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Eco-friendly Management of *Meloidogyne incognita* Infecting Eggplant under Greenhouse Conditions

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ABSTRACT

The impact of magnetic iron, *Bacillus thuringiensis* (B.t) and dry leaf powder of moringa singly or integrated with its half dose each together as dual or triple treatments comparing with oxamyl on adjusting *Meloidogyne incognita* infecting eggplant cv. Black long was investigated under greenhouse conditions (19±3°C). Results revealed that all tested treatments significantly ameliorated eggplant growth criteria and reduced tested nematode parameters as well. Among the single tested materials, moringa powder achieved maximum increments of plant growth criteria that averaged 104.6, 57.5, 93.8, 100.0 and 70.0% for plant length, number of branches, total plant fresh weight, number of flowers and shoot dry weight, respectively. It also accomplished the highest percentage reduction of final nematode population (87.4%), number of galls (78.2%) and egg-masses (85.4%), followed by magnetic iron with values of 87.4, 78.2 and 85.4%, respectively. The dual application of {½ (moringa + magnetic iron)} overwhelmed other tested integrated materials in improving tested plant growth criteria with values of 124.8, 89.7, 141.0, 136.0 and 143.3%, respectively, whereas the triple one gave the least values in this respect. However, the triple treatment {½(moringa + magnetic iron + B.t)} ranked first in diminishing nematode criteria, followed by the double treatment {½(moringa + magnetic iron)} in this respect. Meanwhile, treatments containing either {½(moringa + B.t)} or {½ (magnetic iron + B.t)} showed low values for nematode parameters. Nematode reproduction factor (RF) under the stress of magnetic iron, (B.t) and moringa powder solely or mixed at its half dose each were adversely affected. Such rates ranged between 0.19-0.34 vs. 2.24 for nematode alone, where the triple treatment had the lowest value (0.19), B.t. showed the highest one (0.34) and oxamyl had the lower value (0.16) in this study.

Key words: Integrated management, *Meloidogyne incognita*, eggplant, *Bacillus thuringiensis*, magnetic iron, moringa dry leaf powder

INTRODUCTION

The sedentary endoparasite, *Meloidogyne incognita* is an important plant parasitic nematode that infect more than 2000 plant species causing significant yield losses (Olowe, 1992; Mangala and Mauria, 2006). Recently, the root-knot nematode, *Meloidogyne* spp. were among the five nematode genera with high population densities, frequency of occurrence and prominence values that found in ten governorates of Egypt during the period of 2000 and 2003 (Korayem *et al*., 2011). Application of chemical nematicides does not always prove effective and economic

(Pakeerathan *et al.*, 2009). In addition, poor target specificity of chemicals pose environmental and human toxicity hazards (Barker and Koenning, 1998). Moringa is an important food source in some parts of the world, because it can be grown cheaply and easily and the leaves retain lots of vitamins and minerals, when dried. Moringa is used in India and Africa in feeding programs to fight malnutrition. Extracts from moringa plant has been reported to contain pesticidal properties that inhibit egg-hatch and development of *Meloidogyne* spp. (Salawu, 1992). Magnetic iron as soil fertilizers that causes a revolution in the agriculture. Magentic iron granules have very accurate with high magnetic when contact with water, the resulting electromagnetic field helps the passage of useful elements to plant and eliminate nematodes and microbes from the rhizoshere of plant roots. However, nematode management using several control techniques i.e., powder of certain plant products and magnetic iron etc., as abiotic factors and microbial agents i.e., bacteria *Bacillus thuriengensis* (B.t) as biotic factors with minimal use of nematicides received great attention among the nematologists. These materials showed effective control measures against the target nematode, keep the nematode low at the safe level and avoiding environmental pollution. The objective of this study is to evaluate the eco-friendly components for adjusting *M. incognita* by magnetic iron, *Bacillus thuringiensis* (B.t) and dry leaf powder of moringa singly or mixed at its half dose in comparison with oxamyl under greenhouse conditions (19±3°C).

MATERIALS AND METHODS

Preparation of nematodes inocula: Second stage juveniles (J2) of *Meloidogyne incognita* (Kofoid and White) Chitwood were obtained from a pure culture of *M. incognita* that was initiated by 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, where this study was carried-out. *M. incognita* second stage juveniles (J2) inocula prepared by extracting them from soil of infected coleus plants through sieving modified Baermann technique (Goodey, 1957) and determined according to the design of the experiment in this investigation.

