

Management of *Meloidogyne incognita* infecting eggplant by ginger and clove grinded seeds singly integrated with two bio-agents under greenhouse conditions

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ABSTRACT

A greenhouse experiment was conducted to assess the potential of ginger and clove grinded seeds singly or integrated with two bio-agents namely bio-arc (*Bacillus megaterium*), bio-zed (*Trichoderma album*), under the stress of *Meloidogyne incognita* infecting eggplants cv. Black king Japanese (27±5 °C). Results indicated that integration of two components of such bio-agents gave better results in eggplant growth parameters than did single ones or dual in concomitant with bio-arc and bio-zed and oxamyl induced significant ($P \leq 0.05$) and maximum improvement in total plant fresh weight and shoot dry weight. The greatest suppression in nematodes population (95.7%), root galling (83.0%), and number of egg masses (100%) was also sustained at the soil amended with ginger +clove + bio-arc + bio-zed+ oxamyl since incorporation of such organic materials into soil might enhance *B. megaterium* and *Trichoderma album* activity that initiates antibiotics towards nematode population. Meanwhile, the dual applications of (Ginger+ bio-arc) surpassed other applications in percentage increase values of N, P, and K as well as total chlorophyll that averaged 70.56, 83.25, 95.45, and 85.73 %, respectively, followed by the dual application with two compositae pants (Clove+ bio-zed)

with values percentage increase that amounted to 67.74, 76.55, 84.34, and 76.69% for the same parameters.

Introduction

Root-knot nematode (RKN), *Meloidogyne incognita* seems to be well for damaging vegetable crops severely in tropical and subtropical regions, making it one of the most harmful phytopathogens in the entire world (Trudgill and Blok, 2001 & Sikora and Fernandez, 2005). Biological control and other eco-friendly disease control measures have gained increasing interest among researchers after the environmental restrictions on nematicidal use for controlling plant parasitic nematodes. Incorporation of plant parts/extracts into the soil alone or with bio-control agents have been suggested as an alternative, safe and effective control method for the management of plant parasitic nematodes (Siddiqui and Alam, 1985). Ginger (*Zingiber officinale* Roscoe) is a biennial plant, bearing sessile aromatic tubers. It has been used in British as well as Indian Pharmacopoeia as a hot remedy for various diseases. It is also reported to cause motor paralysis of central origin after subcutaneous injection in frog (Watt and Breyer-Brandwijk, 1962). Plant-derived compounds are a potential source for such nematicides, and essential oils are one type of botanical product that has been studied for management of weeds, arthropods, nematodes, and microbial pathogens. These oils are active against a number of pest organisms, are removed fairly rapidly from the environment, and often have low toxicity to mammals. The clove oil used in our research was prepared in an aqueous solution with lecithin from soybean (*Glycine max*) and a nonionic surfactant. In micro-well assays, this formulation was toxic to eggs and second-stage juveniles (J2) of RKN (Meyer *et al.*, 2008). Therefore, environmentally friendly alternatives are required for nematode control. Biological control is one possible safe alternative to pesticides for disease management and is likely to be free from toxic residual effects. There are numerous microbial antagonists of root-knot nematodes and their application resulting in significant decrease in the nematode populations (Khan, 2007). The approach of combining biological control agents to many various soil borne pathogens including plant parasitic nematodes has been investigated extensively (Hojat *et al.*, 1998, Duponnis *et al.*, 1999 and Al-Ghnam, 2011). In 2004, Siddiqui and Shaukat reported that the using of *Trichoderma harzianum* plus *Pseudomonas fluorescens* caused maximum reduction in *M. javanica* population densities on tomato roots in un-sterilizing sandy soil. *Bacillus megaterium* (Bio-arc) and *Trichoderma album* (Biozeid) are among the most used bioagents against plant nematodes (El-Sherif *et al.*, 2011). However, integrated nematode management using several control techniques as abiotic factors and microbial agents i.e. bacteria and fungi as biotic factors

with minimal use of nematicides received great attention among the nematologists providing effective control measures against the target nematode, keep the nematode low at the safe level and avoiding environmental pollution. Therefore, the objectives of this study were conducted to determine the potential of ginger and clove grinded seeds singly or integrated with two bio-agents namely bio-arc (*Bacillus megaterium*), bio-zed (*Trichoderma album*), on the *M. incognita* infecting eggplants cv. Black king Japanese under greenhouse conditions (27 ± 5 °C).

