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Potentials of potassium humate, ammonium humate, and vermicompost tea in controlling root-knot nematode, *Meloidogyne arena*ria and improving biochemical components in eggplant

Sahar H. Abdel-Baset[1], Eman Abdelrazik*[2], and Ashraf S. Shehata[3]
1- Department of Nematology, Plants Pathology Res. Inst., ARC, Giza, Egypt.
2- Botany and Microbiology Department, Faculty of Science, Suez University, Suez, Egypt.
3- Soil improvement and conservation Dept., Soils, Water and Environment Res. Inst., ARC, Giza, Egypt
*abdelrazikeman1@gmail.com

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ABSTRACT

The impact of potassium humate, ammonium humate and vermicompost tea on the root-knot nematode, *Meloidogyne arenaria*, and their effect on plant growth and some biochemical parameters in eggplant were determined *in vitro* and *in vivo*. The *in vitro* results showed that egg hatching and juvenile mortality of *M. arenaria* were significantly ($P \leq 0.05$) affected by treatments. Ammonium humate 3 and 2% were more effective in inhibiting egg hatching after 4 days by 61.6 and 82.6% respectively. Also, in ammonium humate 3%, the increase of exposure period from 24 to 48 hrs. raised mortality from 73.3 to 82%. *In vivo* experiment treatments were applied under greenhouse conditions with two different application methods (soil drenching, and foliar spray) and two concentrations (2 and 3%) on the root-knot nematode, *M. arenaria* population, and their impact on eggplant. All tested treatments significantly decreased nematode number to different levels compared to control. The highest suppression in total nematode population was recorded with drenching by 3% ammonium humate. A similar trend was noticed with reproduction factor and number of galls or egg- masses per root system. Plants grown in soil drenched by 3 % ammonium humate recorded the highest value in fresh and dry weight, as well as shoot and root length. Also, these plants gave the highest values of carotenoids, phenolic compounds and total amino acids. While, the highest value of proline content appeared with control. Also, the results showed that the total chlorophyll, total protein and reducing sugar content increased in all treatments compared to control.

Keywords: *Solanum melongena* L., *Meloidogyne arenaria*, organic soil amendments, non-enzymatic antioxidant, total chlorophyll.

INTRODUCTION

Root-knot nematodes, *Meloidogyne* spp. are categorized as important plant-parasitic nematodes, infecting over 2000 plant species, with significant world yield loss estimated to be USD 100 billion per year (Oka *et al*., 2000; Mangala and Mauria, 2006). In Egypt, root-knot nematodes, are becoming real threats to almost all vegetable crops, particularly in the newly reclaimed areas and are considered as limiting factors in crop production (Ibrahim *et al*., 1992). The average estimates of their corresponding annual yield losses of vegetables are 5.12 billion $. Eggplant is known to be highly susceptible to root-knot nematodes *Meloidogyne* spp., caused annual losses of 20% (Abd-El gawad 2014). Although a pesticide application may enhance economic potential for increasing food production, it caused serious health implications to human and the environment (Forget, 1993).

The use of organic amendments in management of plant-parasitic nematodes

have been demonstrated in a large number of studies (Litterick *et al.*, 2004; Oka, 2010). There have been many mechanisms to explain the beneficial effects on plants of observed organic amendments in the presence of nematodes. Many studies mentioned nematicidal compounds released from decomposition materials, stimulation of natural nematode enemies, improved plant growth and the tolerance of nematodes (Akhtar and Malik, 2000; Thoden *et al.*, 2011). Humus is the final product resulting from physical, chemical, and microbiological processing. (humification) of organic matter. Humic substances are component of humus and widely dispersed over the earth's surface (Rizal *et al.*, 2010). Humic substances were classified into three general classes such ; humic acid, fulvic acid, and humin (Solange and Rezende, 2008). Humate is used in agricultural production as a natural resource for sustainability, it affects positively on the physical, chemical, and bio- properties of the soil, as well as controlling soil-borne diseases, improving soil health and nutrient uptake, activities of plant enzyme, root growth, and plant yield (Mikkelsen, 2005; Mauromicale *et al.*, 2011). Various salts of humic substances viz., calcium humate, and ammonium humate were used to increase soil fertility and stimulates plant growth (Lotosh, 1991; Buckau *et al*., 2000). Potassium humate is known to exhibit properties of soil amelioration growth stimulation, enhancement of crop yield, and restoration of soil biodiversity and also resistant to nematode attack (Daneel *et al*., 2000). Dawood *et al*. (2019) reported that the humic acid caused significant increases in carbohydrate content, total phenolic content, proline, and free amino acids relative to control.

Vermicomposting has long been recognized as a low cost and environmentally sound process for treatment of many organic wastes such as animal manures, sewage-sludge, crop residues, and industrial wastes (Arancon *et al*., 2008; Lazcano and Dominguez, 2011).

Vermicompost promotes plant growth, enhances germination and increases yield in various vegetables, field crops, and ornamentals as well as, it is a sustainable source of macro, and micro-nutrients and has extensive potentials for enhanced plant growth when used as soil components (Sahni *et al*., 2008).

