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Utilization of organic and bio fertilizers against root-knot nematode (*Meloidogyne incognita*) infecting faba bean (*Vicia faba* L.)

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Abstract

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Two experiments were conducted under greenhouse conditions to evaluate susceptibility of five cultivars of faba bean (*Vicia faba* L.) to root-knot nematode (*Meloidogyne incognita*). Also, the influences of *Rhizobium leguminosarium* (R), arbuscular mycorrhiza fungi (AMF), inoculation separately and in combinations, potassium humate and compost tea on the activity of *Meloidogyne incognita* in faba bean host plants and plant growth parameters were studied. The results indicated that nematode succeeded in developing and multiplying on almost all of tested cultivars of faba bean. According to rating scale based on damage index (DI) on nematode, Giza 40, Giza 716, Sakha 3 and Sakha 283 cultivars were considered as susceptible hosts to nematode infection, while Misr-1 cultivar was classified as a moderately resistant host. The tested organic and bio fertilizer treatments significantly reduced the number of galls/root system with 50.00-84.64% and reduced the final nematode population in soil (J2) by 77.5-91.3% compared to control 1 (nematode only). The highest reduction was achieved, when the soil was treated with potassium humate at the higher concentration (humate 2), then AMF treatment, which were not significantly different from the nematicide Vydate 24% SL. Also, all treatments significantly increased the dry weights of shoots and number of flowers, except in case of R (*Rhizobium leguminosarium)* with nematode or low concentration of potassium humate, and enhanced length of plants and leaf content of total mineral including N, P, K, compared with control 1. Present findings may promote organic and bio fertilizers and eco-friendly management of *M. incognita*.

Keywords: faba bean; *Meloidogyne incognita*; organic fertilizers; bio fertilizers

Introduction

Faba bean (*Vicia faba* L.) is a major grain legume widely cultivated in many countries for food and feed purposes (Sillero et al., 2010). It is one of the oldest legume crops grown in Egypt (Nassib et al., 1991). Egypt now is the largest importer country all over the world of faba bean; its annual requirement of half million tones accounts for over half of global imports (GRDC, 2014).

Root-knot nematode, *Meloidogyne* spp., is the major plant-parasitic nematode attacking many crops. The root-knot nematode species significantly reduce the yield of leguminous crops grown in infested soils (Montasser et al., 2017). *M. incognita*, one of the most economically important species of root-knot nematodes, adversely affects plant growth and yield causing an estimated $100 billion loss per year (Mukhtar *et al*., 2014). Soliman (2002) tested the susceptibility of five cultivars of broad bean against *M. incognita* and *M. javanica* under greenhouse conditions. He found that three cultivars (Giza 1, Giza 3 and Giza 716) were resistant to both nematode species, only one (Giza 402) was susceptible and one cultivar (Giza 463) was highly susceptible to both nematodes. Montasser et al. (2017) found that Giza 3, Giza 40, Misr 3 and Wady 1broad bean cultivars were classified as less susceptible hosts to root-knot nematode *M. incognita*, whereas Misr-1 cultivar was classified as moderately susceptible, but Giza-843 was regarded as a resistant host to nematode infection.

The most effective method of nematode disease control is the use of synthetic chemical nematicides. However, health hazards and the adverse effects of these chemicals on the beneficial non-target organisms and on the environment are serious constraints. Attention on environmental safety has led to the search of alternative strategies including the use of organic and bio-fertilizers as management strategy that can suppress nematode population and increase soil health and plant vigor

Bio-fertilizers, an alternative source of N, P and K fertilizers, especially rhizobia and arbuscular mycorrhizal fungi (AMF) in legume symbiosis, are a promising technology (Abd-Alla et al., 2014). Rhizobium forms an endosymbiotic nitrogen-fixing association with roots of legumes and it can also act as a biocontrol agent on root-knot nematodes exhibiting the capacity to colonize plant roots and the nematode galls (Siddiqui & Akhtar, 2009; Tabatabaei & Saeedizadeh, 2017).

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil organisms that can form mutualistic associations with the roots of the majority of vascular plant species (Linderman, 1988). Symbiotic associations between plants and AMF have beneficial effects on plants, ensuring proper absorption of nutrients, especially phosphorus (Sharma et al., 2007). Many researchers have reported the effectiveness of AMF in the control of root-knot nematodes. (Udo et al., 2013; Akram et al., 2016; Tehabi et al., 2016).