Materials and Methods

Preparation of nematodes inocula:

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

Nematicide:

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

Tested bio-agents

(A) Bio-arc: (*Bacillus megaterium*).

It is a local bactericide which contains 6% active ingredient with 25 millions bacterial cells per one gram of the compound and enrolled by the Ministry of Egyptian Agriculture Under No.1087.

(B) Bio-zeid: (*Trichoderma album*).

It is a local fungicide which contains 2.5% active ingredient with ten million spores per one gram of the compound and enrolled by the Ministry of Egyptian Agriculture Under No.1088.

Experimental design:

Integrated studies were carried out between two compositae grinded seed powders i.e., ginger and clove as single or plus bio arc and bio zed as double applications compared with oxamyl at the recommended dose against the root-knot nematode *M. incognita* infecting eggplant cv. Black king Japanese under greenhouse conditions (27 ± 5 °C). Forty-four plastic pots (13cm-diam). containing 850 g steam sterilized sand loamy soil (1:1, v:v) with one eggplant seedling 30 days-old each was used in this study. On the same day after eggplant seedlings transplanting, 1000 J2 of *M.*

incognita were inoculated to forty seedlings each and left four seedlings (pots) without nematode to serve as a check. One week later, the tested materials were added to four seedlings each and mixed with soil. In the meantime, four seedlings, with nematode juveniles received oxamyl at the rate of 0.3 ml per pot (seedling) in addition other four seedlings (pots) with nematode only were served as control. Each treatment was replicated four times. The experiment was designed as follows:

1-N+ Bio-Arc (5g)

2-N+ Bio-Zed (5g)

3-N+ Ginger (5g)

4-N+ Clove (5g)

5- N+Bio-Arc (5g)+Ginger (5g)

6- N+Bio-Arc (5g) + Clove (5g)

7- N+Bio-Zed (5g) +Ginger (5g)

8- N+Bio-Zed (5g) + Clove (5g)

9- N+ Oxamyl

10- Plant free of N and Untreated and

11-N alone

Pots were irrigated with water as needed, treated horticulturally the same, and were arranged in a randomized complete block design in a greenhouse bench at $27\pm 5^{\circ}\text{C}$. During the period of the experiment, plants were protected against mites and insect pests. Eggplant plants were harvested after 45 days of nematode inoculation. Plant growth criteria as well as nematode parameters were estimated and recorded as previously mentioned. Data were subjected to statistical analysis and chemical analysis was determined as formerly mentioned.

Data Analysis:

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 grinded dried leaves of such treatment, wet, digested and their nitrogen (N), phosphorus (P), potassium (K) contents were determined according to kjeldahl methods (A.O.A.C, 1980).

Chlorophyll content:

Represent sample from the upper fourth leaf were obtained at 75 days after sowing and both chlorophyll a and b were determined mg\g F.W following equation that were used for the calculation pigments content according to Goodwin (1965).

Table (1): Mechanical analysis of soil:-

Mechanical analysis				
C. sand	F. sand	Silt	Clay	T. class
5.87	20.44	31.10	33.68	Clay loamy

Results and Discussion

Data in Tables (2) and Fig. (1) illustrated the efficacy of two compositae grinded seed powders i.e., *Zingiber officinale* and *Syzygium aromaticum* as single or plus bio arc and bio zed as double applications comparing with oxamyl at the recommended dose against the root-knot nematode *M. incognita* infecting eggplant cv. Black king Japanese under greenhouse conditions ($27\pm 5^{\circ}\text{C}$). Results indicated that all treatments tested were found to be more effective in improving plant growth parameters as compared with nematode alone.

