academicJournals

Vol. 7(4), pp. 30-38, May, 2015
DOI: 10.5897/JEN2015.0125
Article Number: 6B47BEE53308
ISSN 2006-9855

http://www.academicjournals.org/JEN

Journal of Entomology and Nematology

Full Length Research Paper

Evaluation of calcium sulphate, potassium silicate and moringa dry leaf powder on *Meloidogyne incognita* infecting tomato plant with reference to N, P, K, total phenol and cholorophyl status under greenhouse condition

El-Sherif, A. G.*, Gad, S. B. and Saadoon, S. M.

Nematology Research Unit, Agricultural Zoology Department,. Faculty of Agriculture, Mansoura University, Egypt.

Received 30 March, 2015; Accepted 20 May, 2015

A pot randomized complete block design experiment was carried-out to evaluate the impact of calcium sulphate or potassium silicate or moringa dry-leaf powder either alone or combined as double or triple treatments along with oxamyl or the three items + oxamyl 24% L as tetra application in comparison with oxamyl at the recommended dose against *Meloidogyne incognita* infecting tomato plant cv. 9065 FI under greenhouse conditions (25±3°C). All tested treatments improved plant growth criteria and reduced nematode parameters as well. Moringa dry leaf powder singly surpassed other tested single treatments in the increment values of total plant length, number of leaves and branches per plant; total plant fresh weight and shoot dry weight, and also accomplished the highest percentage reduction of nematode parameters with the maximum values of final nematode population, number of galls and egg mass/plant, respectively. Among the dual treatments, moringa dry leaf powder plus calcium sulphate at their half doses overwhelmed other double treatments in the percentage increase values of such plant growth characters of tomato and achieved the highest reduction values of final nematode population, number of galls and egg masses. Likewise, the same trend was evident as the two compounds of moringa dry leaf powder plus calcium sulphate mixed with oxamyl at 1⁄3 each. Moreover, a similar trend was also strongly observed in the case of applying oxamyl to that tested three components as 1⁄4 each which gave the high synergistic action by showing the highest recorded values of plant growth criteria and also recorded a high synergistic action in diminishing nematode criteria as well as the highest percentage increase value of total phenol (23.70%). The C/N ratio of the tetra treatment gave the least value of this item (16.03:1), while oxamyl had 19.67:1 vs. 22.88:1 for nematode alone, respectively.

Key words: Tomato plant, *Meloidogyne incognita*, oxamyl, moringa, total phenol, calcium sulphate, potassium silicate, integrated control.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are one of the most wide spread and damaging agricultural pests in the world causing an estimated US $100 billion loss/year worldwide (Oka et al., 2000). They are widely distributed in cultivated areas of Egypt causing remarkable crop losses. Tomato is preferable host to several species of root-knot nematodes. Biocontrol agents applied singly are not likely to perform consistently against all parasitic nematodes under soil environmental conditions. Different mechanisms of control may be dissimilarly influenced by environmental conditions; and it is possible that if multiple mechanisms are involved under a certain set of conditions, one mechanism may compensate for the other (Guetsky et al., 2002). So, the control achieved by biocontrol agents with several distinct mechanisms of control may be additive or synergistic. The approach of combining biocontrol agents to manage various soil borne pathogens including plant parasitic nematodes has been investigated extensively **(**Hojat et al., 1998; Pierson and Weller, 1994; and Siddiqui and Mahmood, 1993). Integrated nematode management using several control techniques that is soil amendments and bioagents with minimal use of nematicides received recently great attention among nematologists, providing effective control measures against the target nematode by keeping nematode population densities at the safe level and avoiding environmental pollution (AL-Ghnam, 2011). The objective of the present work was to study the impact of calcium sulphate or potassium silicate or moringa dry-leaf powder in comparison with oxamyl on plant growth response of tomato plants infected with M. *incognita* under greenhouse conditions.

MATERIALS AND METHODS

Source of nematodes

Second stage juveniles (J2) of *M. incognita* (Kofoid & White) Chitwood, were obtained from a pure culture of *M. incognita* that was initiated by a single eggmass propagated on coleus plants, *Coleus blumei* in the greenhouse of Nematology Research Unit (NERU) Agricultural Zoology Department, Faculty of Agriculture, Mansoura University, Egypt, where this work was carried-out. Second stage juveniles (J2) were extracted from soil of coleus plants by sieving and modified Baermann technique (Goodey, 1957) counted in a Hawksely counting slide under x 10 magnification then calculated for each 1 ml of the nematode suspension and recorded for preparing nematode inoculation of this work.

Nematicide

Oxamyl (Vydate) 24% L. Methyl-N'N'- dimethyl-N [(methyl) carbamoyl-oxy]-1- thioxamidate, which was used at the rate of 0.3 ml / plant.

