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Nematicidal performance of certain organic and inorganic compounds against *Meloidogyne incognita* infecting okra plants

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Abstract

The present study was carried out to evaluate the nematicidal activity of some organic and inorganic compounds, as well as fosthiazate as a standard nematicide for their hatching inhibitory and juvenile mortality potential and to ascertain their role as organic amendments for the management of root-knot nematode (*Meloidogyne incognita*) affecting okra plants *in vitro* as well as *in vivo* conditions. Five concentrations of all chemicals were prepared. Data of *in vitro* studies showed a marked nematicidal and nematode hatching inhibitory activity against root-knot nematode (*M. incognita*). However, the nematicidal activity differed between treatments as compared to control. Fosthiazate was found as the most toxic compound against J_2 of *M. incognita* after 24 and 48 hour exposure time as well as inhibited the egg hatching significantly after 2, 5 and 7 days of exposure. Moreover, the juvenile mortality increased with increase in concentration of chemical compounds and the exposure time. *In vivo* studies data showed that all treatments reduced root galls, egg-masses, number of J_2 in soil, total eggs, final population and reproduction rate significantly. Meanwhile, the applied treatments exhibited enhancement in plant growth parameters and decreased the host infection by *M. incognita* over control. Among all the treatments Fosthiazate proved to be the best treatment while cattle manure was found as the least significant. The biochemical response of treated plants showed that infected plants with *M. incognita* recorded the least increase of total phenols (0.42 mg/g) and highest suppression in total proteins (10.41 mg/g), total sugars (7.86 mg/g) and reduced sugars (3.09 mg/g) as compared with untreated check. Moreover, citric acid was the most effective treatment which exhibited the highest values of total phenols (0.69 mg/g), total protein (18.08 mg/g), total sugar (17.89 mg/g) and reduced sugar (10.05 mg/g), whereas cattle manure gave the least values of total phenols (0.47 mg/g), total protein (11.70 mg/g), total sugar (9.74 mg/g) and reduced sugar (4.39 mg/g).

Keywords: *Meloidogyne incognita*, okra plants, organic acids, amino acids, growth regulators, mineral fertilizer, organic matters, Fosthiazate.

Okra (*Abelmoschus esculentus* L.) is an important vegetable crop of the world belonging to the family Malvaceae. Okra is one of the most popular, widely cultivated summer vegetables in Egypt. It is considered as a valuable source of calcium, iron and vitamins. The total world planting area of okra was1.83 million hectares in 2014, while the total area in Egypt was 47.47 hectares (FAO STAT, 2014). Okra crop is infested by several diseases and pests which cause great damages. Root-knot nematodes (*Meloidogyne* sp.) are considered one of the serious pathogens that cause quality defects and reduced the crop value. Root-knot nematodes cause annual losses of about US $100 billion worldwide (Brand *et al*., 2010). They are

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considered an economically important group of soil borne pathogens because they are difficult to control due to their wide host range which includes more than 2000 hosts such as vegetables, fruits, cereals, oil and fibre crops and high rate of reproduction. Since root-knot nematodes are of great economic importance, much attention has been directed to their control. Among various management strategies, the use of synthetic nematicides has been found to be highly effective and reliable in controlling a wide range of nematodes. Continuous use of these nematicides causes phytotoxicity, environmental pollution problems, soil infertility and adverse effects on non-target organisms. Chemical nematicides have also adverse effects on human life. Moreover, there has been a considerable pressure to eliminate the use of the chemical pesticides. Therefore, there is an urgent need to find alternatives, environment friendly and easily degradable compounds for effective nematode control (Noling & Becker, 1994). The usage of mineral salts, organic acids, amino acids, growth regulators, mineral fertilizer, and organic matters are probably one of the possible novel alternatives and environmentally safe management practices against plant parasitic nematode infestations, as well as promoting plant growth and increasing its resistance (Pankaj & Sharma, 2003; Al-Ghonaimy & Zawam, 2016; Radwan *et al*., 2017). In addition the utilization of organic amendments such as cattle, chicken, sheep and horse manures and others were very effective tools against plant parasitic nematodes at different crops (Ismail & Mohamed, 2012; Renco, 2013; Abdel-Bary *et al*., 2014). This investigation aimed to study the nematicidal impacts of some mineral salts, organic acids, amino acids, growth regulators, mineral fertilizer and organic matters against root-knot nematode, *Meloidogyne incognita in vitro* and *in vivo* conditions.