The aim of this study is to investigate the effect of some organic soil amendments *viz*., potassium humate, ammonium humate and vermicompost tea on the root-knot nematode, *M. arenaria* reproduction, and their effect on growth and some biochemical parameters of eggplant under greenhouse conditions.

MATERIALS AND METHODS

The experiment was conducted at Ismailia Agriculture Research Station during summer season, 2019 to evaluate the efficacy of organic soil amendments *viz*., potassium humate, ammonium humate and vermicompost tea on the root-knot nematode, *Meloidogyne arenaria* reproduction, and their impact on eggplant growth and some biochemical parameters under greenhouse conditions.

Identification and propagation of pure cultures of root-knot nematode, *M. arenaria*:

Galled eggplant roots were washed carefully with a gentle flow of water to remove adhering soil particles. A single egg- mass was collected using a special needle for this technique. The pure culture of *M. arenaria* from single egg-mass has been maintained on tomato seedling (*Solanum Lycopersicum* L. cv. G.S) in a greenhouse at 25± 2°C in order to obtain sufficient numbers of eggs and second stage juveniles. Species identification was based on second stage juvenile measurements and examination of the perineal pattern system of adult females, according to Eisenback *et al*., (1981) and Jepson (1987).

Source of vermicompst and compost:

Vermicompst was obtained from Central Laboratory of Organic Agriculture, Agricultural Research Center, Giza, Egypt. Compost was obtained from Agricultural Microbiology Research, Department, Soils, Water, and Environment Research Institute (SWRI), Giza, Egypt. Some chemical properties of vermicompost and compost are shown in Table (1).

Vermicompost tea was prepared by soaking the finely ground vermicompost in distilled water at 1:1 (v/v) ratio and mixed thoroughly for 48 hours. The suspension was filtered using muslin tissue and kept in a clean glass flask to be used as a standard solution (S).

Table 1: Chemical analysis of vermicompost and compost used in experiments.

Value	C/N ratio	Organic matter %	Total-N (%)	Total-P (%)	Total-K (%)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Weed seeds	Nematodes
Vermi-compost	1:20	21	0.71	0.28	0.92	1.58	0.009	0.008	0	0
Compost	1:16	24.3	0.81	0.37	1.32	2.06	0.006	0.005	0	0

Extraction of potassium, and ammonium humate from compost:

Extraction of potassium humate:

Extraction of potassium humate was run according to the method described by Sanchez-Monedero *et al*., (2002). The compost was treated with 1.0 N KOH and 2 N NH_4OH. A mixture of 40 g of compost and 800 ml of solution was shaked 120 rpm under N_2 gas atmosphere in sealed bottles for 12 hours, then centrifuged for 15 minutes at 6000 rpm and the supernatant was removed then pH was adjusted to 2.0 or less by addition of 2 M H_2SO_4. The formed precipitate, of humic acid (HA) was allowed to coagulate for 24 hours at 4 °C then separated from the soluble fulvic acids by centrifuging for 15 minutes at 6000 rpm. The coagulated precipitate was washed twice with 0.1 M H_2SO_4 and one time with deionized water. One part of HA was freeze-dried for storage **(**unpurified humic acid).

Neutralized solution of extracting potassium humate (standard solution):

It was subjected for determination of total potassium content by flame photometer**.**

Extraction of ammonium humate (standard solution):

Soluble nitrogen forms were determined according to the method of (Page *et al.,* 1982). The extracted ammonium humate was subjected to steam distillation one time in presence of MgO_2 and another time in presence of Devarda's alloy, at both times, the evolved ammonia was collected at 4% H_3BO_4 using mixed indicator of methyl red and promo cresol green and determined by titration against 0.01N H_2SO_4 solution (Shehata, 2018).

Laboratory Assay:

Hatching test:

Suspensions of eggs were prepared in distilled water according to Hussey and Barker (1973). One ml of egg suspension about (100 egg/ml) and 10 ml in concentrations 2, and 3 % from standard solution (pH=7) of soil organic amendments, whether potassium humate or ammonium humate and vermicompost tea, were transferred in glass cavity blocks and kept at room temperature (≈25^{o}C). Each treatment was replicated three times. The glass cavity containing 1 ml egg

suspension and 10 ml distilled water served as control. After four days of exposure, the number of hatched eggs was counted under a low power (6X) stereomicroscope. Percentage of hatching inhibition was calculated in comparison with the control treatment, according to the following equation:

Egg hatching inhibition % = {(control - treatment) /control}×100.

Effect on juvenile mortality:

Nine ml of each concentration was separately poured into Petri dishes from every treatment, then added 1 ml of suspensions containing 100 freshly hatched juveniles were added to each Petri dish. The Petri dishes with distilled water were taken as control. All treatments were replicated three times. The Petri dishes were incubated at room temperature (≈25^{o}C). Percent mortality was calculated after 24 and 48 hours.

The mortality percentages were calculated as the following equation:

Mortality (%) = Dead number of juveniles in treatment / Total number of juveniles in treatment ×100.