Data indicated that concomitant treatments achieved better results than single treatments did (Table 2). The double application (*Zingiber officinale* + bio arc) significantly increased plant length, whole plant fresh weight, and shoot dry weight with values of increase percentage that were 96.95%, 88.88%, and 200.0%, respectively as compared with nematode alone. Meanwhile, bio zed plus the compositae grinded seed powders i.e., *Syzygium aromaticum* gave a moderate percentage increase in whole plant fresh weight, which amounted to 78.04, 79.36 and 188.8 % for plant length, whole plant fresh weight, number of leaves and shoot dry weight, respectively as compared to nematode alone.

It was evident that pots that received oxamyl accomplished considerable plant growth parameters, since the percentages of increase in total plant length, total plant fresh weight, as well as shoot dry weight averaged 99.32, 96.82, and 200.0% respectively. Moreover, plants free of nematodes that received none of the tested components show slight percentages of increase in total plant length (118.58%) and fresh weight (114.28%), as well as shoot dry weight (211.1%), respectively when compared to that of nematode alone.

Table (2): Impact of two compositae grinded seeds, ginger and clove singly or integrated with bio-arc and bio-zed comparing to oxamyl on growth of eggplant plants cv. Black king Japanese infected with *M. incognita* under greenhouse conditions ($27 \pm 5^\circ\text{C}$).

Treatments	Plant Growth Response									
	Length(cm)		Plant length (cm)	Inc. %	Fresh weight (g)		Plant F.wt (g)	Inc.%	Shoot Dry Wt. (g)	Inc. %
	Shoot	Root			Shoot	Root				
BioArc	25.3e	18.7a-d	44	48.64	7.4a-d	2.8d-f	10.2	61.90	1.5b	66.6
BioZed	20.0f	16.0b-d	36	21.62	5.8b-e	2.7e.g	8.5	34.92	1.3bc	44.4
Ginger	19.0f	15.7b-d	34.7	17.22	5.0c-e	2.5fg	7.5	19.04	1.2bc	33.3
Clove	16.7f	15.0cd	31.7	7.09	4.5de	2.3fg	6.8	7.93	1.1c	22.2
BioArc + Ginger	37.3ab	21.0a-c	58.3	96.95	8.3ab	3.6ab	11.9	88.88	2.7a	200.0
BioArc + Clove	33.0bc	19.7a-c	52.7	78.04	8.0a-c	3.3bc	11.3	79.36	2.6a	188.8
BioZed + Ginger	31.0cd	17.0b-d	48	62.16	6.7a-e	3.3bc	10	58.73	2.7a	200.0
BioZed + Clove	28.0de	16.7b-d	44.7	51.01	6.2b-e	3.1c-e	9.3	47.62	2.4a	166.6
Oxamyle	37.7ab	21.3ab	59	99.32	8.5ab	3.9a	12.4	96.82	2.7a	200.0
Plant free of N and Untreated	40.7a	24.0a	64.7	118.58	9.5a	4.0a	13.5	114.28	2.8a	211.1
Nematode only (N)	16.3f	13.3d	29.6	-----	4.1e	2.2g	6.3	-----	0.9c	----
LSD	4.9	6.2	-----	-----	3.0	0.8	-----	-----	0.4	-----

N= 1000 juveniles (J_2) of *M. incognita*.

$$** \text{ Increase \%} = \frac{\text{Treatment} - \text{N alone (Untreated)}}{\text{N alone (Untreated)}} \times 100$$

**Each value is the mean of four replicates. *Means in each column followed by the same letter (s) did not differ at $P < 0.05$ according to Duncan multiple- range test