Humate and compost extracts as organic substances are among the means available to achieve sustainability in agricultural production. They play a vital role because of their beneficial effects on physical, chemical and biological characteristics of soil (Afifi, 2010). Compounds of humic acid have been recorded as effective against plant-parasitic nematodes (El-Mougy et al., 2013; Tolba & Mohamed, 2016). Many studies reported that nematode populations were greatly suppressed after compost application (Meyer et al., 2011; Abdel-Bary, 2014; Ravindra et al., 2014). Compost performed as an amendment for reducing populations of *Meloidogyne incognita* and increasing plant vigor.

The aim of this investigation was to study the effects of some organic and bio fertilizer products to control root-knot nematode *Meloidogyne incognita* on faba bean plants.

Materials and Methods

Source of experimental materials

Seeds of faba bean were obtained from Legume Crops Research Department, Sakha Agricultural Research Station. The bio-fertilizer, Okaden that contains (*Rhizobium leguminosarium* 1×10^9 cfu/g), and compost tea were obtained from Soil, Water and Environment Research Institute, Unit of Bio-fertilizers Production, Agricultural Research Station, Sakha, Egypt, the bio and chemical properties of compost tea were determined (Table 1). Potassium Humate (13%humic acid) and the bio-fertilizer mycorrhizeen having three AMF genera (Glomus, Gigaspora and Acaelspora) were obtained from Soil, Water and Environment Research Institute, Unit of Bio-fertilizers Production, Giza, Egypt.

Table 1. Bio and chemical properties analysis of compost used in the experiments

Item	Analysis value
N (%)	1.9
P (%)	0.59
K (%)	0.75
O.M (%)	20.2
C/N ratio	15.4 : 1
Ash (%)	19.7
pH	7.6
EC (dSm^{-1})	4.3
Microbial activity (mg CO_2/100 g soil/day)	98.7
Total number of fungi per gram of compost (Log 10)	6.8
Total number of bacteria per gram of compost (Log 10)	7.1

Nematode culture

The inoculum required of *M. incognita* was obtained from stock pure culture maintained and propagated on tomato cv. Super strain B in greenhouse at the Department of plant Pathology, Sakha Agricultural Research Station.

Susceptibility of faba bean cultivars to *Meloidogyne incognita* infection under greenhouse conditions

Five faba bean cultivars – Giza40, Giza716, Misr1, Sakha 3 and Sakha 283 were tested for infection of *Meloidogyne incognita* at the greenhouse of Sakha Agricultural Research Station. In 2015/2016 season, faba bean seeds were sown in 30 cm diameter pots filled with a mixture of clay and sand soil (2:1, v:v). Two weeks after sowing, the plants were thinned into two seedlings per pot. Then, each pot was inoculated with approximately 5000 eggs and newly hatching second stage juveniles of *M. incognita* per plant. The inoculum was put inside of three holes in the soil, around the base of stems, and then the pots were irrigated. Each cultivar was represented by four pots. The uninoculated pots served as a check. All pots were arranged in the greenhouse in a randomized block design, and kept at 20±5°C. Sixty days after inoculation, the faba bean plants were uprooted, and the roots were washed with a gently stream of water. Then, the having nematode galls were rated on 1-9 scale of gall index (GI), according to Sharma et al. (1994) as follows: 1 = no galls , 2 = 1 to 5 galls, 3 = 6 to 10 galls, 4 = 11 to 20 galls, 5 = 21 to 30 galls, 6=31 to 50 galls, 7 = 51 to 70 galls, 8 = 71 to 100 galls, and 9 = >100 galls per root system. Gall size (GS) and percent galls area (GA) are also, rated on a 1-9 scale. For GS: 1 =no galls, 3 = very small galls (about 10% increase in root area at the galled region over non-galled normal root area), 5 = small galls (about 30% increase), 7 = medium galls (about 31 to 50% increase), and 9 = big galls (about 51 to 100% increase). For GA: 1 = no galls, 3 = 1 to 10% root area galled, 5 = 11 to 30 % root area galled,7 = 31 to 50% root area galled, and 9 = >50% root area galled (Sharma et al., 1994). A damage index (DI) is calculated by dividing the sum of GI, GS, and GA by 3 for each replicate (Sharma et al., 1994). Based on DI, the host susceptibility (designation of resistance) of each plant variety is determined according to the following scheme: plants with DI = 1 is designated as highly resistant; DI = 2 to 3, resistant; DI = 4 to 5, moderately resistant; DI = 6 to 7, susceptible; DI = 8 to 9, highly susceptible (Sharma et al*.,* 1994).