Greenhouse Experiment:

Efficacy of potassium humate, ammonium humate, and vermicompost tea on *M. arenaria* reproduction and eggplant growth:

Twenty five days old seedlings of eggplant (*Solanum melongena* cv. Travita) were used as the host plant for *M. arenaria.* Seedlings were transplanted into30 cm diameter clay pots (one seedling/pot) filled with 4 kg a sterilized mixture of clay and sand (4:1 w/w), pots were regularly watered with tap water**.**

Time and methods of application:

The treatments were prepared fresh for each treatment, and added at different times;

1- The first application at transplanting time.

2- The second application with nematode inoculations was applied five days after transplanting (the plants were inoculated with 2000 eggs/plant of *M. arenaria* into three holes around plant root).

3- Every 10 days after the inoculation of nematodes, 3rd, 4th, and 5th applications were added as recommended @ 400 liter per Fed (80ml/ plant) for each treatment.

The treatments were as follows:

T1= potassium humate (2%) as foliar spray.
T2= potassium humate (3%) as foliar spray.
T3= potassium humate (2%) as soil drenching.
T4 = potassium humate (3%) as soil application.
T5= ammonium humate (2%) as foliar spray.
T6 = ammonium humate (3%) as foliar spray.
T7=ammonium humate (2%) as soil drenching.
T8=ammonium humate (3%) as soil drenching.
T9 = vermicompost tea as foliar spray (2%).
T10= vermicompost tea as foliar spray (3%).
T11=vermicompost tea (2%) as soil drenching.
T12=vermicompost tea (3%) as soil drenching.
T13=Nematicide treatment (Vydate® (oxamyl) 24% L.
T14 = Control (nematode only).

Control treatment (without any treatment or nematicide, inoculated by *M. arenaria* as mentioned before). A comparison treatment with the nematicide Vydate® (oxamyl) 24% L, was added at the rate of 4 L/Fed (0. 2 ml /plant) as recommended, after two days from nematode inoculation.

All treatments were replicated three times, pots were arranged in a completely randomized design, and watered regally. Experiments terminated after 45 days of nematode inoculation.

The growth parameters included length as well as fresh and dry weight of shoots and roots were measured.

Increasing percentages of total plant fresh weight, and shoot fresh weight % = {(treatment – control) / control} x 100.

Nematode populations in soil per pot were determined according to Hopper *et al*. (2005). Roots were stained by acid fuchsin in acetic acid according to Byrd *et al*. (1983) to examine for counting the number of developmental stages, females/root, number of galls, and egg-masses. Eggs/egg-mass per plant were extracted by using sodium hypochlorite

(NaOCl) method as described by Hussey and Barker (1973).

Root gall index (RGI) or egg -mass index (EI) was determined according to Taylor and Sasser (1978) as follows: 0: = no galls, 1= 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5= more than 100 galls or egg-masses per root system.

The final nematode populations (Pf), and reproduction factor (RF) were calculated according to Oostenbrink (1966) as follows: Reproduction factor= Pf/Pi, where Pf is a final population of nematodes, and Pi is a primary population of nematodes (the nematode inoculum).

Final nematode population (Pf) =No. of eggs/root system + No. of females + No. of developmental stages + No. of juveniles in the soil/pot.

Biochemical Determinations:

Photosynthetic pigments (mg 100 g^{-1} FW):

In the fresh leaves, total chlorophyll and carotenoids were extracted with 85% of acetone and estimated spectrophotometrically at 662, 644 and 440.5 nm (Lichenthaler and Wellburn, 1983).

Total protein (mg/g FW):

It was estimated by Bradford method (Bradford, 1976) at 595 nm. 0.2g fresh leaves were homogenized in a pre-chilled mortar with 1ml of 0.1M phosphate buffer (pH 7). Then, the suspension obtained was filtered through one layer of muslin cloth and then centrifuged at 10000 rpm for 15 min., 4^{o}C (Urbanek *et al*., 1991). 2 ml of Bradford reagent was added to 200 µl leaves extract.

Proline (mg g^{-1}FW):

It was determined with ninhydrin reagent as described by Bates *et al. (*1973). Fresh leaf was digested by 3% aqueous sulphosalicylic acid. The homogenate was filtered through Whatmann filter paper. For estimation, 2ml of the filtrate was added to 2mL of glacial acetic acid and 2ml of acid ninhydrin (1.25g ninhydrin was warmed in 30 ml of glacial acetic acid and 20 ml of 6M phosphoric acid, with agitation till dissolved). The mixture was heated in a boiling water bath for 1 h. The reaction was terminated by placing the tubes in ice bath. Add 4ml toluene to the reaction mixture and stirred well for 20-30 sec. The toluene layer was separated and warmed to room temperature. The red color intensity was measured at 520 nm against the toluene blank.

Total free amino acids (mg g^{-1} FW):

Ethanolic extract (0.1 ml) was added to 5 ml methanol +1 ml ninhydrin reagent (4 g ninhydrine +300 ml acetone) and heated in water bath at 60 ^{o}C for 20 min. The blue colored samples were measured against blank sample at 570 nm (Rosen, 1957). For determination of total free amino acids, free phenolic compounds and reducing sugar alcohol extraction of leaves were prepared according to Abdel-Rahman *et al.* (1975).