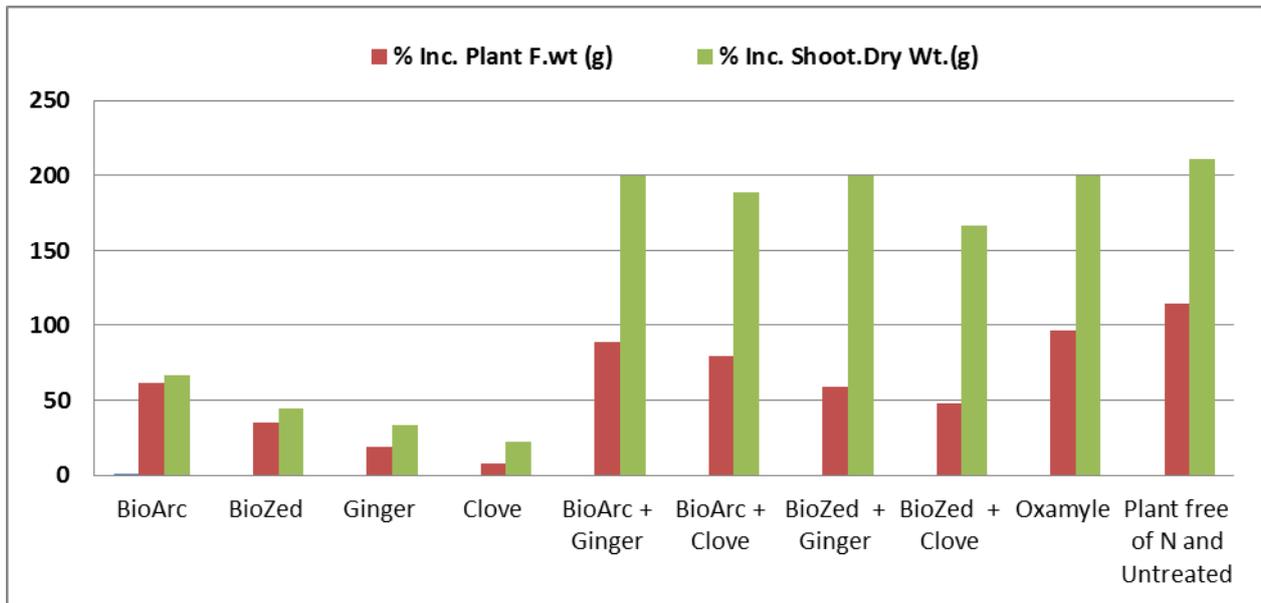


Fig. (1): Increase percent of whole plant fresh and shoot dry weights of eggplant plants infected with *M. incognita* treated by two compositae grinded seeds, ginger and clove singly or integrated with bio-arc and bio-zed comparing to oxamyl under greenhouse conditions (27 ± 5 °C).

Data in Table (3) showed the impact of two compositae grinded seeds, ginger, and clove singly or dual each application with two agents (bio arc and bio zed) compared to oxamyl at the recommended dose on the growth of eggplant plants infected with *M. incognita* in terms of reduction percentage of final nematode population on such host plant under greenhouse conditions ($27\pm 5^\circ\text{C}$). It was obvious that nematode population density and rate of nematode reproduction as well were significantly affected by the tested materials. All tested materials showed antagonistic potential against *Meloidogyne* spp. infecting eggplant. The integration of *B. megaterium* (bio-arc) and *Trichoderma album* (bio-zeid), with the ginger, and clove, in comparison with oxamyl induced systemic resistance towards the challenger *Meloidogyne* spp. in eggplant. Nematode population densities within 250 g soil and the number of females (1 g /root) were significantly suppressed with single and concomitant applications by a reduction percentage in the final nematode population ranging from 57.50 to 86.36% (Table 3). The greatest reduction in the nematode population was sustained by the application of Bio-arc (86.36%). Root galling (83.0%) and egg masses number (100.0%) by Bio-zeid treatment were significantly suppressed for such treatment. It was evident that pots that received oxamyl accomplished considerable plant growth parameters, since the percentages of increase in total plant length, total plant fresh weight, as well as shoot dry weight averaged 99.32, 96.82, and 200.0% respectively. Moreover, plants free of nematodes that received none of the tested components show slight percentages of increase in total plant length (118.58%)

and fresh weight (114.28%), as well as shoot dry weight (211.1%), respectively when compared to that of nematode alone.