Effect of some organic and bio-fertilizers against root-knot nematode (*M. incognita*) infecting faba bean plants under greenhouse conditions

This experiment was conducted in 2016/2017 season to determine the efficiency of rhizobia (R) at the rate of 2 mg/pot and arbuscular mycorrhiza fungi (AMF) at the rate of 3 g/pot as bio fertilizers with recommended doses. Also, potassium humate (13% humic acid) and compost tea were used as organic fertilizers against root-knot nematode (*M. incognita*) infecting faba bean plants.

Experimental treatments

The pots were formulated and planted with seeds of Sakha 3 cultivar and inoculated with nematode, as above, after that treatments each with four replicates were arranged in a randomized block design and maintained in the greenhouse at 20±5°C. Plants were treated as follows:

– ***With M. incognita***: *Rhizobium leguminosarium* (R), Arbuscular mycorrhiza fungi (AMF), R+AMF, Humate 1 (0.1 g/pot), Humate 2 (0.2 g/pot), Compost 1 (0.5 ml/pot), Compost 2 (1.0 ml/pot),Vydate (Oxamyl) SL. 24% with recommended dose at the rate of 0.01 ml/pot and nematode alone (control 1). Potassium humate and compost tea treatments were added three times at 15 day intervals.

– ***Without M. incognita***: *Rhizobium leguminosarium* (R), Arbuscular mycorrhiza fungi (AMF), R+AMF and non-infested pots (control 2).

After two months, plants were uprooted and number of galls, final nematode population in 250 cm³ of soil, bacterial nodulation, mycorrhizal colonization, fresh and dry weights of shoots, length of plants, number of flowers and mineral elements (N, P, K) were determined.

Assessment of mycorrhizal colonization

The percent of mycorrhizal colonization in root was calculated by the gridine intersect method (Govannetti & Mosse, 1980) after staining with ink and vinegas (Vierheilig et al., 1988).

$$\text{Mycorrhizal colonization (\%)} = \frac{\text{Total number of root segments colonized}}{\text{Total number of root segments studied}} \times 100$$

Mineral solute analysis

For nitrogen (N), phosphorus (P) and potassium (K) analysis, shoot samples were washed by distilled water and dried in an oven at 70°C for 48 h, ground, mixed and wet digested using hot sulfuric acid with repeated additions of 30% hydrogen peroxide (H_2O_2) as described by Wolf (1982) and analyzed as follows: nitrogen content was determined by micro-Kjeldahl method (Jackson, 1967), phosphorus was determined using hydroquinine method (Snell & Snell, 1967), potassium content was determined using flame photometer (Jackson, 1967).

Statistical analysis

Data collected were statistically analyzed using randomized block design. Averages were compared according to Duncan's multiple range test (Duncan, 1955). Analysis was performed using the computer program (ASSISTAT).

Results

Susceptibility of faba bean cultivars to *Meloidogyne incognita* under greenhouse conditions

According to rating scale, based on damage index (DI), four tested cultivars (Table 2) were susceptible to *M. incognita*, with Sakha 3 being the most susceptible (6.8). However, only Misr-1 cultivar (4.8 DI) could be considered as moderately resistant.

Effect of treatments on nematode

Data in Table 3 show that all treatments significantly reduced the number of galls on root and final nematode population (J_2) in the soil. The nematicide Vydate

Table 2. Susceptibility of faba bean cultivars to root-knot nematode *Meloidogyne incognita* infection

Cultivar	Parameter					
	No. of galls/ root system	Gall index (GI)	Gall size (GS)	Percent gall area (GA)	Damage index (DI)	Host Type
Giza40	40.1ab	5.3	6.0	9.0	6.7	S
Giza 716	55.0ab	6.5	5.6	8.0	6.7	S
Misr 1	25.0c	4.6	5.0	5.0	4.8	MR
Sakha 3	60.0a	6.6	5.3	8.6	6.8	S
Sakha 283	32.5bc	4.7	6.0	7.5	6.0	S

MR – moderately resistant, S – susceptible

achieved the highest reduction in galls (88.8%). However, AMF+nematode, R+AMF+nematode, Humate 2 induced insignificant differences from the Vydate concerning number of galls achieving 84.62%, 82.63% and 84.62% reduction, respectively. Humate 2 treatment achieved the highest reduction in nematode population then AMF+nematode, compost 2, R+AMF+nematode and Humate 1. The corresponding values of J_2 reduction were 91.3%, 90.0%, 84.7%, 82.8% and 82.5%, respectively, these treatments induced insignificant differences from the Vydate concerning number of J_2 (90.6% reduction).