Free phenolic compounds (mg g^{-1} FW):

Free phenolic compounds were estimated by a modified Folin-Ciocalteu method and measured at 650 nm according to Horwitz *et al.* (1970). For determination, 1 ml of ethanolic extract was added to 1 ml of 2 N Folin-Ciocalteu reagent, 1 ml of Na_2CO_3 solution (14%) and 7 ml distilled water in test tube. Test tubes were heated to about 70°C in water bath.

Reducing sugars (mg g^{-1} FW):

Reducing sugars were determined by Nelson's method with alkaline copper and arsenomolybdate reagents and measured spectrophotometricaly at 540 nm (Moore, 1974).

Statistical analysis:

All experiments were performed twice in a completely randomized design with three replicates in each treatment. Data were subjected to analysis of variance

(ANOVA) using MSTAT-C program version 2.10 (Anonymous, 1991). Means were compared by Duncan's multiple range test at P ≤ 0.05 probability (Duncan, 1955).

RESULTS

Efficacy of two concentrations of potassium, ammonium humate and vermicompost tea on egg hatching and juvenile mortality of root-knot nematode, *M. arenaria in vitro*:

The effect of two concentrations (2 and 3%) of potassium humate, ammonium humate and vermicompost tea on egg hatching and juvenile mortality of root-knot nematode, *M. arenaria* was studied *in vitro*. Data in Table (2) revealed that egg hatching and juvenile mortality of *M. arenaria* were affected significantly (P≤0.05) by treatments. Ammonium humate at 3 and 2% were more effective in inhibiting egg hatching after 4 days with values 61.6, and 82.6% respectively. While, the less effective treatment in inhibiting egg hatching was vermicompost tea 2% with 30.3%. There was a difference in the mortality rate among different treatments tested in both intervals (24 and 48 hours). In all treatments, the mortality rates of juveniles increased with an increase in exposure time and concentrations. As the exposure period increased mortality increase from 73.3 to 82% in ammonium humate 3%.

Table (2): Impact of two concentrations of potassium humate, ammonium humate and vermicompost tea on egg hatch, and juvenile mortality of root-knot nematode, *M. arenaria.*

Treatment	Rate	Number of inhibited eggs	Egg hatching	Number of dead juveniles		Juvenile mortality (%) after	
			Hatch inhibition After4days(%)	24 hours	48 hours	24 hours	48 hours
Potassium humate	2%	48.6d	48.6	33.3d	41d	33.3	41
Potassium humate	3%	56.3 c	56.3	48.6c	59.6c	48.6	59.6
Ammonium humate	2%	61.6 b	61.6	64.3b	70.3b	64.3	70.3
Ammonium humate	3%	82.6a	82.6	73.3a	82a	73.3	82
Vermicompost tea	2%	30.3 f	30.3	13.6f	22.3e	13.6	22.3
Vermicompost tea	3%	35.3e	35.3	19 e	22.6e	19	22.6
Control (distilled water)		0.1 g	0.1	0.1 g	0.1 f	0.1	0.1
LSD 0.05		4.63		5.02	4.35		

Data are average of 3 replicates.
*Different letter(s) indicate significant differences among treatments within the same column according to Duncan's multiple range test (P ≤ 0.05).

Efficacy of two concentrations of potassium humate, ammonium humate and vermicompost tea on eggplant growth and root-knot nematode, *M. arenaria* reproduction under greenhouse conditions:

The results in Table (3) indicated that all of the tested treatments decreased (P ⩽ 0.05) nematode reproduction to different levels compared to control treatment. The highest suppression in total nematode population was recorded with treatment of drenching ammonium humate 3%with reproduction factor (RF) 0.15.A similar trend was noticed for the number of galls (18.8) and egg- masses (3.3) per root system. The same result was obtained with drenching ammonium humate 2% with reproduction factor (RF) 0.21, number of galls (25.9) and egg- masses (3.3) per root system. While, the less effective treatment was detected with vermicompost tea 10 ml as a foliar application with reproduction factor (RF) 0.86, root gall index (RGI) 4, and egg-mass index (EI) 3.

Table (3): Impact of two concentrations of potassium, ammonium humate and vermicompost tea on reproduction of *M. arenaria* infecting eggplant under greenhouse conditions.