Table (3): Development and reproduction of *M. incognita* infecting eggplant plants cv. Black king Japanese as affected by two compositae grinded seeds of ginger and clove singly or dual each application with bio-arc and bio-zed in comparison with Oxamyl under greenhouse conditions ($27 \pm 5^\circ\text{C}$).

Treatments	Nematode population in soil	Red. %	Rf*	No Galls	Red. %	RGI	No egg masses	Red. %	E.I.*
BioArc	590.0b	86.36	0.6	5.0	79.75	2.0	1.3b-d	69.76	1.3
BioZed	633.3b	66.07	0.6	7.0	71.65	2.0	1.3b-d	69.76	1.3
Ginger	793.3b	57.50	0.8	11.3	54.25	2.3	2.7b	37.21	2.0
Clove	730.0 b	60.87	0.7	9.0	63.56	2.3	1.7bc	60.46	1.6
BioArc +	413.3bc			2.0		1.3	0.3cd		0.3
Ginger		77.86	0.4		91.90			93.02	
BioArc +	340.0b			3.7		2.0	0.7cd		0.6
Clove		81.77	0.3		85.02			83.72	
BioZed +	390.0bc			2.7		1.3	0.0d	100.0	0.0
Ginger		79.09	0.4		89.06			=	
BioZed +	495.0bc			3.3		1.3	0.7cd		0.6
Clove		73.47	0.5		86.63			83.72	
Oxamyl	245.0bc	86.87	0.2	1.0	95.95	0.3	0.0d	100.0	0.0
Nematode only (N)	1866.6a	-----		24.7	-----	4.0	4.3a		2.0
			0.6					-----	
LSD	568.8	-----	-----	4.4	-----	----	1.4	-----	----