Nematode management test: In order to examine the eco-friendly management of *M. incognita* infecting eggplant (*Solanum melongena*) cv Black Long by magnetic iron, *Bacillus thuringiensis* (B.t) (from agricultural ministry of Egypt) and leaf powder of moringa (*Moringa oleifera* Lam, Fam: Moringaceae) singly or integrated with its half dose each comparing with oxamyl at the recommended dose under greenhouse conditions (19±3°C). Forty plastic pots (10 cm-dia) containing 1 kg steam sterilized clay loamy soil (1:1, v:v) with one eggplant seedlings 30 days-old each were used in this study. One week after eggplant seedlings transplanting, 1000 *M. incognita* juveniles (J2) were to thirty six seedlings each and left four seedlings (pots) without nematode to serve as check. One week later, the tested materials were added to four seedlings each and mixed with soil, while four seedlings (pots) with nematode only were left without any treatment. Each treatment was replicated four times. Treatments were as follows:

- Nematode + moringa dry leaf powder (5 g/pot)
- Nematode + magnetic iron (5 g/pot)
- Nematode + B.t. (0.15 g/pot)
- Nematode + {½ moringa dry leaf powder (2.5 g) +½ magnetic iron (2.5 g)}
- Nematode + {½ moringa dry leaf powder (2.5 g) +½ B.t. (0.075 g)}

- Nematode + {½ magnetic iron (2.5 g) +½ B.t. (0.075)}
- Nematode + {½ moringa dry leaf powder (2.5 g) +½ magnetic iron (2.5) +½ B.t.(0.075)}
- Nematode + oxamyl (0.3 mL/plant)
- Nematode alone
- Plant free of nematode and any treatment

Plastic pots were then arranged in a randomized complete block design in greenhouse and irrigated with tap water when needed. Plants were harvested after 45 days from nematode inoculation and plant growth criteria i.e., shoot and root lengths and fresh weights, as well as shoot dry weights were determined and recorded. Number of *M. incognita* (J2) in 250 g soil/pot were extracted by sieving and modified Baermann technique (Goodey, 1957) counted by Hawksely counting slide under x 10 magnification then calculated for each pot and recorded. Infected roots of each plant were washed with tap water, fixed in 4% formalin for 24 h and stained in 0.01 lactic acid-fuchsin (Bybd *et al.*, 1983) and then examined for the number of galls, developmental stages, females and egg-masses. The root gall index (RGI) and Egg mass Index (EI) were estimated according to the scale 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. The obtained data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984) followed by Duncan's Multiple Range Test (DMRT) to compare means (Duncan, 1955).

RESULTS AND DISCUSSION

Table 1 and 2 summarize the impact of magnetic iron, *Bacillus thuringiensis* (B.t) and dry leaf powder of moringa solely or mixed at its half dose each in comparison with oxamyl at full recommended dose under the stress of *M. incognita* infecting eggplant, *Solanum melongena* cv Black Long under greenhouse conditions. In general, all tested materials either solely or integrated at its half dose each ameliorated the increments percentage increase values of plant growth parameters and diminished nematode criteria as well, comparing to nematode alone (Table 1 and 2). It is evident that the concomitant treatments showed the maximum results in the increments of growth characters of eggplant and in suppressing the root-knot nematode *M. incognita* criteria (Table 1 and 2). Obviously, plant receiving moringa dry leaf powder application solely ranked first in the single treatments with the maximum increments values that averaged 104.6, 57.5, 93.8, 100.0 and 70.0% for plant length, number of branches, total plant fresh weight, number of flowers and shoot dry weight respectively, followed by that of B.t. treatment with values of 95.2, 55.2, 95.0, 76.0 and 43.3% for the same parameters, respectively. Moreover, plant treated by magnetic iron represented the last position with the minimum increase percentage of plant length (80.0%), number of branches (49.4%), total plant fresh weight (67.1%), number of flowers (68.0%) and shoot dry weight (40.0%), respectively. Oxamyl as a systemic nematicide gave considerable percentage increase values that averaged 34.1, 23.0, 54.7, 60.0 and 80.0% for the same parameters, respectively. Among the integrated applications, treatment containing half dose of moringa dry leaf powder plus half dose of magnetic iron {½(moringa + magnetic iron)} was superior over other tested integrated materials in improving plant length, number of branches, total plant fresh weight, number of flowers and shoot dry weight with values that averaged 124.8, 89.7, 141.0, 136.0 and 143.3%, respectively. Meanwhile, plastic pots receiving {½ (moringa + B.t.)} or {½ (magnetic iron + B.t.)} recorded clearly the improvement in plant growth characters with

