compared with nematode alone. Of the tested single applications, fungal filtrate of *T. harzianum* showed the best results in improving total plant fresh weight and shoot dry weight with values of 54.5 and 52.9%, respectively, followed by the bioagent, *B. thuringiensis* with values of 24 and 35.0%, respectively, whereas, marigold dry leave powder and

Table 3: Impact of certain organic amendments, *Bacillus thuringiensis* and fungal filtrate of *Trichoderma harzianum* alone or integrated with oxamyl on growth of soybean cv. Giza21 plants infected with *Meloidogyne incognita* under greenhouse conditions

	*Plant growth parameters							
	Length (cm)		Fresh weight (g)					
Treatments	Shoot	Root	Shoot	Root	Fresh wt. of whole plant (g)	Increase (%)	Shoot dry weight (g)	Increase (%)
Camel manure	29.66cd	26.00abc	1.80b	2.1b	3.93bc	14.5	0.53a	3.9
½Camel manure +½ox	38.00ab	30.00abc	3.00ab	3.00ab	6.00abc	74.9	0.70ab	37.0
T. harzianum	38.00ab	32.33ab	2.70ab	2.60ab	5.30abc	54.5	0.78ab	52.9
½ *T. harzianum*+ ½ox	40.66a	33.33ab	3.86a	2.83ab	6.70ab	95.0	0.823b	61.0
Marigold	32.00c	25.00abc	2.33b	1.83b	4.16abc	21.0	0.63ab	23.5
½Marigold+ ½ox	38.66ab	35.66ab	2.92ab	3.00ab	5.92abc	72.5	0.80b	56.8
B.t.	33.33bc	22.66bc	2.33b	1.93b	4.26abc	24.0	0.69ab	35.0
½ B.t. +½ox	30.00cd	39.00a	2.60ab	4.23a	6.83a	99.0	0.96b	88.0
Oxamyl	32.33c	32.00ab	2.56ab	1.66b	4.23abc	23.0	0.80b	56.8
Control	34.00bc	29.6abc	3.30ab	2.00b	5.30abc	54.5	0.81b	58.8
N alone	26.33d	17.00c	2.00b	1.43b	3.43c	-	0.51a	-

N = 2000 second stage juveniles of *M. incognita*. *Each value is a mean of three replicates. Means in each column followed by the same letter(s) didn't differ at $p<0.05$ according to Duncan's multiple -range test

Table 4: Impact of certain organic amendments, *Bacillus thuringiensis* and fungal filtrate of *Trichoderma harzianum* alone or mixed with oxamyl on development of *Meloidogyne incognita* infecting soybean cv. Giza 21 under greenhouse conditions

	Nematode population										
		Root									
Treatments	Soil	Development stage	Female	Total Pf	Red. (%)	No. of galls	Red. (%)	** RGI	No. of eggmasses EI	Red. (%)	**EI
Camel manure	6.01b	80.66b	85.33b	10366.00b	24.8	74.66b	39.0	4	73.33bc	43.0	4
½Camel manure+ ½ox	2.42e	55.33de	60.33c	4512.33e	67.0	60.66cd	50.5	4	60.66cde	52.9	4
T. harzianum	2.10e	74.33b	77.66b	3805.30f	72.0	67.33bc	45.0	4	70.00bcd	45.7	4
½ *T. harzianum*+½ox	0.69f	42.66f	46.00d	1342.60g	90.0	42.66e	65.0	4	48.00e	62.7	4
Marigold	4.69c	71.33bc	77.66b	8598.60c	37.5	71.66b	41.5	4	84.00b	34.8	4
½Marigold+ ½ox	0.81f	57.66de	53.33cd	1589.60g	88.5	49.33de	59.8	4	53.00de	58.9	4
B.t.	2.95d	63.66cd	61.66c	5452.00d	60.5	54.00de	55.9	4	59.66cde	53.7	4
½B.t. +½ox	0.73f	45.00f	50.30cd	1419.00g	89.7	46.00e	62.5	4	50.00e	61.0	4
Oxamyl	0.90f	51.00ef	54.66cd	1748.30g	87.0	53.66de	56.0	4	58.00cde	55.0	4
N alone	7.52a	117.33a	134.30a	13787.00a	-	122.60a	-	5	129.00a	-	5