Greenhouse experiment

A pot trial was set-up to study the effect of moringa dry leaf powder of and two plant mineral nutrients that is calcium sulphate and potassium silicate singly or integrated together as dual or triple or the three elements plus oxamyl as tetra application in comparison with oxamyl at the recommended dose on root-knot nematode *M. incognita* infecting tomato plant cv.9065 F1 (*Solanum lycopersicum* L.) under greenhouse conditions. Sixty four plastic pots (10 cm) containing 900 g steam sterilized sand loamy soil (1:1, v:v) with one 30 day-old tomato seedlings each were used in this study. One week after tomato seedlings transplanting, 2000 J_2 of *M. incognita* were inoculated to 60 seedlings each and four non inoculated were used as control. One week later, treatments were added to four seedlings each and mixed with soil, while four seedlings (pots) with nematode received oxamyl at the rate of 0.3 ml / pot. Another four seedlings (pots) with nematode only were left without any treatment. Treatments were as follows: 1. N +calcium sulphate(5 g /pot); 2. N + Potassium silicate (5 g /pot); 3. N +moringa dry leaf powder (5 g / pot)]; 4. N + [½ moringa dry leaf powder (2.5 g) + ½ Potassium silicate (2.5g)]; 5. N + [½ calcium sulphate (2.5 g) + ½ Potassium silicate (2.5 g)]; 6. N + [½ moringa dry leaf powder (2.5 g) + ½ calcium sulphate (2.5 g)]; 7. N + [1/3 calcium sulphate(1.6 g)+1/3 moringa dry leaf powder(1.6 g)+1/3 oxamyl(0.1mL/plant)]; 8. N + [1/3 Potassium silicate (1.6 g) +1/3 moringa dry leaf powder (1.6 g)+ oxamyl (0.1 ml/plant)]; 9. N + [1/3 Potassium silicate (1.6 g) +1/3 calcium sulphate (1.6 g)+1/3 oxmyl(0.1 mL/plant)]; 10. N + [1/3 calcium sulphate (1.6 g)+1/3 moringa dry leaf powder(1.6 g)+1/3 calcium sulphate (1.6 g)]; 11. N + [1/4 oxamyl (0.75)+1/4 calcium sulphate(1.25 g)+1/4 Potassium silicate(1.25 g)+1/4 moringa dry leaf powder(1.25 g)]; 12. N + Oxamyl (0.3 ml /plant); 13. Nematode alone and 14. Plant free of nematode and any treatments.

Each treatment was replicated four times. Plastic pots were arranged in a randomized complete block design and irrigated with tap water as needed. Plants were harvested 45 days after nematode inoculation, and plant growth criteria that is shoot and root lengths, number of leaves and branches per plant and fresh weights, as well as shoot dry weights were determined and recorded. Number of *M. incognita* (J_2) in 250 g of soil/pot were extracted by sieving and modified Baermann technique (Goodey, 1957) counted in a Hawksely counting slide under x 10 magnification then calculated for each pot and recorded. Infected roots of each plant per treatment were washed with tap water, fixed in 4% formalin for 24 h and stained in 0.01 lactic acid-fuchsin (Byrd 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 given by 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. The obtained data were subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984) followed by Duncan's multiple ranges to compare means (Duncan, 1955).

Chemical analysis

Samples of tomato dried leaves were ground, wet, digested and their nitrogen (N), phosphorus (P), potassium (K) contents were determined according to Kjeldahl methods (A.O.A.C,1980).

Chlorophyll content

Representing sample from the upper fourth leaf of tomato plant /

*Corresponding author. E-mail: elsherifmohammed@yahoo.com.

treatment were obtained at 75 days after sowing and both chlorophyll a and b were determined in mg/g F.W following the equations used for the calculation of pigments content according to Goodwin (1965).

$$\text{Chlorophyll } (a) = \frac{12.7\, D\, 663 - 2.69 D 645 \times V \times 10}{d \times 1000 \times 10}$$

$$\text{Chlorophyll } (b) = \frac{22.9 D\, 645 - 4.68 D\, 663 \times V \times 10}{d \times 1000 \times 0.5}$$

Where, V= Acetone volume; D= Optical density reading at the wave length (cm) and D =⁄cm.

Determination of total phenols

Total phenols were determined after harvesting tomato fresh leaves bases using the Folin-Ciocalteu reagent method (Kaur and Kapoor, 2001). Total content of phenolic compounds in plant ethanolic extracts was calculated as catechol equivalents by the following equation:

$$T = \frac{C \times V}{m \times 100}$$

Where, T=Total content of phenolic compounds, in mg of catechool/100 g of fresh weight material.
C = The concentration of catechol established from the calibration curve, in mg/ml and V= the volume of extract in ml; m =The weight of pure plant ethanolic extract in g.

RESULTS AND DISCUSSION

Tables 1 and 2 verify the impact of calcium sulphate or potassium silicate or moringa dry-leaf powder either alone or mixed as double or triple treatments along with oxamyl 24% L or the three elements + oxamyl as tetra application in comparison with oxamyl at the recommended dose against *M. incognita* infecting tomato plant cv. 9065 FI under greenhouse conditions (25±3°C). Obviously, results indicated that all tested treatments improved plant growth criteria and reduced nematode parameters as well.

Among single applications, plant that received moringa dry leaf powder surpassed other tested single treatments in the increment values for total plant length (57.8%), number of leaves (13.3)% and branches per plant (28.6)%; total plant fresh weight (41.12%) and shoot dry weight (40%) followed by calcium sulphate in this respect compared to nematode alone (Table 1).