Materials and Methods

Root-knot nematode culture and inoculum: Root-knot nematode inoculum used in the present study was obtained from infested roots of eggplant (*Solanum melongena* cv. Black Beauty) which were collected from El-Nobarya region, El-Behaira Governorate, Egypt. Culture of *Meloidogyne incognita* (Kofoid & White) was maintained on eggplants (*Solanum melongena* cv. Black Beauty) growing in 20 cm diameter clay pots filled with steam sterilized soil (1 clay: 2 sand (v /v)).

Infected plants were placed on a bench in outdoor conditions, and watered regularly. Two months later, the infected roots were uprooted and cut down into pieces and used as sources of inoculation for other healthy seedlings of eggplant and for the experimental purposes. Nematodes eggs were obtained from galled roots of egg plants. The galled roots were washed from the adhering soil practices by running water and the roots were cut into small pieces, and then shaken for 3 minutes in 0.5% sodium hypochlorite (NaOCl) solution to dissolve the gelatinous matrix of egg-masses and to get free nematode eggs from the mass matrices (Hussey & Barker, 1973). To obtain the second stage juveniles (J_2s), the eggs were hatched under laboratory conditions using Baermann plates technique (Ayoub, 1980). The collected J_2 were used in laboratory experiments

Source of materials for experimentation: In the investigation certain mineral fertilizers (di-potassium hydrogen orthophosphate, di-sodium hydrogen orthophosphate, potassium sulphate, urea and super phosphate), organic acids (salicylic acid and citric acid), amino acids (glycine and cysteine) and growth regulators (gibberellic acid and indole 3- butyric acid) were obtained from the Central Agricultural Pesticide Laboratory, Research Center, Giza, Egypt.

On the other hand, fosthiazate (Nemathoren®10% G) was obtained from Syngenta Agro., while cattle and chicken manures were obtained from the Farm at Etay El-Baroud zone, El-Baheria Governorate, Egypt. The above mentioned organic matters were dried, grinded and stored.

Evaluation of best control concentration *in vitro* studies: For *in vitro* studies efficacy of the tested compounds was evaluated. Five concentrations of each compound were prepared viz., 312.5, 625, 1250, 2500 and 5000 mg/l. A volume of 10 ml from each concentration was transferred to Petri-dishes which contained 1000 eggs or 200 newly hatched J_2s of *M. incognita*.

Four replicates per each concentration were used and free distilled water served as untreated check. In case of the Nemathorin® treatment, the tested concentrations were; 6.25, 12.5, 25, 50 and 100 mg/l.

In larval mortality test the dead and alive juveniles were counted after 24 and 48 hrs of exposure. Whereas, the egg hatching was estimated after 2, 5 and 7 days. The percentages of mortality were corrected according to Abbott formula (1925).

***In vivo studies*:** A pot experiment was carried out to manage the *M. incognita* on okra as host plant using some organic acids, amino acids, growth regulators, mineral fertilizers and animal manures in comparison with fosthiazate. The clay pots (15 cm in diameter) were filled with autoclaved loamy sand soil.