N = 2000 second stage juveniles of *M. incognita*. *Each value is a mean of three replicates. Means in each column followed by the same letter(s) didn't differ at p<0.05 according to Duncan's multiple -range test. Pf: Final nematode population. **Root- gall index (RGI) or egg-masses index (EI) was determined according to Taylor and Sasser (1978) as follows: 0 = no galls or egg-masses, 1 = 1-2 galls or egg-masses, 2 = 3-10 galls or egg-masses, 3 = 11-30 galls or egg-masses, 4 = 31-100 galls or egg-masses and 5 = more than 100 galls or egg-masses

camel manure showed the lowest values for the same plant growth parameters which averaged 21 and 23.5%; 14 and 3.9%, respectively comparing to nematode alone. Moreover, oxamyl as a nematicide at the recommended dose achieved a considerable percentage increase of total plant fresh weight and shoot dry weight with values of 23 and 56.8%, respectively comparing to nematode alone. Table 4 indicated the influence of camel manure, fungal filtrate of *T. harzianum*, marigold dry leave powder, bioproduct, of B.t. (protecto) singly or mixed with oxamyl on nematode *M. incognita* infecting soybean cv Giza 21 under greenhouse conditions 22±3°C. Data revealed that the concomitant applications obviously showed better results than single ones did (Table 4). Of the tested concomitant treatments, pots received fungal filtrate of *T. harzianum* plus oxamyl at half dose of both components ranked first in suppressing final nematode population (90%), root galling (65%) and eggmasses number (62.7%), respectively, followed by B.t. plus oxamyl, marigold powder plus oxamyl and then camel manure+ oxamyl, where their reduction percentage values averaged 89.7, 62.5 and 61, 88.5, 59.87 and 58.9% and 67.0, 50.5 and 52.9%, respectively comparing to nematode alone (Table 4). Among the single treatments, pots received the fungus culture filtrate of *T. harzianum* achieved the best results in reducing final nematode population of *M. incognita* with values of 72%, followed by B.t. (60.5%) whereas, camel manure treatment gave the lowest reduction percentage of final nematode population and root galling number with values of 24.8 and 39.%, respectively. B.t. treatment gave the highest reduction percentage of eggmasses number with values of 53.7% over those of other single treatments except oxamyl since their values for this nematode parameter were 34.88, 43.0, 45.7 and 55% for marigold powder, camel manure, *T. harzianum* and oxamyl, respectively as compared with nematode alone. Like wise, similar results were obtained for reduction percentage of root galls numbers with values of 41.5, 45, 55.9 and 56% for marigold powder, *T. harzianum*, B.t. and oxamyl, respectively as comparing to nematode alone. Significant results were also recorded among treatments with the indices of root galls as well as eggmasses number where the lowest indices for those two nematode parameters was achieved by all test components with equal values of (4) comparing nematode alone (5) (Table 4). Data in Table 5 showed the influence of two organic amendments i.e., camel manure, marigold dried leaf powder, *T. harzianum* 100% filtrate and *B. thuringiensis* alone or in combination with oxamyl on nitrogen (N), phosphorus (P) and potassium (K) concentrations as well as total chlorophyll content in soybean infected with *M. incognita*. It was evident that N, P and K concentrations were obviously diminished, while total chlorophyll contents increased by nematode infection. All tested components showed remarkable increase in N, P and K concentrations as well as reduction values of total chlorophyll content comparing to nematode alone (Table 5). Of the tested application, B.t. either mixed with oxamyl or alone achieved the highest increments in nitrogen, phosphorus and potassium concentrations, followed by *T. harzianum*+oxamyl, whereas camel manure plus oxamyl resulted the least concentrations of N, P and K. These values were amounted to 3.16, 0.481 and 1.94 or 2.78, 0.455 and 1.8; 2.63, 0.423 and 1.74; and 2.17, 0.332 and 1.44, respectively. Of the tested singly applications *T. harzianum* 100% ranked second to B.t. and then followed by marigold powder and camel manure in the increment values of N, P and K concentrations, since their values were averaged 2.48, 0.38 and 1.36; 2.27, 0.347 and 1.49; and 1.94, 0.311 and 1.36, respectively. Meanwhile, oxamyl application achieved the second rank to B.t. plus oxamyl in increments in nitrogen, phosphorus and potassium concentrations with values of 2.87, 0.471 and 1.86.