Moreover, among the dual treatments tested in this study, moringa dry leaf powder plus calcium sulphate at their half doses overwhelmed other double treatments in the percentage increase values of such plant growth characters of tomato plant that is total plant length (94.25%), number of leaves (55.5%) and branches (60.71%), total plant fresh weight (70.71%) and shoot dry weight (80.0%), followed by that of ½ (calcium sulphate + potassium silicate) and then ½ moringa dry leaf powder + potassium silicate), respectively. Likewise, the same trend was evident as the two compounds of moringa dry leaf powder plus calcium sulphate mixed with oxamyl at 1⁄3 each since this integrated treatment achieved the highest percentage increase values of total plant length (124.26%), number of leaves (61.6%), and branches (67. 85%), total plant fresh weight (110.13%) and shoot dry weight (105.2%) followed by that of ⅓ (potassium silicate + moringa dry leaf powder + oxamyl) and then 1⁄3(potassium silicate + calcium sulphate +oxamyl)in such plant growth parameters, respectively. It is interesting to note that a synergistic effect to the increments values of tomato plant growth positively occurred for the three tested items added at ⅓ each with values of 111.46, 61.6, 71.42, 113.26 and 114.8% for plant length, number of leaves and branches⁄plant, total plant fresh weight and shoot dry weight, respectively compared with nematode alone. Moreover, similar trend was also strongly observed in the case of applying oxamyl to that tested three components at ¼ each as 1⁄4 (moringa dry leaf powder + calcium sulphate + potassium silicate + oxamyl) treatment which gave the high synergistic action by showing the highest recorded values of such tomato plant growth criteria in this study, since its values were 146.84, 66.6, 114.28, 127.04 and 122.8% for plant length, number of leaves and branches/plant ,total plant fresh weight and shoot dry weight, respectively (Table 1). It is worthy to note that oxamyl as a systemic nematicide gave considerable values of percentage increase values of tomato plant growth characters, since its values amounted to 93.6, 34.6, 28.57, 78.53 and 81.2% for plant length, number of leaves and branches / plant, total plant fresh weight and shoot dry weight, respectively, comparing to nematode alone (Table 1). Moreover, plant free of nematode and receiving none of the tested components gave reasonable values of such tested tomato growth characters, since its values were 12.89, 4.4, 7.14, 4.32 and 8.0% for plant length, number of leaves and branches ⁄ plant, total plant fresh weight and shoot dry weight, respectively (Table 1) .

Data presented in Table 2 reveal the influence of calcium sulphate, potassium silicate and moringa dry leaf powder either alone or mixed as dual or triple treatments along with oxamyl or three items plus oxamyl as tetra application compared to oxamyl at the recommended dose on reproduction and development of *M. incognita* infecting tomato plant cv. 9065 F1 under greenhouse conditions. In general, results indicate that all tested treatments obviously diminished *M. incognita* tested criteria that is number of juveniles in soil, developmental stages, root galling, females and egg masses on root system as compared to those of the inoculated untreated cheek. It is interesting to observe that the tested double or triple or tetra application treatments gave better results than single ones did. In the meantime among the single applications, moringa dry leaf powder accomplished the highest percentage

Table 1. Impact of calcium sulfate or potassium silicate or moringa dry leaf powder either alone or mixed as double or triple treatments or with oxamyl or as tetra application in comparison with oxamyl on plant growth response of tomato cv. 9065 FI infected with 2000 second stage juveniles (J2) of *Meloidogyne incognita* under greenhouse conditions

Treatment	*Plant growth response													
	Length (cm)		Total plant length (cm)	** Inc.%	No. of leaves\ plant	** Inc.%	No.of branches\ plant	** Inc.%	Total plant Fresh weight (g)		Total plant F.Wt (g)	** Inc.%	Shoot dry weight (g)	** Inc.%
	Shoot	Root							Shoot	Root				
Potassium silicate	53.00f	20.26g	73.26h	40.18	50.0fg	11.1	8.00ef	14.28	12.50f	4.43e	16.93f	26.15	2.93f	17.2
Calcium sulphate	59.62e	22.25f	81.87g	56.65	50.5fg	12.2	8.50def	21.4	13.00f	4.15e	17.15f	27.79	3.05f	22
Moringa dried leaf powder	57.87e	24.60e	82.47g	57.8	51.0f	13.3	9.00cdef	28.6	14.50ef	4.45e	18.95ef	41.12	3.50e	40
1\2 (Moringa dried leaf powder + Potassium silicate)	68.50d	26.25d	94.75f	81.3	57.0e	26.6	10.00bcde	42.85	15.00ef	5.24cd	20.24de	50.81	3.91d	56.4
1\2 (Calcium sulphate + Potassium silicate)	72.00d	22.90f	94.90f	81.59	65.3c	45.1	10.30bcde	47.14	16.00e	5.93b	21.93cd	63.41	4.08d	63.2
1\2 (Moringa dried leaf powder + calcium sulphate)	69.46d	32.06b	101.52e	94.25	70.0b	55.5	11.25bc	60.71	17.00de	5.91b	22.91bc	70.71	4.50c	80
1\3 (calcium sulphate + moringa dried leaf powder + Ox)	87.70b	29.50c	117.20b	124.26	72.8ab	61.6	11.75b	67.85	22.22b	5.98b	28.2a	110.13	5.13b	105.2
1\3 (Potassium silicate + moringa dried leaf powder + Ox)	83.56b	30.00c	113.56bc	117.29	70.0b	55.5	10.75bcd	53.57	19.85bc	5.53bcd	25.33b	88.74	4.57c	82.8
1\3 (Potassium silicate + calcium sulphate + Ox)	85.00b	24.83e	109.83cd	110.16	66.0c	46.6	11.00bcd	57.14	20.00bc	5.79bc	25.79b	92.17	4.68c	87.2
1\3 (Potassium silicate + moringa dried leaf powder+ calcium sulphate)	87.25b	23.26f	107.51d	111.46	72.8ab	61.6	12.00b	71.42	21.90b	6.72a	28.62a	113.26	5.37ab	114.8
1\4 (Calcium sulphate + Potassium silicate + moringa dried leaf powder+ Ox)	95.00a	34.00a	129.00a	146.84	75.0a	66.6	15.00a	114.28	25.00a	5.47bcd	30.47a	127.04	5.57a	122.8
Ox amyl 24% L	75.93c	25.25de	101.18e	93.6	60.6d	34.6	9.00cdef	28.57	18.95cd	5.01d	23.96bc	78.53	4.53c	81.2
N alone	36.00h	16.25i	52.25j	_	45.0h	_	7.00f	_	10.00g	3.42f	13.42g	_	2.50g	_
Plant free of any treatment	40.00g	19.00h	59.00i	12.89	47.0gh	4.4	7.50f	7.14	10.00g	4.00e	14.0g	4.32	2.70fg	8
LSD	3.463	1.054	4.072		2.954		1.619		2.025	0.451	2.203		0.342	