The pots were then arranged in a complete randomized block design on a bench in outdoor conditions. Each pot was sown with two seeds of okra plants and later thinned to one plant per pot at one week after sowing. The experimental treatments with both inoculated and un-inoculated checks (each replicated four times) were conducted as follows:

1- Uninoculated check (control)
2- Inoculated with *M. incognita* alone
3- *M. incognita* + Salicylic acid
4- *M. incognita* + Citric acid
5- *M. incognita* + Gibberellic acid
6- *M. incognita* + Indole 3- butyric acid
7- *M. incognita* + Glycine
8- *M. incognita* + Cysteine
9-*M.incognita* + dipotassium hydrogen orthrophosphate
10- *M. incognita* + disodium hydrogen orthrophosphate
11- *M. incognita* + Potassium sulphate
12- *M. incognita* + Urea
13- M. incognita + Super phosphate
14- *M. incognita* + Cattle manure
15- *M. incognita* + Chicken manure
16- *M. incognita* + Fosthiazate

All treatments were applied as soil drench two days before inoculation. Two week old seedlings of okra were inoculated with 5000 nematode eggs after two days from treatments. The chemical compounds were applied at the rate of 0.5 g/pot except mineral fertilizers Potassium sulphate, urea and Super phosphate were applied at the rate of 1, 1.1 and 4g/ kg soil, and nematicide was applied at rate of 0.01 g/ kg soil.

The organic matters were applied at the rate of 50 g/ kg soil. On the termination of experiments, observations were recorded 45 days of inoculations by determining the number of galls per root system, number of egg-masses, number of total eggs/ root system and number of 2^{nd} stage juveniles / kg soil. Moreover, the okra plant growth indices such as shoot and root length (cm) and shoot and root dry weights g / plant were also recorded.

Biochemical response of host plant in response to different treatments

Preparation of root extracts: At the end of experiment, 1g fresh roots were obtained from all treatments. Each sample was finely chopped and immersed in hot ethanol (95%) for 10 minutes to kill tissue. Root extracts were subjected to Soxhlet Apparatus using Ethyl Alcohol (75%). Alcoholic extractions were filtrated and evaporated on mild water bath at 60°C. The dried residue was re-dissolved in 5 ml of 50% isopropanol. These extracts were then used for determination of total sugars, and phenol content as follows:

Determination of total sugars: The determination of total sugar was carried out

according to Malik & Singh (1980). Sugars were extracted from 5g fresh weight and determined by phenol sulfuric and Nelson arsenate-molybadate colorimetric methods for total and reducing sugars, respectively. The non-reducing sugars were calculated by difference between total sugars and reducing sugars.

Determination of phenolic compounds: Phenolic compounds were determined using the colorimetric method of Folin-Denis reagent described by Snell & Snell (1953). The colour intensity was recorded using spectrophotometer in the presence of a blank (containing all reagents without the extracts) at the wave length of 560 nm. The concentrations of free and total phenols were calculated as mg caticol/g of the fresh weight. Values were obtained from standard curve constructed for caticol in an identical way.

Statistical analysis: Obtained data results were analyzed using proc ANOVA in SAS. Mean Separation were conducted using Duncan's multiple range test in the same program.

Results and Discussion

The toxicity of the tested inorganic and organic compounds namely; salicylic acid, citric acid, glycine, cysteine, Gibberellic acid (GA), Indole 3- butyric acid (IBA), Di-potassium hydrogen orthophosphate (DKO), Di-sodium hydrogen orthophosphate (DSO), Potassium sulphate (KS), urea and Super phosphate (SP), as well as the nematicide fosthiazate were evaluated against the second stage juveniles (J_2) of *Meloidogyne incognita* under laboratory conditions during different intervals (24 and 48 hrs). The tested concentrations of all chemical compounds were 312.5, 625, 1250, 2500 and 5000 mg/l, while fosthiazate was assessed at the concentrations of 6.2, 12.5, 25, 50, and 100 mg/l. Data of *in vitro* studies showed a marked nematicidal and nematode hatching inhibitory activity against root-knot nematode (*M. incognita*). However, the nematicidal activity differed between treatments as compared to control. Fosthiazate was found as the most toxic compound against J_2 of *M. incognita* after 24 and 48 hour exposure time as well as inhibit the egg hatching significantly after 2, 5 and 7 days of exposure. Moreover, the juvenile mortality increased with increased in concentration of chemical compounds and the exposure time. *In vivo* studies data showed that all treatments reduced root galls, egg-masses, numbers of J_2 in soil, total eggs, final population and reproduction rate significantly. Meanwhile, the applied treatments exhibited enhancement in plant growth parameters and decrease the host infection by *M. incognita* over control. Among all the treatments Fosthiazate proved to be the best treatment while cattle manure was found as the least significant. The results proved that the toxicity of tested compounds increased gradually with the increment of concentration and the exposure time (Table 1).