Nodulation of rhizobia

The experiment revealed that number of nodules of faba bean plants significantly increased on treatments with organic and bio-fertilizers, especially, that inoculated with *Rhizobium leguminosasum* than the uninoculated treatments, compared with control 1 and control 2. It is very interesting to note that the nodulation of rhizobia was decreased, when soil treating with nematicide, which was significantly different from control 1 (Table 3). The highest nodulation was recorded in the soil inoculated with R alone.

Mycorrhizal colonization

Data in Table 3 show that the maximum percent of mycorrhizal colonization (92.6%) was obtained when the infested with nematode soil was treated with AMF then R+AMF (78.6%), which are significantly different from R+AMF with nematode treating (76.8%). On the other hand, AMF treatment achieved the minimum mycorrhizal colonization (64.4%). The higher proportion of mycorrhizal colonization was consistent across mycorrhizal structure such as arbuscules, vesicles and external mycelium (Fig. 1).

Table 3. Effects of organic and bio fertilizers on root-galling, nematode final population of (*Meloidogyne incognita*), mycorrhizal colonization and bacterial nodulation on faba bean (*Vicia faba*) plants, under greenhouse conditions

Treatments	Gall index (GI)	Galls/root system		J_2/250 cm^3 of soil		Mycorrhizal colonization (%)	No. of bacterial nodules
		No.	Reduction%	No.	Reduction%		
With *M. incognita*							
R	4.0	11.5c	82.3	16.8b	79.1	0.0	18.3bc
AMF	4.0	10.0e	84.6	8.0c	90.0	92.6a	23.8ab
R+AMF	4.0	11.3e	82.7	13.8bc	82.8	76.8b	22.8ab
Humate 1	5.8	32.5b	50.0	14.0bc	82.5	0.0	17.0cd
Humate 2	3.0	10.0e	84.6	7.0c	91.3	0.0	19.5bc
Compost 1	5.0	26.0c	60.0	18.0b	77.5	0.0	17.5bc
Compost 2	4.5	21.3d	67.3	12.3bc	84.7	0.0	19.3bc
Vydate 24% SL.	2.5	7.3e	88.8	7.5c	90.6	0.0	13.0ef
Nematode alone (control 1)	7.0	65.0a	0.0	80.0a	0.0	0.0	11.8f
Without *M. incognita*							
R	0.0	0.0	0.0	0.0	0.00	0.00	27.5a
AMF	0.0	0.0	0.0	0.0	0.00	64.4c	20.0bc
R+AMF	0.0	0.0	0.0	0.0	0.00	78.6b	16.8cd
Plant alone (control 2)	0.0	0.0	0.0	0.0	0.0	0.0	14.3de

Means values followed by a common letter(s) are not significantly different at the 5% level by DMRT;
R: *Rhizobium leguminosarum*, AMF: Arbuscular mycorrhizal fungi