Values are the mean of four replicates. Ox = oxamyl. *Means in each column followed by the same letter(s) did not differ at p<0.05 according to Duncan's multiple-range test.

reduction of nematode parameters with the maximum values of 66.5, 75.96 and 77.77% for final nematode population, number of galls and egg mass ⁄ plant, respectively comparing with nematode alone (Table 2).

Moreover, plant receiving calcium sulphate as a single treatment ranked second to moringa dry leaf powder in reducing nematode parameters since. Its values amounted to 66.4, 72.22, and 75.55%, for the same parameters whilst potassium silicate showed the least values in this respect which were appointed to 61.2, 62.27 and 74.07% for final nematode population, number of gall and egg masses ⁄root system, respectively compared to nematode alone (Table 2). As far for the dual applications, moringa dry leaf powder plus calcium sulphate at its half doses achieved the highest reduction values of final nematode population (78.2%), number of galls (83.33%) and egg masses (85.18%), followed by that of 1⁄2 (calcium sulphate + potassium silicate) than treatment that contained 1⁄2 (moringa dried leaf + potassium silicate), respectively as compared to nematode alone. It is interesting to observe that

Table 2. Nematode parameters of *Meloidogyne incognita* infecting tomato plant cv. 9065 F1 as affected by calcium sulphate or potassium silicate or moringa dry leaf powder either alone or mixed as double or triple applications or plus oxamyl as tetra treatments in comparison with oxamyl under greenhouse condition (25 ± 3°C).

Treatment	Nematode population in											
	Soil (j_2)	Roots: Females	Roots: Dev. stages	Final population (pf)	Red %	RF	No. galls	Red %	RGI %	No .of Egg-masses	Red %	EI
Potassium silicate	1800.0b	37.0b	51.00b	1888.0b	61.2	0.94	50.0b	65.27	4	35.0b	74.07	4
Calcium sulphate	1578.0c	35.0bc	34.25d	1647.25c	66.4	0.82	40.0c	72.22	4	33.0bc	75.55	4
Moringa dried- leaf powder	1561.25d	32.5c	32.00d	1625.75d	66.5	0.81	35.0d	75.69	4	30.0c	77.77	4
½ (Moringa dried- leaf powder + Potassium silicate)	1136.0e	36.25b	42.00c	1214.25e	75	0.6	37.0cd	74.3	4	25.0d	81.48	3
½ (calcium sulphate + Potassium silicate)	1032.0f	27.0d	29.50e	1088.5f	77.6	0.54	34.0d	76.38	4	24.0d	82.22	3
½(moringa dried- leaf powder + calcium sulphate)	1024.0g	27.25d	9.75g	1060.75g	78.2	0.53	24.0e	83.33	3	20.0e	85.18	3
1/3 (Calcium sulphate+ moringa dried- leaf powder + Ox)	420.0j	15.0f	10.00g	450.0k	90.5	0.22	16.0f	88.9	3	9.0f	88.9	2
1/3 (Potassium silicate + moringa dried-leaf powder + Ox)	422.0j	19.0e	17.50f	458.5j	90.6	0.23	18.0f	87.5	3	10.0f	92.59	2
1/3 (Potassium silicate + calcium sulphate + Ox)	481.25i	17.5ef	10.25g	508.25i	89.5	0.25	12.25g	91.49	3	10.0f	92.59	2
1/3 (Potassium silicate + moringa dried-leaf powder+ calcium sulphate)	590.0h	15.25f	11.50g	616.75h	87.3	0.31	12.0g	91.66	3	10.0f	92.59	2
1/4 (calcium salphate + Potassium silicate + moringa dried- leaf powder + Ox)	400.0k	8.5g	4.25h	412.75l	91.5	0.21	10.75g	92.53	2	7.0f	94.81	2
Oxamyl 24% L	312.0L	1.5h	2.25h	315.75m	93.5	0.16	4.25h	97.04	2	2.0g	98.51	1
N alone	4593.6a	150.5a	122.00a	4866.1a	_	2.43	144.0a	_	5	135.0a	_	5
L.S.D	4.01	2.648	2.314	6.924			3.301			3.291		