The LC_{50} values indicated that fosthiazate was the most toxic compound based on J_2 mortality with 36.08 mg/l after 24 hrs of exposure. While SP was the least toxic compound with values of 10861.39 mg/l after 24 hrs. The remaining treatments recorded values ranged from 3221.31 to 9905.73 mg/l. On the other hand, after 48 hrs of exposure the LC_{50} values showed that fosthiazate was the most toxic compound with value of 10.06 mg/l, while the amino acid Cysteine was the least toxic compound with value of 7351.36 mg/l. Rest of the treatments recorded LC_{50} values ranging from 2169.90 to 6360.56 mg/l after 48 hrs of exposure (Table 1).

Data presented in Table 2 clearly indicate that the fosthiazate was the most efficient treatment which inhibited egg hatching after 2, 5 and 7 days of exposure with LC_{50} values of 24.82, 2.48 and 0.33 mg/l, respectively. The corresponding LC_{50} values of IBA, SP and SP were the least effective treatments having 9010.66, 8912.69 and 5866.06 mg/l after 2, 5 and 7 days of exposure, respectively. The results presented in Table 3 illustrate the impact of certain chemical compounds namely; salicylic acid, citric acid, glycine, cysteine, Gibberellic acid (GA), Indole 3- butyric acid (IBA), Di-potassium hydrogen

Table 1. LC_{50}, slop, Fiducial Limits and Regression equation of inorganic and organic compounds tested against *Meloidogyne incognita* juveniles for 24 and 48 hrs under laboratory conditions.

Treatments	Exposure time (hrs.) of juveniles			
	24 hrs			
	LC_{50} (mg/l)	Fiducially Limits (Upper - Lower)	Slop ± variance	Regression equation
Citric acid	3221.31	3784.14 - 2743.14	1.64 ± 0.02	Y = -5.74 + 1.64 X
Salicylic acid	4806.20	5930.98 - 3897.01	1.62 ± 2.39	Y = -5.95 + 1.62 X
GA*	8456.60	12347.59 - 5802.40	1.21 ±1.90	Y = -4.76 + 1.21 X
IBA	9905.73	15253.97 - 6447.06	1.21± 2.15	Y = -4.82 + 1.21 X
Glycine	8250.80	11495.40 - 5928.05	1.64 ± 4.55	Y = -6.43 + 1.64 X
Cysteine	8355.09	11661.15 -5991.75	1.81± 6.48	Y = -7.09 + 1.81 X
DKO	5162.58	6493.01 - 4107.60	1.56 ± 2.51	Y = -5.80 + 1.56 X
DSO	6492.79	8597.59 - 4907.99	1.50 ± 2.68	Y = -5.70 + 1.50 X
KS	5996.21	7726.24 - 4657.06	1.63 ± 3.15	Y = -6.14 + 1.63 X
Urea	3539.71	3964.38 - 3160.80	3.18 ± 0.10	Y = -11.30 + 3.18 X
SP	10861.39	17157.82 - 6889.45	1.37 ± 3.74	Y = -5.53 + 1.37 X
Fosthiazate	36.08	40.95 - 31.79	1.67± 1.44	Y = -2.61 + 1.67 X
	48 hrs			
Citric acid	2169.90	2478.69 - 1899.89	1.69 ± 0.02	Y = -5.63 + 1.69 X
Salicylic acid	3131.20	3676.54 - 2667.62	1.64 ± 1.91	Y = -5.74 + 1.64 X
GA	4517.48	5672.48 - 3600.35	1.42 ± 1.91	Y = -5.19 + 1.42 X
IBA	4935.59	6228.04 - 3914.28	1.53 ± 2.34	Y = -5.65 + 1.53 X
Glycine	3656.15	4379.39 - 3053.68	1.57 ± 2.01	Y = -5.60 + 1.57 X
Cysteine	7351.36	10186.37 - 5311.90	1.46 ± 3.03	Y = -5.63 + 1.46 X
DKO	3687.40	4543.43 - 2994.60	1.37± 1.66	Y = -4.89 + 1.37 X
DSO	5780.82	7660.64 - 4367.07	1.34 ± 2.09	Y = -5.05 + 1.34 X
KS	3625.72	4412.30 – 2981.00	1.51± 1.98	Y = -5.38 + 1.51 X
Urea	4693.49	5959.257 - 3699.61	1.43 ± 2.04	Y = -5.24 + 1.43 X
SP	6360.56	8640.12 - 4688.19	1.37 ± 2.41	Y = -5.20 + 1.37 X
Fosthiazate	10.06	12.63 - 7.78	1.14 ± 1.20	Y = -1.15 + 1.14 X