Treatment		Nematode population			Eggs/ root system	Total nematode population	R f	Galls/ root system	RGI	Egg masses/ root system	EI
		Soil/ pot	Root								
			Developmental stages	Females							
Potassium humate 2%	Soil drench	86.6^{f}	2.6^{d}	10.3^{cde}	795f^{g}	894.5	0.44	23.1^{e}f^{g}	3	7de	2
Potassium humate 3%		98.6^{ef}	2^{d}	11.5^{bcd}	732^{g}	844.1	0.42	29^{ef}	3	6^{e}	2
Potassium humate 2%	Foliar spray	122.6^{de}f	3.6^{cd}	12.8^{bcd}	1132^{bcd}	1271	0.63	31.9e	3	9 ^{bcd}	2
Potassium humate 3%		130.6^{def}	2.6^{d}	11.5^{bcd}	1135.6^{bcd}	1280.3	064	26.6^{efg}	3	9.3 ^{bc}	2
Ammonium humate 2%	Soil drench	86.5^{f}	1.6d	6.8^{ef}	331.3 ^{h}	426.2	0.21	25.9^{efg}	3	3.3 ^{f}	2
Ammonium humate 3%		81.3^{f}	1.3^{d}	5.6^{f}	230^{h}	318.2	0.15	18.8^{fg}	3	3.3 ^{f}	2
Ammonium humate 2%	Foliar spray	106.6^{ef}	2.3d	9.5^{def}	1023.6^{cde}	1142	0.57	29.8^{ef}	3	10^{bc}	2
Ammonium humate 3%		112 ^{ef}	2.6d	9.5^{def}	987.3^{de}	1111.4	0.55	28.1^{ef}	3	8.6^{cd}	2
vermicompost tea 2%	Soil drench	196^{d}	3d	14.2^{bc}	990.6^{de}	1203.8	0.6	45.4^{d}	4	9 ^{bcd}	2
vermicompost tea 3%		170.6^{de}	2.3^{d}	14.4bc	924.6^{ef}	1111.9	0.55	44.1^{d}	4	8.3 cd	2
vermicompost tea 2%	Foliar spray	388.8^{c}	6.6bc	11.2^{cde}	1241.6^{b}	1648.2	0.82	84.4^{b}	4	8.3 ^{cd}	2
vermicompost tea 3%		519.7^{b}	7.6^{b}	15.9^{b}	1191.6^{bc}	1734.8	0.86	70.5^{c}	4	11^{b}	3
Control (nematode alone)		726.6^{a}	20.3^{a}	43^{a}	4326.6^{a}	5116.5	2.55	131.3^{a}	5	22.3^{a}	3
Vydate (oxamyl) 24% L		85^{f}	1d	9.2^{def}	202.3^{h}	297.5	0.14	16.1^{g}	3	3 ^{f}	2
LSD 0.05		75.61	3.24	4.58	185			11.42		2.12	

Data are average of 3 replicates.
*Different letter(s) indicate significant differences among treatments within the same column according to Duncan's multiple range test ($P \leq 0.05$). *Rf (Reproduction factor) =Final Population (Pf) /Initial Population (Pi), * Root gall index (RGI) or egg- masses index (EI) was determined according to Taylor and Sasser (1978).

Effect of some organic soil amendments on the plant growth:

The influence of organic soil amendments *viz.*, potassium, humate, ammonium humate and vermincompost tea that added five times at two rates with two different applications (soil drenching and foliar spray) on eggplant plants infected with *M. arenaria* under greenhouse conditions. Data presented in Table (4) showed that eggplant growth parameters were significantly ($P<0.005$) increased as the dosage of organic soil amendments increased than in the control plants. Plants treated with, ammonium humate 3% soil recorded the highest increase percentage in total plant fresh weight (g) with 117%, followed by plants treated with, potassium humate 3% soil increased total plant fresh weight by 90%. The third effective treatment was ammonium humate 3% foliar, since it increased percentage total fresh weight by 85 % compared to control plants. The lowest percentage increase in total fresh weight was recorded with compost foliar 2% and 3% with values 33% and 45 %, respectively compared to control plants. For the dry weight of the shoot system (g), the highest percentage increase was recorded in plants treated with ammonium humate 3% soil by 155%, followed by treatment ammonium humate 2% soil by 100%. While, the treatments, compost 3% foliar, and compost 2% soil were recorded the lowest percentage increase in shoot dry weight by 26% for each, compared to control plants.

Table (4): The influence of some organic soil amendments as a soil drenching and foliar spry at two rates on the growth of eggplants infected with *M. arenaria* under greenhouse conditions.

Treatment		Fresh weight (g)		Length (cm)		Total plant fresh weight (g)	Inc .%	Shoot dry weight (g)	Inc. %
		Shoot	Root	Main stem	Tap root				
Potassium humate 2%	Soil drench	24de	15.3bcd	50.6cd	36ab	39.3 ef	56	14f	36
Potassium humate 3%	Soil drench	31.3b	16.6bc	57.3b	40 ab	47.9 b	90	19.6bc	90
Potassium humate 2%	Foliar spray	27cd	12.6de	44.6de	28.3cd	39.6 e	57	16.3cdef	58
Potassium humate 3%	Foliar spray	25.3cde	16.6bc	51.6bc	35.6ab	41.9 d	66	16def	55
Ammonium humate 2%	Soil drench	28.3bc	17.6ab	57b	34.3bc	45.9 c	82	20.6b	100
Ammonium humate 3%	Soil drench	35a	19.6a	65.6a	42.3a	54.6 a	117	26.3a	155
Ammonium humate 2%	Foliar spray	28.3bc	13.6d	52.3bc	35bc	41.9 d	66	17.6bcde	71
Ammonium humate 3%	Foliar spray	31.3b	15.3bcd	54bc	38ab	46.6 c	85	19.6bc	90
Vermicompost tea 2%	Soil drench	24.3de	14.3cd	36.6f	22.3de	38.6 fg	53	13fg	26
Vermicompost tea 3%	Soil drench	26.3cde	15.3bcd	43.6e	24.6de	41.6 d	65	16.3cdef	58
Vermicompost tea 2%	Foliar spray	23e	10.6ef	43.6e	27.3d	33.6 i	33	14.3ef	39
Vermicompost tea 3%	Foliar spray	23e	13.6d	41.6ef	24 de	36.6 h	45	13fg	26
Control (nematode alone)		15.6f	9.6f	26.3g	20.3e	25.2 j	--	10.3g	--
Vydate (oxamyl) 24% L		25.6cde	12.6de	40 ef	27.3d	38.2 g	52	19 bcd	84
LSD 0.05		3.63	2.95	6.31	6.88	0.851		3.65	

Data are average of 3 replicates.*Different letter(s) indicate significant differences among treatments within the same column according to Duncan's multiple range test ($P \leq 0.05$).