Values are the mean of four replicates. Ox = Oxamyl. EI= eggmass index RGI= root gall index. *Means in each column followed by the same letter(s) did not differ at p<0.05 according to Duncan's multiple-range test. RF= nematode reproduction factor.

when the dual applications applied separately along with oxamyl as triple treatment, an obvious synergistic action of such triple application was clear in the resulting more reduction percentage of nematode criteria.

For instance, plant receiving treatment containing ⅓ (moringa dry leaf powder + calcium sulphate + oxamyl) surpassed over other tested triple treatments in reducing final nematode population (90.5%), number of galls (88.90%) and egg masses (93.33%), followed by that of ⅓ (potassium silicate + calcium sulphate + oxamyl), respectively, compared to nematode alone.

However, plant that received the three compounds as triple treatment without oxamyl [⅓ (calcium sulphate + potassium silicate + moinga dry leaf powder)], gave a considerable reduction percentage of final nematode population (87.3%), number of galls (91.66%), and egg masses (92.59%), respectively. Moreover , when oxamyl was added to this triple treatment that contained four components as tetra application [¼ (calcium sulphate + potassium silicate + moringa dry leaf + oxamyl)], a high synergistic action was obviously recorded in diminishing final nematode population, number of galls and egg masses with values of 91.5, 92.53 and 94.81%, respectively compared to nematode alone. It is worthy to note that oxamyl as a systemic nematicide gave the highest, percentage reduction of final nematode population (93.51%), number of galls (97.04%) and egg masses (98.51%), respectively compared to nematode alone and ranked first in this respect. Likewise, signification results were observed between egg masses indices of all tested treatments and nematode alone, since they ranged from (4) for single applications to (3) for the double ones to (2) for the triple treatment to (1) for oxamyl vs 5 for nematode alone. Similar trend was evident in the case of gall indices of tested treatments since they ranged from 4 for single and some of dual ones to 3 for triple treatments and 2 for four components as tetra treatment with oxamyl vs. 5 for nematode alone (Table 2).

Also, nematode reproduction factors under the stress of potassium silicate, calcium sulphate and moringa dry leaf powder solely or mixed as binary or triple alone or along with oxamyl or as tetra treatments in comparisons with oxamyl at the recommended dose on tomato plant were

Table 3. Nitrogen (N) phosphorus (P), potassium (K), total phenol and total chlorophyll contents in leaves of tomato CV.9065 F1 infected with *Meloidogyne incognita* treated with calcium sulphate or potassium silicate or moringa dry leaf powder either alone or mixed as double or triple or with oxamyl as tetra treatments in comparison with oxamyl under greenhouse conditions.

Treatment	*Chemical components											
	Leaves										Total phenol (mg/100 g)	Inc.%
	N%	P %.	K	C%	C / N	O.M%	Chlorophyll content mg /g F.WT.					
							Chlo. a	Chlo.b	a+b	Red.%		
Potassium silicate	1.55	0.346	1.80	34.4	22.19	59.1	0.537l	0.378m	0.915m	14.1	564.4d	17.6
calcium sulphate	1.59	0.354	1.89	34.7	21.82	59.7	0.556j	0.395j	0.951k	14.9	572.1c	19.2
moringa dry leaf powder	1.64	0.370	1.98	35.0	21.34	60.2	0.545k	0.387l	0.932l	16.6	579.8b	20.8
½ (moringa dry leaf powder + Potassium silicate)	1.70	0.365	2.08	35.2	20.7	60.5	0.563i	0.406i	0.969i	13.2	541.5g	12.9
½(calcium sulphate + Potassium silicate)	1.77	0.373	2.17	35.5	20.05	61.1	0.571h	0.414h	0.985h	11.8	549.6f	14.5
½(moringa dry leaf powder + calcium sulphate)	1.81	0.386	2.25	35.9	19.83	61.7	0.582g	0.422g	1.004g	10.1	557.2e	16.1
½(moringa dry leaf powder + calcium sulphate)	2.02	0.413	2.52	36.9	18.26	63.4	0.615c	0.462c	1.077c	3.6	515.3j	7.4
1⁄3 (Potassium silicate + moringa dry leaf powder + Oxamyl)	2.13	0.422	2.63	37.2	17.43	63.9	0.609d	0.450d	1.059d	5.2	506.1k	5.5
1⁄3 (Potassium silicate + calcium sulphate + Oxamyl)	2.25	0.431	2.69	37.69	16.75	64.6	0.627b	0.470b	1.097b	1.8	523.8i	9.2
1⁄3 (Calcium sulphate + moringa dry leaf powder + calcium sulphate)	1.93	0.407	2.43	36.5	18.91	62.8	0.598e	0.442e	1.04e	6.9	495.9l	3.4
1⁄4 (Calcium sulphate + Potassium silicate + moringa dry leaf powder + Oxamyl)	2.37	0.446	2.79	38.0	16.03	65.3	0.590f	0.370n	0.960j	18.1	593.4a	23.7
Oxamyl	1.84	0.398	2.34	36.2	19.67	62.3	0.588f	0.433f	1.021f	8.6	532.7h	11.0
N alone	1.49	0.335	1.73	34.1	22.88	58.6	0.634a	0.483a	1.117a	--	479.8n	--
Plant free of any treatment	2.49	0.458	2.89	38.3	15.38	65.9	0.544k	0.391k	0.935l	16.3	487.2m	1.5
L.S.D							0.005	0.002	0.007		0.204	