Table 1: Impact of moringa dry leaf powder, magnetic iron and *Bacillus thuringiensis* (B.t) on eggplant plant cv. Black Long singly or integrated in comparison with oxamyl under the stress of *Meloidogyne incognita* infection in the greenhouse (19±3°C)

	*Plant growth response							
	Length (cm)						Fresh weigth (g)	
Treatments	Shoot	Root	Total plant length (cm)	Increase (%)	No. of brances	Increase (%)	Shoot	Root
Moringa dry leaf powder	57.5^{b}	27.6^{d}	85.1^{c}	104.6	13.7^{c}	57.5	21.6^{d}	9.6^{b}
Magnetic iron	50.2^{c}	24.7^{e}	74.9^{e}	80	13.0^{c}	49.4	19.6^{e}	7.3^{d}
B.t (*Bacillus thuringiensis*)	55.0^{bc}	26.2^{de}	81.2^{d}	95.2	13.5^{c}	55.2	21.5^{d}	9.9^{b}
{½ moringa dry leaf powder + ½ magnetic iron}	59.7^{a}	33.8^{a}	93.5^{a}	124.8	16.5^{a}	89.7	28.1^{a}	10.7^{a}
{(½ moringa dry leaf powder + ½ B.t	55.3^{bc}	29.5^{c}	84.8^{cd}	103.8	14.5^{b}	66.7	23.1^{c}	9.4^{b}
{(½ magnetic iron+ ½ B.t	55.4^{bc}	32.7^{b}	88.1^{b}	111.8	13.5^{c}	55.2	26.1^{b}	9.5^{b}
(½ moringa dry leaf powder + ½ magnetic iron +½ B.t	47.0^{d}	26.7^{de}	73.7^{ef}	77.2	12.0^{d}	37.9	23.3^{c}	8.3^{c}
Oxamyl	24.5^{f}	31.3^{b}	55.8^{g}	34.1	10.7^{e}	23	15.3^{f}	9.6^{b}
N alone	19.5^{g}	22.1^{f}	41.6^{h}	----	8.7^{f}	0	9.3^{h}	6.8^{e}
Plant free of any treatment	27.5^{e}	30.9^{bc}	58.4^{f}	40.4	12.5^{d}	43.7	10.3^{g}	7.1^{de}

	*Plant growth response					
Treatments	Total plant F. wt (g)	Increase (%)	No. of flowers	Increase (%)	Shoot dry weigth (g)	Increase (%)
Moringa dry leaf powder	31.2^{d}	93.8	5.0^{ab}	100	5.1^{cd}	70
Magnetic iron	26.9^{e}	67.1	4.2^{b}	68	4.2^{e}	40
B.t (*Bacillus thuringiensis*)	31.4^{d}	95	4.4^{b}	76	4.3^{e}	43.3
{½ moringa dry leaf powder + ½ magnetic iron}	38.8^{a}	141	5.9^{a}	136	7.3^{a}	143.3
{(½ moringa dry leaf powder + ½ B.t	32.5^{c}	101.9	4.7^{b}	88	4.5^{d}	50
{(½ magnetic iron+ ½ B.t	35.6^{b}	121.1	4.7^{b}	88	6.4^{b}	113.3
(½ moringa dry leaf powder + ½ magnetic iron +½ B.t	31.6^{cd}	96.3	3.5^{c}	40	5.6^{c}	86.7
Oxamyl	24.9^{f}	54.7	4.0^{b}	60	5.4^{c}	80
N alone	16.1^{h}	----	2.5^{d}	0	3.0^{g}	0
Plant free of any treatment	17.4^{g}	8.1	3.2^{cd}	28	3.4^{f}	13.3

N = 1000 J2 of *M. incognita*, *Each value is the mean of four replicates, Increase % = Treatment - N alone (Untreated)×100 N alone (Untreated)

values of 103.8, 66.7, 101.9, 88.0 and 50.0%, 111.8, 55.2, 121.1, 88.0 and 113.3%, respectively, for the same parameters. However, {½(moringa dry leaf powder + magnetic iron + B.t.)} gave the least values for the same plant characters, since their values averaged 77.2, 37.9, 96.3, 40.0 and 86.7%, respectively. In the meantime, plant free of nematode and receiving non of the tested materials gave non significant increase percentage in plant length, number of branches, total plant fresh weight, number of flowers and shoot dry weight, with values 40.4, 43.7, 8.1, 28.0 and 13.3%, respectively, comparing to nematode alone (Table 1). Moreover, it is worthy to note that the dry leaf powder of moringa either alone or mixed with magnetic iron at half dose each accomplished the highest eggplant growth values in this study (Table 1).