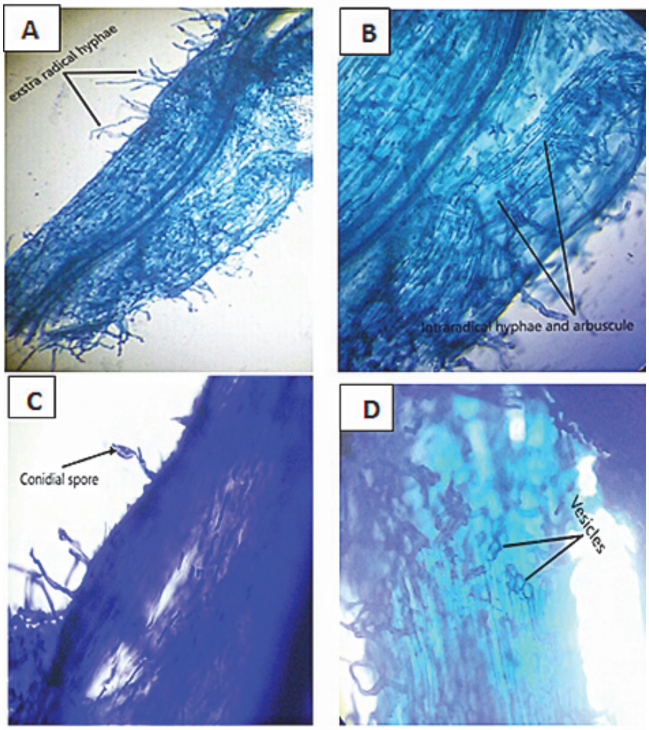

Fig. 1. (A-D) Light micrographs of arbuscular mycorrhizal fungi colonized roots of faba bean plants infected with *M. incognita*: (*A*) extra radical hyphae, (*B*) intra radical hyphae and arbuscule, (*C*) conidial spore, (*D*) vesicles

Effect of treatments on plant growth parameters

Data analysis of plant growth parameters indicated that all treatments achieved significant increases in fresh weight of shoots compared to control 1 (nematode alone), except the treatments with R inoculation either with or without nematode and the low concentrations of humate or compost (Table 4). The greatest increase (26.8 g) was obtained in the treatment of AMF then humate 2 treatment (22.89 g). Also, data in Table 4 show that all treatments significantly increased dry weight of shoots and number of flowers, except the treatment with nematode either R or humate 1 compared with control 1. The highest increasing in dry weight of shoot was achieved, when the soil was treated with compost 2 (4.1 g), humate 2 induced the highest number of flowers (26.5 flowers/plant). On the other hand, all treatments significantly enhanced plant heights (Table 4).The longest plants were achieved when the soil was treated with humate 2 (47.7 cm).

Mineral solute analysis

Fig. 2 demonstrates that all treatments significantly increased the leaf content of total mineral including N, P, K (%) compared with control 1. The treatments significantly increased N% of the leaf compared with control 1 (2.9) and control 2 (3.2). The highest increase in N% of the leaf was achieved when the soil was treated with Humate 2 (4.3), which is significantly different from R+N (4.1) or compost 2 (4.1). All treatments increased P% of the leaf compared with control 1 (0.8) and control 2 (1.0), except R+N or R+N+M treatment but the reduction was not significant. The high-

Table 4. Effects of organic and bio fertilizer on some plant parameters on faba bean (*Vicia faba*) plants infected with root-knot nematode *M. incognita* under greenhouse conditions

Treatments	Fresh weight of shoots (g)	Dry weight of shoots (g)	Plant height (cm)	Number of flowers/plant
With *M. incognita*				
R	14.5e	1.8c	43.3cd	18.0bc
AMF	26.8a	3.8a	47.5a	22.5ab
R+AMF	17.5de	2.8b	43.3cd	16.0d
Humate 1	18.6cd	1.8c	42.6d	17.3cd
Humate 2	22.8b	2.8b	47.7d	26.5a
Compost 1	17.1de	2.4b	41.3d	18.1bc
Compost 2	21.1bc	4.1a	45.3ab	22.5ab
Vydate 24% SL.	20.1bc	2.8b	43.8bc	24.5a
Nematode alone (control 1)	14.0e	1.4c	37.0e	15.6d
Without *M. incognita*				
R	17.1de	3.6b	47.3ab	23.5a
AMF	22.0b	3.7a	46.7ab	22.8ab
R+AMF	20.4bc	3.6a	45.5ab	24.5a
Plant alone (control 2)	23.1b	2.8b	45.25ab	21.5ab

Means values followed by a common letter(s) are not significantly different at the 5% level by DMRT; R: *Rhizobium leguminosarum*, AMF: Arbuscular mycorrhizal fungi est increase was 1.40, when the soil was treated with AMF or compost 1. Also, Fig. 2 shows that all treatments significantly increased K% of the leaf compared to control 1 (1.8) and control 2 (3.2), except when the soil was treated with R or AMF separately. The highest increase in K% of the leaf was achieved when the soil was treated with compost 2 (3.8) then R+N treating (3.7).