Values are the mean of four replicates. Ox = Oxamyl. *Means in each column followed by the same letter(s) did not differ at $p<0.05$ according to Duncan's multiple-range test.

adversely affected. Such rates ranged from 0.94 to 0.21 vs. 2.43 for nematode alone. Namely, the treatment containing ¼ (potassium silicate +potassium sulphate +moringa dry leaf + oxamyl) had the lowest rate of reproduction 0.21 whilst that of potassium silicate alone showed clearly the highest (0.94), respectively, whereas oxamyl had the least value (0.16) in this respect. Promising results were reported among the tested applications of adding moringa dry leaf powder with calcium sulphate and oxamyl plus potassium silicate at ¼ each dose which showed few number of females (8.5), galls (10.75), egg masses (7) and juveniles (400/1 Kg soil) that can be detected on root system and soil of tomato cv. 9065 FI in this study, respectively (Table 2).

Data in Table 3 shows the impact of either potassium silicate or calcium sulphate or moringa dry leaf powder alone or mixed as dual or triple or tetra with oxamyl comparing to oxamyl at the recommended dose on nitrogen (N) phosphorus (P), potassium (K), total phenol and total chlorophyll contents in leaves of tomato plant cv.9065 F1 infected with *M. incognita* under greenhouse conditions (25±3°C). It was evident that N, P and K concentration were obviously reduced by nematode infection. It is interesting to note that all tested treatments gave remarkable increase in N, P, K and total phenol concentrations exceeding that of nematode alone (Table 3). Among the single treatments, moringa dry leaf powder ranked first in increasing N, P, K and total phenol concentrations with values of 1.64, 0.370, 1.98 and 20.8%, followed by calcium sulphate and then potassium silicate, respectively.

In the meantime, among the binary treatments, moringa dry leaf powder + calcium sulphate at its half dose accomplished the highest concentrations of N (1.81%), P (0.386%), K (2.25%) and total phenol (557.2 mg/100 g) (16.1%), followed by ½ (calcium sulphate + potassium silicate) at its half dose and then ½ (moringa dry leaf+ potassium silicate), respectively.

Moreover, an obvious synergetic action occurred in increasing N, P and K concentrations only when oxamyl was added to potassium silicate plus calcium sulphate at ⅓ dose each as triple treatment with values of 2.25, 0.431 and 2.69% and low value of total phenol percent (9.2%), respectively. However, a reasonable concentration of N, P and K was recorded by mixing the three tested components that is potassium silicate, calcium sulphate and moringa dry leaf powder together that exceeded the single treatment at 1/3 dose each with values of 1.93, 0.407 and 2.43% with low value of total phenol (3.4%). It is worthy to note that high synergistic action was evident with tetra application in increasing concentrations of N (2.37%), P (0.446%), K (2.79%) and total phenol (23.7%) that exceeded all treatments in this respect even that the plant free of nematode and any treatment. Moreover, the tetra treatment that contained all items plus oxamyl at 1/4 dose each ranked first in increasing N, P, K and total phenol comparing to nematode alone (Table 3).

It is worthy to note that the tetra application [1⁄4(calcium sulphate + potassium silicate + moringa dry leaf + oxamyl)], gave the highest percentage increase of total phenol (23.7%) and the highest reduction value of total chlorophyll content (18.1%) comparing to nematode alone (Table 3).

Concerning total chlorophyll content in leaves of tomato plants infected with *M. incognita* under the tested treatments, the single ones showed the high reduction percentage values that ranged between 14.1 to 16.6% which was more than other double, triple, oxamyl and plant free of nematode and any treatments, whilst the tetra treatment accomplished its highest reduction percentage (18.1%) comparing to nematode alone (Table 3).

Regarding the C/N ratio, it ranged from 21.34:1 to 22.19:1; 19.83:1 to 20.07:1 and 16.75:1 to 18.91:1 and 16.03:1 for single, dual, triple and tetra treatments, respectively. The C/N ratio of the tetra treatment gave the least value of this item (16.03:1) and oxamyl had 19.67:1 and 22.88:1 for nematode alone (Table 3).