*Gibberellic acid (GA), Indole 3- butyric acid (IBA), Di-potassium hydrogen orthophosphate (DKO), Di-sodium hydrogen orthophosphate (DSO), Potassium sulphate (KS) and Super phosphate (SP).

Table 2. The LC_{50} values, fiducial limits, slop and Regression equation of inorganic and organic compounds against eggs hatching of *Meloidogyne incognita* under laboratory conditions after 2, 5 and 7 days of exposure.

Treatments	Exposure time (days) for eggs			
	2 days			
	LC_{50} (mg/l)	Fiducially Limits (Upper - Lower)	Slop ± variance	Regression equation
Citric acid	2251.06	2499.54 - 2027.55	1.08 ± 3.34	Y = -3.63 + 1.08 X
Salicylic acid	3896.67	4342.10 - 3497.50	1.37 ± 5.05	Y = -4.93 + 1.37 X
***GA**	4092.78	4576.89 - 3660.54	1.34 ± 4.37	Y = -4.83 + 1.34 X
IBA	9010.66	11089.18 - 7325.32	1.21 ± 6.31	Y = -4.80 + 1.21 X
Glycine	4002.16	4532.24 - 3534.93	1.16 ± 3.67	Y = -4.18 + 1.16 X
Cysteine	7302.31	8736.09 -6106.22	1.27 ± 6.48	Y = -4.91 + 1.27 X
DKO	2324.36	2548.62 - 2120.03	1.19 ± 3.33	Y = -4.01 + 1.19 X
DSO	5562.78	6490.66 - 4769.20	1.16 ± 4.52	Y = -4.36 + 1.16 X
KS	6494.57	7682.73 - 5492.37	1.12 ± 3.94	Y = -4.27 + 1.12 X
Urea	7466.17	8872.78 - 6284.73	1.32 ± 6.60	Y = -5.13 + 1.32 X
SP	8982.87	10974.46 - 7355.95	1.30 ± 7.36	Y = -5.15 + 1.30 X
Fosthiazate	24.82	29.79 - 20.69	0.48 ± 2.13	Y = -0.68 + 0.48 X
		5 days		
Citric acid	1245.70	1387.51 - 1118.32	0.90 ± 2.60	Y = -2.79 + 0.90 X
Salicylic acid	2393.08	2656.60 - 2156.04	1.08 ± 3.03	Y = -3.65 + 1.08 X
GA	3172.48	3630.81 - 2772.86	0.94 ± 2.81	Y = -3.28 + 0.94 X
IBA	6961.08	8668.57 - 5594.14	0.86 ± 2.98	Y = -3.31 + 0.86 X
Glycine	3156.24	3491.88 - 2853.23	1.43 ± 5.96	Y = -5.01 + 1.43 X
Cysteine	6114.63	7223.84 - 5177.83	1.11 ± 4.02	Y = -4.19 + 1.11 X
DKO	1542.93	1762.89 -1350.54	0.69 ± 2.28	Y = -2.20 + 0.69 X
DSO	3426.61	3928.56 - 2989.74	0.94 ± 2.84	Y = -3.34 + 0.95 X
KS	3688.32	4218.73 - 3225.58	1.03 ± 3.07	Y = -3.67 + 1.03 X
Urea	4995.27	5761.15 - 4332.57	1.12 ± 3.60	Y = -4.15 + 1.12 X
SP	8912.69	11186.35 - 7106.04	0.99 ± 3.95	Y = -3.92 + 0.99 X
Fosthiazate	2.48	3.56 - 1.71	0.63 ± 2.40	Y = -0.25 + 0.63 X
		7 days		
Citric acid	582.47	676.89 - 501.01	0.75 ± 2.36	Y = -2.01 + 0.76 X
Salicylic acid	1392.97	1527.20 - 1270.56	0.99 ± 0.02	Y = -3.14 + 0.99 X
GA	1797.25	2016.43 - 1602.13	0.81 ± 2.33	Y = -2.65 + 0.81 X
IBA	2964.95	3367.33 - 2611.35	0.89 ± 2.41	Y = -3.11 + 0.89 X
Glycine	1496.25	1639.33 - 1365.72	1.00 ± 2.31	Y = -3.18 + 1.00 X
Cysteine	4771.00	5747.10 - 3963.03	0.83 ± 2.63	Y = -3.04 + 0.83 X
DKO	468.95	557.58 - 394.17	0.71 ± 2.23	Y = -1.91 + 0.71 X
DSO	1747.89	1931.53 -1581.87	0.96 ± 2.53	Y = -3.13 + 0.96 X
KS	2404.99	2700.21 - 2142.51	0.91± 2.27	Y = -3.07 + 0.91 X
Urea	3374.82	4007.61 -2843.44	0.71± 2.33	Y = -2.52 + 0.71 X
SP	5866.06	7146.25 - 4818.29	0.83 ± 2.49	Y = -3.12 + 0.83 X
Fosthiazate	0.33	0.72 - 0.15	0.60 ± 3.24	Y = 0.29 + 0.60 X