Data in Table 2 show the number of galls, egg-masses, females and developmental stages of *M. incognita* on eggplant cv. Black Long roots and nematode population in soil under the stress of

Table 2: Impact of moringa dry leaf powder, magnetic iron and *Bacillus thuringiensis* (B.t) singly or integrated in comparison with oxamyl on *Meloidogyne incognita* infecting on eggplant cv. Black Long under greenhouse conditions (19±3°C)

		*Nematode population in				
		Root				
Treatments	Soil (J2)	Females	Dev. stages	Final population (Pf)	RF	Red (%)
Moringadry leaf powder	252.3^{d}	9.0^{e}	20.0^{d}	281.3^{c}	0.28	87.4
Magnetic iron	266.1^{c}	12.1^{c}	25.1^{c}	303.3^{c}	0.3	86.4
B.t (*Bacillus thuringiensis*)	295.6^{b}	12.5^{c}	29.2^{b}	337.3^{b}	0.34	84.9
{½ moringa dry leaf powder + ½ magnetic iron}	216.5^{f}	10.3^{d}	20.0^{d}	246.8^{e}	0.25	89
{(½ moringadry leaf powder + ½ B.t)}	260.9^{c}	10.1^{d}	15.4^{e}	286.4$^{\cdot}$	0.29	87.2
{(½ magnetic iron+ ½ B.t)}	235.2^{e}	13.5^{b}	20.3^{d}	269.0^{d}	0.27	88
(½ moringadry leaf powder + ½ magnetic iron +½ B.t)}	155.6^{g}	8.1^{f}	23.2^{cd}	186.9^{f}	0.19	91.6
Oxamyl	145.2^{h}	0^{g}	15.6^{e}	160.8^{g}	0.16	92.8
Nalone	2100.1^{a}	55.0^{a}	80.1^{a}	2235.2^{a}	2.24	---

	*Nematode population in					
Treatments	No. of galls	RGI	Red (%)	No. of egg- masses	EI	Red (%)
Moringadry leaf powder	24.0^{d}	3.1^{d}	78.2	13.1^{de}	3.4^{b}	85.4
Magnetic iron	33.1^{bc}	4.0^{b}	69.9	15.2^{c}	3.1^{b}	83.1
B.t (*Bacillus thuringiensis*)	35.3^{b}	4.2^{b}	67.9	16.3^{b}	3.0^{b}	81.9
{½ moringadry leaf powder + ½ magnetic iron}	25.4^{e}	3.1^{d}	76.9	14.1^{d}	3.0^{b}	84.3
{(½ moringadry leaf powder + ½ B.t)}	25.1^{e}	3.6^{c}	77.2	12.2^{e}	3.6^{b}	86.4
{(½ magnetic iron+ ½ B.t)}	24.2^{d}	3.0^{e}	78	16.3^{b}	3.1^{b}	81.9
(½ moringadry leaf powder + ½ magnetic iron +½ B.t.)}	23.3^{e}	3.4^{c}	78.8	12.1^{e}	3.2^{b}	86.6
Oxamyl	13.1^{f}	3.2^{d}	88.1	5.0^{f}	2.0^{c}	94.4
Nalone	110.0^{a}	5.0^{a}	--	90.0^{a}	4.2^{a}	---

*Each value presented the mean of four replicates, N = *M. incognita* (1000 J2/ plant) as the initial population, RF = Final population (Pf)/ initial population (Pi) = Reproduction factor, Means in each column followed the same letter (s) did not differ at p≤0.05 according to Duncan's multiple rang test

magnetic iron, (B.t) and dry leaf powder of moringa solely or mixed at its half dose each in comparison with oxamyl at full recommended dose under greenhouse conditions. Data indicated that concomitant treatments obviously gave better results than single treatments (Table 2). Among single applications it was evident that plant treated by dried-leaf powder of moringa accomplished the highest percentage reduction of final nematode population, number galls and egg-masses followed by magnetic iron with values of 87.4, 78.2 and 85.4%, 86.4, 69.9 and 83.1%, respectively, whereas *Bacillus thuringiensis* showed the lesser values for the same nematode criteria with values of 84.9, 67.9 and 81.9%, respectively.