Effect of some organic soil amendments on chlorophyll, reducing sugar and protein:

The results in Table (5) showed that the total chlorophyll, total protein and reducing sugar content increased in all treatments compared to control. The highest content of total chlorophyll resulted with 3% potassium humate foliar treatment (21.235mg/gFW), compared to control treatment (5.515mg/gFW). While, the highest content of protein recorded with 3% ammonium humate soil treatment (15.065mg/gFW) compared to control treatment (6.845mg/gFW). The plants treated with vermicompost tea 3% as foliar showed the highest concentration of reducing sugar (34.325mg/gFW) compared to control treatment (13.5mg/gFW).

Effect of some organic soil amendments on non-enzymatic antioxidant:

Data in Table (6) expressed that the highest values of carotenoids, phenolic compounds and total amino acids were detected in treatment of 3% ammonium humate in soil. The highest value of the proline content was appeared with control treatment (8.31), while the lowest one was measured with 2% ammonium humate as foliar treatment (0.74).

Table (5): The influence of some organic soil amendments as a soil drenching and foliar spray at two rates on chlorophyll, reducing sugar and protein of eggplants infected with *M. arenaria* under greenhouse conditions.

Treatments		Total chlorophyll (mg 100 g^{-1} FW)	Reducing-sugar (mg g^{-1}FW)	Total protein (mg/g FW)
Potassium humate 2%	Soil drench	20.18 b	13.466 j	8.35 h
Potassium humate 3%		13.5 d	21.253 cde	11.72 cde
Potassium humate 2%	Foliar spray	17.36 c	15.203 i	8.76 gh
Potassium humate 3%		21.23 a	19.213 fgh	11.31 de
Ammonium humate 2%	Soil drench	19.61 b	18.1 h	11.81 cd
Ammonium humate 3%		19.26 b	15.83 i	15.06 a
Ammonium humate 2%	Foliar spray	10.89 f	18.55 gh	8.06 hi
Ammonium humate 3%		20.07 b	28.33 b	10.50 ef
Vermicompost tea 2%	Soil drench	17.42 c	21.81 cd	9.83 fg
Vermicompost tea 3%		12.00 e	20.4 def	11.76 cd
Vermicompost tea 2%	Foliar spray	9.10 g	19.92 efg	12.55 bc
Vermicompost tea 3%		17.14 c	34.32 a	10.65 def
Control (nematode alone)		5.51 h	13.5 j	6.84 i
Vydate (oxamyl) 24% L		19.35 b	22.013 c	13.08 b
LSD 0.05		0.964	1.460	1.220

Data are average of 3 replicates.
*Different letter(s) indicate significant differences among treatments within the same column according to Duncan's multiple range test ($P \leq 0.05$).

Table (6): The influence of some organic soil amendments as a soil drenching and foliar spry at two rates on non-enzymatic antioxidant of eggplants infected with *M. arenaria* under greenhouse conditions.

Treatments		Free phenolic compounds (mg g^{-1}FW)	Free amino acids (mg g^{-1}FW)	Proline (mg g^{-1}FW)	CAR (mg 100 g^{-1} FW)
Potassium humate 2%	Soil drench	3.62 b	10.61 b	2.91 ef	7.42 b
Potassium humate 3%		2.22 h	7.61 i	1.33 gh	1.99 g
Potassium humate 2%	Foliar spray	2.60 f	9.1 g	1.47 gh	6.65 c
Potassium humate 3%		3.14 d	9.87 cd	1.24 hi	5.68 d
Ammonium humate 2%	Soil drench	2.47 g	9.2 fg	1.78 g	5.65 d
Ammonium humate 3%		4.34 a	12.19 a	0.76 ij	10.11 a
Ammonium humate 2%	Foliar spray	1.90 i	6.67 j	0.74 j	1.91 g
Ammonium humate 3%		3.31 c	8.8 h	2.68 f	5.88 d
Vermicompost tea 2%	Soil drench	2.19 h	7.52 i	3.34 de	3.63 f
Vermicompost tea 3%		1.67 j	9.9 c	3.77 d	2.23 g
Vermicompost tea 2%	Foliar spray	2.80 e	10 c	7.16 b	2.35 g
Vermicompost tea 3%		3.61b	9.40 ef	5.88 c	4.33 e
Control (nematode alone)		1.96 i	4.74 k	8.31 a	0.89 h
Vydate (oxamyl) 24% L		2.72 e	9.63 de	1.1 hij	5.63 d
LSD 0.05		0.109	0.265	0.492	0.469

Data are average of 3 replicates. *Different letter(s) indicate significant differences among treatments within the same column according to Duncan's multiple range test ($P \leq 0.05$).