*Gibberellic acid (GA), Indole 3- butyric acid (IBA), Di-potassium hydrogen orthophosphate (DKO), Di-sodium hydrogen orthophosphate (DSO), Potassium sulphate (KS) and Super phosphate (SP).

orthophosphate (DKO), Di-sodium hydrogen orthophosphate (DSO), Potassium sulphate (KS), urea, Super phosphate (SP), cattle and chicken manures, as well as the nematicide, fosthiazate against *Meloidogyne incognita* infecting okra plants. All treatments showed different degree of nematicidal activities comparing to infected control.

The infected check untreated recorded that the highest mean numbers in galls (291.75), second stage juveniles (11345.5), egg-masses (248.25), total eggs (147163), final population (158756.75) and reproduction rate (31.75). As general trend fosthiazate was the superior treatment which significantly suppressed the numbers of galls (58), second stage juveniles (955.25), egg-masses (23), total eggs (4315.5) and final population (5293.75). Meanwhile, the least reproduction rate was recorded with fosthiazate (1.06). Moreover, the cattle manure was the least effective treatment which reduced the numbers of galls (196.75), second stage juveniles (9072), egg-masses (163.75), total eggs (91081.25) and final population (100317), as well as the reproduction rate was 16.42. The number of root galls were significantly suppressed and ranged from 83.25 to 185.25, while the numbers of second stage juveniles (J_2) ranged from 2184.25 to 8366.25. Furthermore, the numbers of egg-masses was reduced and ranged from 40 to 139.5. The total numbers of eggs/root were from 73598.5 to 11106. Also, the number of final population was ranged from 82104.25 to 13330.25. The calculated reproduction rate showed range from 16.42 to 2.66.

The present findings are in agreement with those of Charehgani *et al*., (2010) who found that chemical fertilizers as nitrogen, phosphorus, iron and zinc decreased eggs, egg-masses and galls of *Meloidogyne javanica* on cucumber under greenhouse conditions. Moreover, Castro *et al*., (1990) documented that simple inorganic salts of the ions K^+, NH^+_4, Cs^+, NO_3, and Cl were strongly repellent to infective second-stage larvae of the root-knot nematode, *Meloidogyne incognita*. These salts are known to be beneficial to plant growth.