Using calcium sulphat or potassium silicate or moringa dry leaf powder either alone or mixed as dual or triple or tetra treatments along with oxamyl at ½ or ⅓ or ¼ doses compared to oxamyl at the recommended dose on reproduction and development of *M. incognita* play an important role in diminishing root knot nematode on tomato plants and its reproduction factors (RF), where the double, triple and tetra treatments tested gave better results than single ones did. In the meantime, among the single applications, moringa dry leaf powder accomplished the highest percentage reduction of nematode parameters. Results of this work are supported by the findings of Sowley et al. (2014) who said that the infestation of root-knot nematodes were significantly lower in the moringa leaf powder-treated plots than the control. They added although significant differences were not observed in all the parameters evaluated among the moringa leaf powder treatments, sweet pepper plants treated with 80 g/L of moringa leaf powder per plot recorded the highest mean value of plant height, number of leaves, number of fruits per plant, fruit weight per plant total yield per plot and the thickest plant girth. Similarly, the sweet pepper plants treated with 80 g/L of moringa leaf powder had the lowest infection index (root gall) and nematode population. Application of moringa leaf powder at 40, 60 and 80 g/L increased sweet pepper yield and decreased nematode population confirming their potential in management of root-knot nematodes.

Claudius-Cole et al. (2010) recorded that *Moringa oleifera*, is widely used in water treatment as a good inhibitor of nematode egg hatch and juvenile survival. It was also effective in reducing nematode population in plants with a subsequent increase in plant growth and yield. Guzman (1984) found that water extracts of moringa leaves were to be as toxic to *M. incognita* as standard pesticides. The nematicidal effect of the tested materials may possibly be attributed to their high contents 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 positive increments values of N, P and K was correlated within any tested single, dual, triple and tetra applications of such materials along with oxamyl added, a situation that is supported by the findings of El-Sherif and Ismail (2009) who reported that ½ (B.t. + Ox) treatment exceeded that of either B.t. or ox amyl alone in values of N, P and K cons. in soybean plant infected with *M. incognita*. Meanwhile, the same trend occurred in the case of total phenol content on tomato plant infected with *M. incognita*, in this study and is supported by the findings of Kesba (2010) in respect to treatments of humic and fulvic acids that significantly improved the levels of non-enzymatic antioxidants molecules including total phenol in the roots infected grape rootstocks roots with *Rotylenchulus reniformis* or *Tylenchulus semipenetrans*, especially at the higher concentration of the organic acids. Increasing levels of total phenol may serve as defense compounds against pathogens (Kosuge, 1969). However, there were negative correlations between the single and concomitant applications of the tested components regarding the reduction of total chlorophyll content in the present study compared to nematode alone, a condition which is in agreement with those reported by El-Sherif and Ismail (2009) in respect to *M. incognita* infecting soybean plant.

Undoubtedly, the abiotic factors used in the present investigation as tool for the integrated control of *M. incognita* on tomato plants through the tested one or two or more components along with oxamyl as systemic nematicide at their proper doses succeeded to generate a sort of inducing resistance in a susceptible host plant against such pathogenic nematode, since tetra application in this work showed very low eggmasses (7.0) or females (8.5) on root system of tomato plant infected with *M. incognita*. Moreover, the nematicidal activities of the tested materials as biofertilizers as well as their thermo stable toxin in the integrated management of *M. incognita* on tomato plants along with oxamyl can be varied from component to another. These variations may be attributed to the differences in the chemical nature, compound present in these tested material and method of application used. The safety of such materials and its low cost is one of its advantages. These observations agreed with those of Oteifa (1953) who stated that root-gall nematode damage on cabbage increased with amounts of potassium available to the host plant because potassium increased the rate of reproduction of nematode. Huber (1991) also recorded that root gall nematode damage on lima bean decreased with increased ammonium supplied to the plant.

In addition, these results are also in agreement with those reported by Oteifa and El-Gindi (1962) in respect to plants with fewer root galls that would translocate more nutrients to vegetative organs than heavily galled roots. Regarding the C/N ratio, it ranged between 21.34 to 22.19, 19.83 to 18.26, 16.03 for single, dual, triple and tetra treatments vs. 22.88 for nematode alone, respectively, and agrees with the findings of Miller and Donahue (1990) who reported that organic residues with C/N ratio 20:1 or narrow have sufficient nitrogen to supply the decomposing microorganisms and also to release for plant use.

However, more research is needed to be done in this direction under field conditions before drawing such recommendations for new trend safe and effective integrated nematode management alternative (s) based on the combined use of natural and synthetic compounds.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENT

This work is a part of M.Sc. thesis of Saadoon M. Saadoon (Post graduate student) and was supported by a grant from the government of Iraq. The authors thank the staff members of the Nematology Research Unit (NERU), Agricultural Zoology Department, Faculty of Agriculture, Mansoura University, Egypt for their encouragement to carried-out this work.

REFERENCES

AOAC (1980). Association of official Agriculture Chemist, official methods of Analysis. 13th ed. Washington, D.C.

AL-Ghnam AH (2011). Integrated management of citrus nematode, Tylenchulus semipenetrans. Ph. D. Agric. Zool. Dept. Fac. Of Agric. Mansoura Univ. 155p.

Byrd DW, Kirkpatrick T, Barker K (1983). An improved technique for clearing and staining plant tissues for detection nematodes. J. Nematol.15(3):142-143.