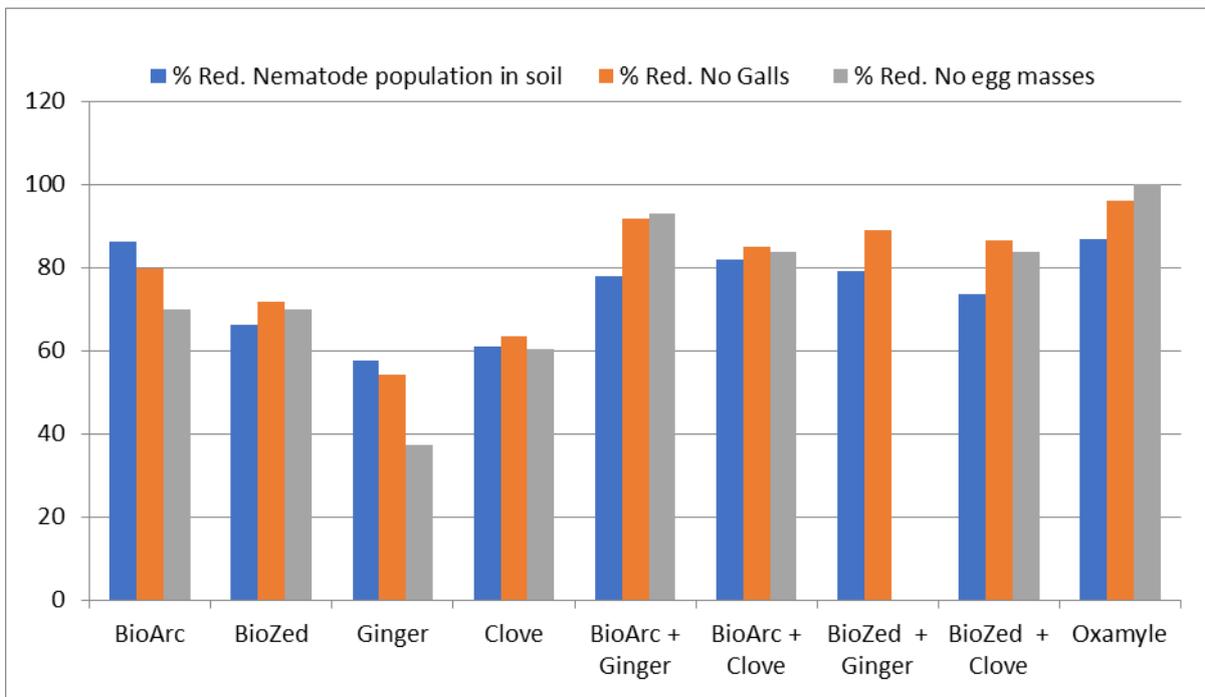


Fig. (2): % Reduction of final nematode population, number of galls and egg masses of *M. incognita* infecting eggplant plants cv. Black king Japanese as affected by two compositae grinded seeds of ginger and clove singly or dual with bio-arc and bio-zed in comparison with oxamyl at the recommended dose under greenhouse conditions ($27 \pm 5^\circ\text{C}$).

Data in Table (4) reveal the percentage increase or decrease of N, P, and K as well as total chlorophyll and phenol contents in leaves of eggplant plants cv. Black king Japanese infected with *M. incognita* under the stress of two compositae grinded seeds, ginger, and clove singly or integrated with bio arc and bio zed in comparison with Oxamyl under greenhouse conditions ($27 \pm 5^\circ\text{C}$). In general, all tested components significantly increased percentage values of eggplant leaves parameters i.e., N, P, K as well as total chlorophyll in the infected plants. Among the tested components, The dual applications of (Ginger+ bio-arc) surpassed other applications in percentage increase values of N, P, and K as well as total chlorophyll that averaged 70.56, 83.25, 95.45, and 85.73 %, respectively, followed by the dual application with two compositae pants (Clove+ bio-zed) with values percentage increase that amounted to 67.74, 76.55, 84.34, and 76.69% for the same parameters, respectively, compared to nematode alone. These results indicated that the integrated applications ranked first in percentage increase values of N, P, and K and total chlorophyll and phenol contents, followed by those of single one, whereas plants received a single application with two compositae pants Ginger or Clove had the lowest values in this respect with values of 30.24, 8.13, 0.50 and 1.27% & 12.90, 1.91, -14.34, and -0.56 % for N, P, and K and total chlorophyll

respectively as compared to nematode alone. Oxamyl as a systemic nematicide gave higher values of percentage increase of N (78.62%), P (90.90%), and K (101.0%) than such tested components with the lowest value of total chlorophyll that averaged (89.83%), respectively comparing to nematode alone (Table 4).

Table (4): N, P, and K concentrations and chlorophyll content in leaves, of eggplant plants cv. Black king Japanese infected with *M. incognita* under stress of two compositae grinded seeds, ginger and clove singly or dual integrated with bio-arc and bio-zed in comparison with Oxamyl under greenhouse conditions ($27 \pm 5^\circ\text{C}$).

Treatments	Chemical components									
	Leaves						Chlorophyll content m/g			
	N %	Inc. %	P %	Inc. %	K%	Inc. %	Chlo . A	Chlo . B	Tota l (A+ B)	Inc.%
BioArc	3.54	42.74	0.288	37.79	3.42	72.72	0.725	0.440	1.165	64.54
BioZed	3.44	38.71	0.235	12.44	2.13	7.57	0.588	0.385	0.973	37.42
Ginger	3.23	30.24	0.226	8.13	1.99	0.50	0.401	0.316	0.717	1.27
Clove	2.80	12.90	0.213	1.91	1.70	-14.34	0.401	0.303	0.704	-0.56
BioArc + Ginger	4.23	70.56	0.383	83.25	3.87	95.45	0.790	0.525	1.315	85.73
BioArc + Clove	4.16	67.74	0.369	76.55	3.65	84.34	0.761	0.490	1.251	76.69
BioZed + Ginger	3.85	55.24	0.324	55.02	3.51	77.27	0.763	0.486	1.249	76.41
BioZed + Clove	3.70	49.19	0.313	49.76	3.22	62.62	0.701	0.490	1.191	68.22
Oxamyl	4.43	78.62	0.399	90.90	3.98	101.0	0.795	0.549	1.344	89.83
Plant free of N and untreated	4.98	100.8	0.450	115.3	4.30	117.1	0.812	0.599	1.411	70.3
Nematode alone	2.48	-----	0.209	-----	1.98	-----	0.396	0.312	0.708	-----