Moreover, the triple treatment {½(moringa + magnetic iron + B.t)} ranked first and represented the maximum values in diminishing final nematode population, number of galls and egg-masses that amounted to 91.6, 78.8 and 86.6%, respectively, followed by the double treatment

{½ (moringa + magnetic iron)} with values 89.0, 76.9 and 84.3% for final nematode population, number of galls and egg-masses, respectively. Meanwhile, treatments containing either {½ (moringa + B.t)} or {½ (magnetic iron + B.t)} showed less values which averaged 87.2, 77.2 and 86.4%, 88.0, 78.0 and 81.9% for same nematode parameters, respectively. Oxamyl as a systemic nematicide gave the highest values of diminishing number of galls (88.1%), final nematode population (92.8%) and egg-masses (94.4%), respectively, when compared to nematode alone. Moreover, results of this experiment are interesting, especially with the indices of root galls and egg-masses as well where the latter was more affected by tested applications either singly or binary ones than the former. For instance, the indices of egg-masses ranged between 3.0-3.6 vs. 4.2 for nematode alone, whereas those of triple application recorded 3.2 vs. 4.2 for nematode alone, respectively. Similar trend was observed for the root gall indices which ranged between 3.1-4.2; 3.0-3.4 and 3.6 vs. 5.0 for single, double and triple against nematode alone, respectively (Table 2). Also, nematode reproduction factors under the stress of magnetic iron, (B.t), dry leaf powder and moringa solely or mixed at its half dose each in comparison with oxamyl were adversely affected. Such rates ranged between 0.19-0.34 vs. 2.24 for nematode alone. Namely, triple treatment {½ (moringa + magnetic iron + B.t)} had the lowest rate of reproduction, (0.19) while that of *Bacillus thuringiensis* (B.t) showed relatively the highest (0.34), respectively, whereas oxamyl appointed the lowest value (0.16) in this respect.

Apparently, results of the present investigation indicated that nematode criteria and host plant growth parameters were obviously affected by tested materials. The tested biotic and abiotic factors in the present study as tool for the integrated control of *M. incognita* on eggplant succeeded to generate a sort of inducing resistance in a susceptible host plant against such pathogenic nematode, since the double treatment {½(moringa + magnetic iron)} ranked first and represented the maximum values in improving plant growth parameters i.e., plant length, number of branches, total plant fresh weight, number of flowers and shoot dry weight with values that averaged 124.8, 89.7, 141.0, 136.0 and 143.3%, respectively under the stress of *M. incognita* infection, whereas the triple showed the lesser values in this respect, even it gave the highest values in diminishing nematode criteria. Meanwhile, the nematicidial activities of the tested abiotic agents i.e., magnetic iron and moringa dry leaf powder as well as biotic agents i.e., B.t as soil amendments in the integrated adjustment of *M. incognita* on eggplant can be varied from component to another. These variations may be attributed to the differences in the chemical nature, compound present in these tested materials and method of application used. The safely of such materials and its low cost is one of its advantages. Moringa dried leaf powder was not phytotoxic, improved plant growth and vigour by suppressing nematode population (Makkar and Becker, 1996). Ethanol extract of moringa leaf contained growth enhancing principles belonging to the cytokinine group which was effective in reducing nematode population in plants with a subsequent increase in plant growth and number of leaves (Guzman, 1984). The nematicidal effect of the moringa leaf powder could be attributed to its high content of certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (Knobloch *et al*., 1989). The present study are in accordance with Mohammed *et al*. (2008) who recorded that the culture fluid, cell-free suspension and cell pelleted residues of the four selected B.t isolates clearly showed a suppressive effect on the occurrence of root galling of *M. incognita*. Results also showed that all treatments increased the root fresh weight in comparison with the control. Improving in growth characters of such infected plants has been recorded due to the application of most tested biotic and

abiotic resistance inducers. However, the triple treatments in spite of its suppressive effect on nematode development and reproduction ranked second to oxamyl treatment in this respect, it had phytotoxic effect on the plant growth when applied together as broadcasting treatment. Because of *M. incognita* endo parasitic and sedentary nature in root, all tested biotic and abiotic materials especially treatment of moringa dried leaf powder alone or mixed with magnetic iron are effective in suppressing it to great extent in this study. This is not surprising since tested moringa powder enrich with protein, vitamin A, vitamin B, vitamin C and minerals. (calcium and potassium) that showed nematicidal properties against such pathogenic nematode. On the other hand the present data are in agreement with Ismail *et al.* (2010) who reported that the low bio-NK (BC) rate surpassed magnetic iron ore (MIO) and metal compound fertilizer (MCF) three times by controlling *M. incognita* populations in both soil and roots of grapevine cv. Superior through two successive seasons. However, the high rate of MIO and BC was superior for two experimental periods, as well as the recommended and high rates of MCF. The low rate of MCF was the best formulation for soil and roots of grape cv. Thompson seedless. However, more study is needed to be done in this direction under field conditions before drawing such recommendations for this new trend that is safe and effective integrated nematode management program (s).

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