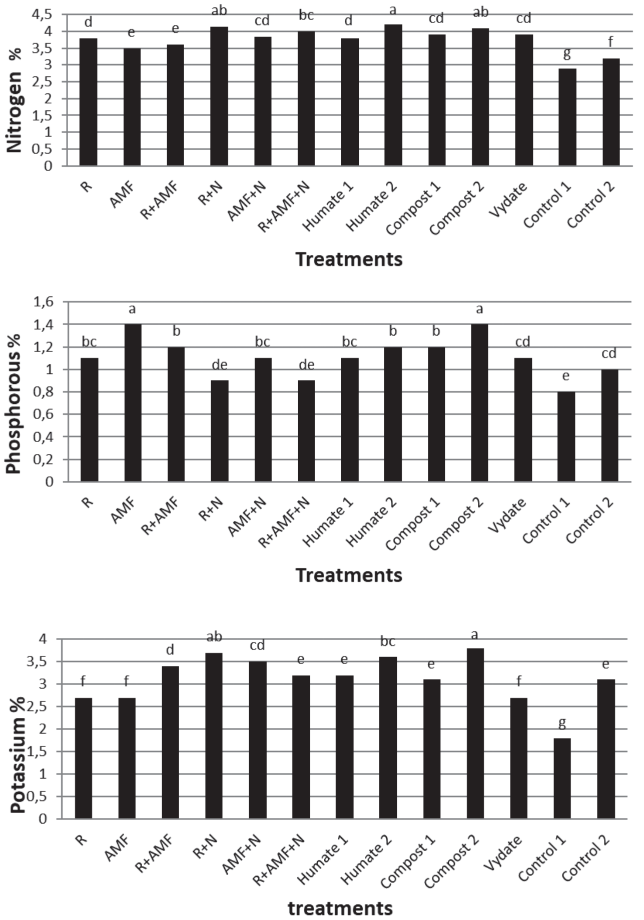

Fig. 2. Nitrogen (N), phosphorous (P) and potassium (K) percentages in shoot system of faba bean treated plants

Discussion

Our results indicate that all the tested cultivars of faba been were susceptible to root-knot nematode, *M. incognita*, except Misr-1 cultivar that was classified as moderately susceptible. Montasser et al. (2017) suggested that the behavior of root-knot nematode varied greatly according to the cultivar type and the nematode species that succeeds in developing and multiplying on almost all the broad bean cultivars. Also, the results show that the treatments either single or dual with R and AMF as bio-fertilizers can reduce *M. incognita* activity on faba bean plants and improve growth parameters of plants compared to the uninoculated ones. Similar results have been reported in *Vicia faba* (Abd-Alla et al., 2014; Tabatabaei & Saeedizadeh, 2017). Rhizobia and AMF have been reported to produce toxic metabolites inhibitory to many plant pathogens (MacGuidwin et al., 1985; Hemissi et al., 2011). There has been induced systemic resistance due to improved host nutrition, changes in the root morphology, histopathological and bio-chemical changes (Masadeh et al., 2004). Also, treating with R or AMF leads to improvements of plant vigor and growth by a symbiotic N_2 fixation and enhances the uptake of phosphorus and other nutrients (Hussy & Roncadori, 1982). Inoculation with *Rhizobium* alone can reduce *Meloidogyne* activity on faba bean plants while the nematode reduces the bacterial nodules on the roots of the plants, and consequently reduces nitrogen fixation in plants. These results are in agreement with Tabatabaei and Saeedizadeh(2017). On contrary, the nematode increases mycorrhizal colonization when the infested soil is treated with AMF may be to wounds caused by nematode due to increasing of burrowed AM fungi.

Results indicated that the co-inoculation with R+AMF in faba bean plants had a less effect on nematode activity and plant growth compared to the inoculation with nematode only. Many authors studied the interaction between R and AMF, the underlying mechanisms behind these association are not yet well understood (Chalk et al., 2006). On the other hand, the treatments of humate and compost were more effective in reducing the nematode (*M. incognit*a) and improved growth of plants, especially in the higher concentration. Several studies have reported the positive effect of humate and compost tea as plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases and pests (Scheuerell & Mahaffee, 2006). These material may increase competition between fungivorous and baderioruos nematode resulting from increased availability of food sources after applications (Abdel-Barry et al., 2014). In addition, these micronutrients play pivotal roles in plant resistance by regulating the levels of auxin in plant tissues by activating the auxin oxidase system, what leads to increase of total phenol, calcium content and activity of catechol oxidase, these materials protect plants against pathogen stress (Chowdhury, 2003).

In conclusion, we can say that according to our results, usage of *Rhizobium. Leguminosarum* and AM fungi separately or in combinations, as commercial/bio fertilizers products also, potassium humate and compost tea as organic fertilizers can be more effective against *Meloidogyne incognita,* reducing the level of its pathogenic activity.

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