Table 5: Nitrogen, Phosphorus and Potassium concentrations as well as chlorophyll in fresh shoot of soybean cv. Giza21 influenced by *Meloidogyne incognita* treated with camel manure, marigold dried leaf powder, *Trichoderma harzianum* filtrate, *Bacillus thuringiensis* singly or in combination with oxamyl under greenhouse conditions

	*Plant growth parameters					
				Chlorophyll content		
Treatments	N (%)	P (%)	K (%)	A (mg g^{-1})	B (mg g^{-1})	Total chlorophyll
Camel manure	1.94ef	0.311e	1.36cd	0.774a	0.609a	1.383a
½Camel manure +½ox	2.17def	0.332e	1.44bcd	0.748a	0.5653a	1.330a
T. harzianum	2.48bcde	0.380cde	1.63abcd	0.761a	0.597a	1.358a
½ *T. harzianum*+½ox	2.63abc	0.423bcd	1.74abcd	0.660a	0.495a	1.152a
Marigold	2.27cde	0.347de	1.49bcd	0.802a	0.629a	1.431a
½Marigold+ ½ox	2.35bcdef	0.365de	1.55abcd	0.698a	0.533a	1.231a
B.t.	2.78abc	0.455abc	1.80abc	0.735a	0.571a	1.306a
½B.t. +½ox	3.16a	0.481ab	1.94a	0.683a	0.521a	1.204a
Oxamyl	2.87ab	0.471ab	1.86ab	0.712a	0.546a	1.258a
Control	3.07a	0.520a	1.97a	0.669a	0.500a	0.843a
N alone	1.87f	0.310e	1.33d	0.820a	0.640a	1.450a

N = 2000 second stage juveniles of *M. incognita*. *Each value is a mean of three replicates. Means in each column followed by the same letter(s) didn't differ at $p<0.05$ according to Duncan's multiple -range test. B.t.= *Bacillus thuringiensis* (protecto)

Concerning the total chlorophyll content in soybean cv. Giza 21 under the infection by *M. incognita*, results indicated that nematode infection without any materials added revealed the highest percentage increase with value of 41.86% over the untreated uninoculated plants. Moreover, there was negatively correlations between the singly and concomitant applications of the tested components regarding the reduction percentages of chlorophyll content comparing to nematode alone, since their values were ranged from 4.62 to 8.27% or from 6.34 to 20.55%; or from 1.3 to 15.1% and or from 9.93 to 16.89% for both treatments of camel or *T. harzianum* or marigold or *B. thuringiensis*, respectively. In addition, oxamyl treatment alone gave the value of 13.24% (Table 5) comparing to nematode alone.

From these data, it was clear that concomitant applications obviously showed better results than single ones did. Improvement of the plant growth of soybean may be attributed to nematode alimentation and to the improvement of soil nutritive status (Akhtar and Mahmood, 1996; Firoza and Maqbool, 1996; Vats *et al*., 1996; Youssef and Amin, 1997; El-Sherif *et al*., 2007), in addition, many fungi including *Trichoderma* group are known to produce nematicidal or nematistatic compounds (Anke *et al*., 1995; Hallmann and Sikora, 1996; Chen *et al*., 2000; Meyer *et al*., 2000; Köpcke *et al*., 2001; El-Sherif and Ismail, 2007), a situation which may illustrate the superior effects of *T. harzianum* filtrate at 100% in improving plant growth parameters of such plant and suppressing root-knot nematode *M. incognita* infecting soybean in this study. Organic amendments enhance soil fertility, improve biological and physiological properties of soil, help in controlling root-knot nematodes and increase plant growth since the majority of nitrogen in horse or camel manures is in form of uric acid and can be rapidly converted to ammonium nitrogen if temperature, pH and moisture are suitable for microbial activity (Sims and Wolf, 1994). The ammonium produced has been showed to kill parasitic nematodes (Eno *et al*., 1955). The organic matters of animal manures showed positive effect on *M. incognita* development. These findings are in accordance with those reported by Akhtar and Mahmoud (1997) and El-Sherif *et al*. (2008) in respect to ammonia. The nematicidal activity of the tested soil organic amendments, the bacterium *B. thuringiensis* and fungus filtrate, *T. harzianum* at 100% as biofertilizers as well as their thermostable toxin against *M. incognita* on soybean plants can be varied from component to another. These variations may be attributed to the differences in the chemical nature, composition, concentration of toxin compounds present in these tested materials and methods of application used. The safely of such materials and its low cost is one of its advantages. However, additional research work are needed using the integrated of more than one component including *T. harzianum* or *B. thuringiensis* or such organic matters both in microplot and field experiments before recommendations can be made for Integrated Pest Management (IPM).

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