Pakeerathan *et al*., (2009) found that goat manure and poultry manure minimized the gall formation of *M. incognita* by 89.09 and 64.72%, respectively. Whereas Faruk *et al*., (2011) evaluated the efficacy of poultry refuse to manage *M. incognita* on tomato. Poultry refuse at 3 and 5 ton/ feddan exhibited the reduction of galls by 48.77 and 58.92% for the first year, respectively, while in the second year the reduction increased to 58.53 and 72.41%, respectively. Similarly, it was reported that cattle manure, sheep manure and chicken manure as soil amendments at different rates of 2, 4 and 6 ton/ feddan reduced females, galls and egg-mass numbers of *M. arenaria* infesting sugar beet as compared to un-amended plants (Ismail & Mohamed, 2012). Moreover, Abdel-Bary *et al*., (2014) found that animal compost reduced final population of *Meloidogyne* sp., ranged from 73.57 to 92.18% and the plant compost caused reduction ranged from 71.92 to 92.54%. The usage of soil amendments is a conventional agricultural practice to control pathogens in soil, improve physical and chemical soil properties, soil structure, temperature and humidity conditions as well as nutrients content which are needful for plants growth. Application of organic materials to soil can cause a change in soil microflora and microfauna including soil nematodes (Renco, 2013). Although, the exact mechanism(s) of action for organic matter as soil amendment is not exactly known, but several authors stated that many mechanisms can be involved in plant parasitic nematodes suppression such as direct toxicity of degradation products, change the composition of the soil microbial community, modifying the relationships among microorganisms, both competitive and/or antagonistic, release of biocidal substances which have nematicidal activity or even enhance natural innate resistance within plants toward nematodes (Akhtar & Malik, 2000; Steinberg *et al*., 2004; Renčo *et al*., 2011). The influence of organic acids such as salicylic and citric acids