DISCUSSION

The major alternative strategies to control Phytonematodes are applying organic soil amendments. Many organic soil amendments are known to have nematicidal effect, with constituent compounds directly affecting nematodes (Szakiel *et al*., 2008; Warnke *et al*., 2008).

In the current study the influence of organic soil amendments *viz.*, potassium humate, ammonium humate, and vermicompost tea on egg hatching and mortality of second-stage juvenile j_2 of root-knot nematode, *M.arenaria* at two concentrations were studied *in vitro* assays. Ammonium humate and potassium humate showed significant effect in reducing egg hatching and increasing juvenile mortality of *M. arenaria* (j_2). Mortality increased with increasing concentrations, 3% ammonium humate concentration had a higher inhibition of egg hatch and J_2 mortality. These results are in agreement with those of Saravanapriya and Subramanian (2007) who evaluated four concentrations of humic acid (0.1%, 0.2%, 0.3% and 0.4%). They found that 0.4% achieved higher egg hatch inhibition. Also, these results are in line with Jothi *et al.* (2009) who found that 0.4% humic acid concentration can led to 93% mortality of *M. incognita* J_2. Similarly, *in vivo* experiment soil drenched with ammonium humate and potassium humate 3% was effective in reducing nematode reproduction on eggplant. This result is in agreement with Jothi and Poornima (2017) who reported that application of two grams of potassium humate per kg soil was effective in reducing the gall index and nematode population in tomato infected with *M. incognita* in greenhouse. Humic acid is released during the decomposition of organic amendments, and is toxic to nematodes (Khan *et al.,* 1974). The observed nematicidal efficacy could be due to active principles present in humic acids such as carboxyl, phenolic, alcoholic, hydroxyl and carbonyl groups, especially the carboxyl and phenolic groups (Chitwood, 2002).

The present results indicated that all concentrations of humic acid significantly reduced the nematode soil density and in roots compared to control pots. Generally, the effectiveness of these materials on nematode suppression is usually dependent on the C: N ratio and decomposition status (Stirling, 1991). Properly the decomposed materials release minerals to a soil solution, which increase soil solution's osmotic potential (Stirling, 1991). Mian and Rodriguez-Kabana (1982) reported that nematode suppression by organic amendments is directly related to N content or inversely related to the C/N ratioOrganic materials with C:N ratio less than 20:1 have higher degradation rates and often with nematicidal activities (McSorley and Gallaher, 1995).

The obtained results revealed that the application of all treatments increased plant growth parameters compared to control. Ammonium humate 3, and 2 % as a soil drench significantly increased total plant fresh weight and plant dry weight, followed by foliar application of ammonium humate 3%, and potassium humate 3% as a soil drench. These results are in good line with several reports which indicated that these treatments increased total plant fresh weight, and plant dry weight. Various studies reported that HS enhanced plant growth, and stimulated nutrient uptake (Piccolo *et al*., 1993), and seems to regulate the mechanisms involved in plant growth stimulation (Dobbss *et al*., 2007). Oka and Pivonia (2002) studied the nematicidal activities of ammonia-releasing and ammonium compounds against the root-knot nematode *Meloidogyne javanica*. The compost has 1.32% of potassium (Table1), when potassium move into the cells, the cells collect water and swell. K plays essential

roles in enzyme activation, protein synthesis, photosynthesis, osmoregulation, stomatal movement, energy transfer, phloem transport, cation-anion balance and stress resistance (Marschner, 2012). Perrenoud (1990) found that the use of K significantly decreased the incidence of nematodes by 33%. Other positive results from the foliar application of potassium humate on potato tubers before planting and spraying it on the sprouts increased root system and so caused an increment of up to 22% in tuber yield (Ajalli*et al*., 2013).The applications of soluble humate granules at rates up to 20 kg/ha provided shoot and root growth in lucerne and rye grass, with a maximum benefit gained at 20 kg/ha, five times (Little, 2015). While in cotton the application of ammonium humate at a rate of 400 kg/ha recorded the highest growth and uptake of P (Annaeva *et al*., 1987).

The current data showed that all the treatments increased the total chlorophyll, reducing sugar and protein content in leaves compared to control treatment. The chlorophyll content is the most important component of plants since it makes food, which is necessary for the growth and development of all parts of the plant. Humic acid used as growth regulate hormone, enhances plant growth and improves stress tolerance (Albayrak and Camas, 2005). Humic acid improves soil structure and change physical properties of soil, promote the chelation of many elements and make these available to plants, aid in correcting plant chlorosis, enhancement of photosynthesis density, plant root respiration, enzymatic activities and protein synthesis (Biondi *et al*., 1994; Nardi *et al*., 2002). Also, application of humate lead to rise plant membrane permeability, resulted in enhance growth of some beneficial microorganisms, accelerate cell division, improved root growth and all plant organs for a number of horticultural crops, turf grasses and some trees (Russo and Berlyn,1990; Pioncelot, 1993). Vermicompost and compost contain iron by 1.56 and 2.06 ppm respectively (Table 1), iron plays a significant role in basic biological processes such as photosynthesis, chlorophyll synthesis, respiration and nitrogen fixation (Kim and Rees, 1992). It is also an active cofactor of many enzymes that are necessary for plant hormone synthesis (Siedow, 1991).