Results indicated that integration of two components of such bio-agents gave better results in eggplant growth parameters than did single ones or dual in concomitant with bio-arc and bio-zed

and oxamyl induced significant ($P \leq 0.05$) and maximum improvement in total plant fresh weight and shoot dry weight. The greatest suppression in nematodes population (95.7%), root galling (83.0%), and number of egg masses (100%) was also sustained at the soil amended with ginger + clove + bio-arc + bio-zed+ oxamyl since incorporation of such organic materials into soil might enhance *B. megaterium* and *Trichoderma album* activity that initiates antibiotics towards nematode population. Results of this study support the findings of Mostafa *et al.* (2014) and El Destiny (2016) in respect of the microbial activity, i.e., *B. megaterium* in the soil is enhanced on the incorporation of organic matter that initiated antibiosis towards the nematode activity.

Padgham and Sikora (2007) reported that *B. megaterium* caused a repellence of *M. graminicola* from rice roots. Production of repellent substances or modification of the plant's exudates by the antagonistic bacteria were suggested as mechanisms for this effect (Sikora *et al.* 2007) plant length, whole plant fresh weight, number of leaves, and shoot dry weight, respectively as compared to nematode alone. Meanwhile, the dual applications of (Ginger+ bio-arc) surpassed other applications in percentage increase values of N, P, and K as well as total chlorophyll that averaged 70.56, 83.25, 95.45, and 85.73 %, respectively, followed by the dual application with two compositae plants (Clove+ bio-zed) with values percentage increase that amounted to 67.74, 76.55, 84.34, and 76.69% for the same parameters, a situation that agreed with those of Radwan, (1983) who reported that *B. megaterium* is a considerable microorganism capable of dissolving the unavailable phosphorus compounds in soil rendering them a variable for growing crops.

Increased phosphorous concentration may lead to reduction in the root-knot nematode population density on tomato plants. The present findings are also in agreement with those reported by Brown *et al.*, (1985) in respect to (*B. penetrans*) that also showed increased yield of tobacco and soybean with decreasing pathogenicity of *M. incognita*. Undoubtedly the usage of such tested bioagents i.e. Bio-arc, Bio-Zed solely or mixed with grinder or clove play the foremost role in reducing *M. incognita* penetration to roots in tested host plant and in turn its number of root galling, females and eggmasses where *B. megaterium* (Bio-arc) solely or mixed or as double biotic treatment represented the maximum reduction values. However, Di-Tera compound (*M. verrucaria*) ranked second to Bio-arc while Bio-zed (*T. album*) was in the intermediate position of both in reducing such tested nematode criteria in this study which agreed with the finding of Al-Ghnam (2011) in respect to Bio-arc, Bio-zed and Di-Tera treatments on controlling *T. semipenetrans* infecting sour-orange plants. Moreover, the role of tested compound alone or mixed with oxamyl at their half doses each in preventing *M. incognita* to penetrate root system due to the action of *M. verrucaria* as fungal for mutation acted as a contact nematicide. (Khalil *et al.* , 2010), in addition, many fungi including *Trichoderma* group are known to produce nematicidal or nematostatic compounds (Ank *et al.*, 1995;

Hallmann and Sikora , 1996; Chen *et al.*, 2000; Meyer *et al.*, 2000; El-Sherif and Ismail, 2009; and Al-Ghnam, 2011), a condition which may clarify its third position solely in diminishing nematode criteria within those of bio-arc treatments in this present work.

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