Claudius-Cole AO, Aminu AE, Fawole B (2010). Evaluation of plant extracts in the management of root-knot nematode Meloidogyne incognita on cowpea [Vigna unguiculata (L) Walp]. J. Mycopath 8(2):53-60.

Duncan DB (1955). Multiple range and multiple, F-test. Biometrics 11:1-42. http://dx.doi.org/10.2307/3001478

El-Sherif AG, Ismail AF (2009). Integrated management of Meloidogyne incognita infecting soybean by certain organic amendments, Bacillus thuringiensis, Trichoderma harzianum and Oxamyl with reference to NPK and total chlorophyll status. Plant Pathol. J. 8(4):159-164. http://dx.doi.org/10.3923/ppj.2009.159.164

Gomez KA, Gomez AA (1984). Statistical procedures for Agricultural Research. 2nd Ed., John Wiley &Sons: Inc., New York.

Goodey JB (1957). Laboratory methods for work with plant and soil nematodes. Tech. Bull. No. 2. Min. Agric. Fish Ed. London, 47 p.

Goodwin TW (1965). In: Goodwin, T.W. (Ed.), Chemistry and Biochemistry of Plant Pigments. Academic Press, London, UK.

Guetsky R, Shteinberg D, Elad Y, Fischer E, Dinoor A (2002). Improving biological control by combining biological agents each with several mechanisms of disease suppression. Phytopathology 12: 976-985. http://dx.doi.org/10.1094/PHYTO.2002.92.9.976

Guzman RS (1984). Toxicity screening of various plant extracts, Anthocephalus chinensis (Lamb) Rich ex Walp, Desmodium gangeticum (Linn) DC, Artemisia vulgaris Linn, Eichornia crassipes (Mart) Solms, Leucaena leucocephala (Lam) de Wit, Allium cepa Linn, Allium sativum Linn and Moringa oleifera Lam] against Meloidogyne incognita Chitwood and Radopholus similis Cobb and characterization of their nematicidal components. Ph.D Thesis University of the Philippines at Los Banos, College, Laguna Place College, Laguna (Philippines). 197 pp.

Hojat Jalali AA, Segera R, Coosemans I (1998). Biocontrol of Heterodera schachtii using combinations of the sterile fungus, StFcHI-1, Embellisia chlamydospora and Verticillium chlamydosporium. Nematologica 44:345-355. http://dx.doi.org/10.1163/005525998X00025

Huber DM (1991). The use of fertilizers and organic amendments in the control of plant disease. In: Pimentel D, Hanson AA (ed) Handbook of pest management in agriculture. Flrida: CRC. pp. 357-394.

Kaur C, Kapoor HC (2001). Antioxidants in fruits and vegetables in the millennium's health. Int. J. Food Sci. Technol. 36:703-725. http://dx.doi.org/10.1046/j.1365-2621.2001.00513.x

Kesba HH (2010). Biochemical alterations in grape infected with three phytonematode species with emphasis on root-knot nematode control. Egyptian J. Agronematol. 9(2):116-131.

Knobloch K, Pauli A, Iberl N, Weigand N, Weis HM (1989). Antibacterial and antifungal properties of essential oil components. J. Essential Oil Res. 1:119-128. http://dx.doi.org/10.1080/10412905.1989.9697767

Kosuge T (1969). The role of phenols in host response to infection. Annual Review Phytopathol. 7:195-222. http://dx.doi.org/10.1146/annurev.py.07.090169.001211

Miller RW, Donahue RL (1990). Organic matter and container media. Soils: An introduction to soils and plant growth. 6th (Ed). Prentice Hall, Inc; Englewood Cliffs, N.J. U.S.A.181-225.

Oka Y, Necar S, Putievesky E, Ravid V, Yaniv Z, Spiegel Y (2000). Nematicidal activity of essential oils and their components against the root-knot nematode. J. Phytopathol. 90(7):710-715. http://dx.doi.org/10.1094/PHYTO.2000.90.7.710

Oteifa BA (1953). Development of the root-knot nematode, Meloidogyne spp. as affected by potassium nutrition of the host. Phytopathology 43:171-174.

Oteifa BA, El-Gindi DM (1962). Influence of parasitic duration of Meloidogyne javanica on host nutrient uptake. J. Nematol. 8:200-216.

Pierson EA, Weller DM (1994). Use the mixtures of Fluorescent pseudomonas to suppress take-all and improve the growth of wheat. Phytopathology 84:940-947. http://dx.doi.org/10.1094/Phyto-84-940

Siddiqui ZA, Mahmood I (1993). Biological control of Meloidogyne incognita race3 and Macrophomina phaseolina by Paecilomyces lilacinus alone and in combination on chickpea. Fundam Appt. Nematol. 16:215-218.

Sowley ENK, Kankam F, Adomako J (2014). Management of root-knot nematode (Meloidogyne spp.) on sweet pepper Capsicum annuum

L.) with moringa (Moringa oleifera Lam.) leaf powder. Achives Phytopathol. Plant Prot. 47(13):1531-1538. http://dx.doi.org/10.1080/03235408.2013.848710

Taylor AL, Sasser JN (1978). Biology, identification and control of root-knot nematodes (Meloidogyne species). Raleigh, NC: North Carolina State University Graphics.