Data also, revealed that all treatments increased the free phenolic compounds, free amino acids and carotenoids in eggplant compared to control. The increase in phenolic compounds during infection could be attributed to the rapid decomposition of phenols to change for different paths leading to the formation of various compounds such as the polymer that plays a vital role in the resistance reaction. Farkas and Kiraly (1962) studied the occurrence and metabolism of phenolic substances in plants, in response to injury or invasion by pathogens, such as fungi, bacteria and viruses. In nematodes too, a large number of phenolic compounds have been found to have strong nematicidal activity (Mahajan *et al*., 1992). Free phenolic compounds have high physiological and chemical activity, while, the most phenols occur in plant tissues in bound form as glycosides and decompose to free phenols when plants response to stress (Giebel, 1970). Hopkins and Huner (2004) reported that amino acids used as precursors for synthesizing different important compounds in plant such as tryptophan which induced the growth stimulated phytohormone auxine. Carotenoids are vital antioxidants for buffering of singlet oxygen, dissipation of excess light energy through non-photochemical quenching, and harvesting light energy and channeling to the photosystem (Mcelroy and Kopsell, 2009).

The data showed that proline concentration decreased in all treatments compared to control treatment. Proline is a

multi-functional amino acid, which confers tolerance to plants against abiotic stresses and has been correlated to plant defense against pathogens (Senthil-Kumar and Mysore, 2012). While, the all treatments scrimping the harmful effect of nematodes on plants. Also, proline catabolism is enhanced during the early stages of plant defense against pathogens (Cecchini *et al.*, 2011).

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تاثيراستخدام هيومات البوتاسيوم وهيومات الأمونيوم وشاي الفيرميكومبوست في مكافحة نيماتودا تعقد الجذور *Meloidogyne aren*aria وتحسين المكونات الكيميائية الحيوية في الباذنجان

سحر حسن عبدالباسط[1], ايمان عبدالرازق*[2], اشرف صابر شحاته[3]

1 - قسم بحوث النيماتودا- معهد بحوث امراض النباتات- مركز البحوث الزراعية- الجيزة

2 - قسم النبات والميكروبيولوجي - كليةالعلوم- جامعة السويس- مصر

3- قسم بحوث تحسين وصيانه الاراضى - معهد بحوث الاراضى والمياه والبيئة - مركز البحوث الزراعية- الجيزة

*abdelrazikeman1@gmail.com

المستخلص

تم دراسة تاثيراستخدام ثلاث محسنات تربة (هيومات الامونيوم وهيومات البوتاسيوم وشاى الكمبوست الدودى) على مكافحة نيماتودا تعقد الجذور معمليا وداخل الصوبة بالاضافة الى دراسة التاثيرعلى بعض التغيرات البيوكيمائية على نباتات الباذنجان ، واوضحت النتائج المعملية أن جميع المعاملات ادت الى تقليل معدل فقس البيض وزيادة موت الطورالمعدى (اليرقى الثانى) لنيماتودا تعقد الجذور *M. arenaria* بشكل ملحوظ ، وكانت أكثر المعاملات فعالية في تثبيط فقس البيض بعد 4 أيام هى معاملة هيومات الأمونيوم 2 و 3٪ بنسبة بلغت 61.6 و 82.6٪ على التوالي، وكانت المعاملة الأقل فعالية في تثبيط فقس البيض هى شاي الكومبوست الدودى 2٪ بنسبة 30.3٪ وزادت معدلات موت اليرقات بزيادة فترة التعريض (24 و 48 ساعة) وبزيادة التركيزات (2 و 3٪)، كما أدت زيادة فترة التعريض الى زيادة معدل الموث من 73.3 إلى 82٪ في هيومات الأمونيوم 3٪.

ومن ناحية أخرى اجريت المعاملات تحت ظروف الصوبة باستخدام طريقتي (غمرالتربة والرش الورقي) وبتركزين (2 و 3 ٪) على نباتات الباذنجان المصابة بنيماتودا تعقد الجذور *M arenaria* .أوضحت النتائج أن جميع المعاملات قللت بشكل ملحوظ من تكاثر نيماتودا تعقد الجذور مقارنة بالمعاملة الضابطة (الكنترول) ، تم تسجيل أعلى انخفاض فى معدل تكاثر النيماتودا وكذلك اعداد العقد وكتل البيض على الجذر مع هيومات الأمونيوم (معاملة تربة) 3 ٪ ، كما سجلت نباتات الباذنجان المعاملة بهيومات الأمونيوم 3٪ (معاملة تربة) أعلى قيمة في إجمالي الوزن الطازج والجاف للنبات، تليها النباتات التي تم رشها بهيومات البوتاسيوم 3%وأعطت هذه النباتات أعلى قيمة من الكاروتينات والمركبات الفينولية الحرة والأحماض الأمينية الحرة، في حين ظهرت أعلى قيمة لمحتوى البرولين مع نباتات الكنترول ،كما أوضحت النتائج زيادة المحتوى الكلى للكلوروفيل والبروتين و السكر المختزل فى جميع المعاملات مقارنة بالكنترول.