against plant parasitic nematodes were investigated in various studies (Zinov'eva *et al*., 2011; El-Sherif *et al*., 2015; Radwan *et al*., 2017). Zinov'eva *et al*., (2011) reported that the pre-planting salicylic acid treatment of tomato seeds resulted in an increased resistance of susceptible tomato cultivars to *M. incognita*. This protective effect is higher in the case of SA combined with chitosan. They mentioned that the increase in the resistance of tomato plants is related to the increased activity of phenylalanine ammonia-lyase and an increased SA content in plant tissues. It was found that organic acids such as salicylic, citric, humic and oxalic acids at two rates (0.1 and 0.05%) as foliar spray greatly suppressed the final population (Pf), egg-masses, galls, gall index, females and developmental stages of *Meloidogyne incognita* infecting tomato plants under greenhouse conditions. All applied treatments of organic acids induced the total phenol (El-Sherif *et al*., 2015). Recently, Radwan *et al*., (2017) stated that acetylsalicylic, salicylic and citric acids reduced the soil populations (J_2) by mean reduction of 93.71, 64.55 and 78.88%, respectively. They also found that the foliar spray was the most effective method in comparison with soil drench to induce resistance against *M. incognita*. Application of salicylic acid reduced tomato root galls and eggs of *M. incognita* (Mukherjee *et al*., 2012). Seed treatment or soil drench of 50 µM salicylic acid caused marked reduction in egg-masses of *M. javanica* on the roots of tomatoes (Mostafanezhad *et al*., 2014). Many researchers documented the efficacy of fosthiazate against root-knot nematodes, *Meloidogyne* spp. (Ibrahim *et al*., 2010; Radwan *et al*., 2012; Raddy *et al*., 2013; Saad *et al*., 2017). Saad *et al*., (2011) found that fosthiazate was effective in reducing the population of *M. incognita* in the soil and tomato root galls by 97.8 and 71.3%, respectively. Khalil (2012) found that fosthiazate significantly reduced J_2 of *M. incognita* in the soil and galls by 74.7 and 57.9%, respectively. Moreover, the author declared that the combination between fosthiazate and *Glomus intraradices* (AM), each at half dose reduced the population and galls formation compared to the control treatment. Liu *et al*., (2014) studied the potential of two nematicides (fosthiazate and Dazomet), a biocontrol agent (*Paecilomyces lilacinus*, YES-2), and their combination for controlling root-knot nematodes on tomato plants and their effects on the rhizosphere microbial community. The individual applied treatments of fosthiazate, Dazomet or *P. lilacinum* had significantly reduced the gall index and root-knot nematode population in the soil. Otherwise, the microbial community in the soil increased in the treatments of *P. lilacinus* alone or its combination with chemicals. Data presented in Table 4 clarified the biochemical response of okra plants to infection by *M. incognita* and to the applied compounds. The uninoculated plants represented the normal levels of estimated biochemicals i.e. total phenols, total protein, total sugar and reduced sugar with values of 0.36 mg/g, 24.13 mg/g, 21.79 mg/g and 13.99 mg/g, respectively. Furthermore, it was noticed that plant infected with *M. incognita* caused the least values in all measured parameters as total phenols (0.42 mg/g), total protein (10.41 mg/g), total sugar (7.86 mg/g) and reduced sugar (3.09 mg/g) in comparison with all other treatments. The plants treated with citric acid recorded the highest value of total phenols (0.69 mg/g), while SP and cattle manure were recorded the least induction in total phenol with value of 0.47 mg/g for both. The rest treatments recorded values ranging from 0.65 to 0.47 mg/g. These results are in agreement with El-Sherif *et al*., (2015) who found that application of some organic acids such as salicylic, citric, oxalyic and humic acids induced the total phenol content in tomato plants ranged from 1.81 to 10.52% over control.

In respect to total protein, it ranged from 18.08 to 11.70 mg/g. The plants grown in soil treated with citric acid recorded the highest value with 18.08 mg/g of total protein. Moreover, cattle manure recorded the less value with 11.70 mg/g. In the same context, citric acid showed the highest values in total sugars by 17.89 mg/g, whilst cattle manure treatment recorded the least level of total sugars with value

Table 3. Effect of certain mineral salts, organic acids, amino acids, growth regulators, organic matters, mineral fertilizers and fosthiazate on the reproduction of *M. incognita* infecting okra plants.

Treatment	Dose/ pot (g)	No. of galls / root system	No. of J_2/ kg soil	No. of egg mass / root system	Total eggs/ root system	Final population (Pf)	R
Salicylic acid	0.5	105.5h	3498.75i	56.5jk	21391.5kl	24584.25kl	4.92kl
Citric acid	0.5	83.25j	2184.25j	40l	11106m	13330.25m	2.66m
GA*	0.5	105h	3614.75hi	62.75j	24688jk	28365.5jk	5.67jk
IBA	0.5	116.75g	4402g	76.5i	33348.25i	37826.75i	7.56i
Glycine	0.5	114.75g	3992.25gh	71.25i	28379.5j	32443j	6.48j
Cysteine	0.5	133f	5178.25f	85.5h	38706.25h	43970h	8.79h
DKO	0.5	93.5i	2498.75j	54.5k	18132.75l	20686l	4.14l
DSO	0.5	139.75f	5362f	94.5g	44902.25g	50358.75g	10.07g
KS	1	150.25e	5650f	105f	50153.25f	55908.25f	11.18f
Urea	1.1	172.5d	6404.75e	123e	62037.5e	68565.25e	13.71e
SP	4	178.25d	7215.5d	130.5d	67057d	74403d	14.88d
Manure-1	50	196.75b	9072b	163.75b	91081.25b	100317b	20.06b
Manure -2	50	185.25c	8366.25c	139.5c	73598.5c	82104.25c	16.42c
Fosthiazate	0.01	58k	955.25k	23m	4315.5n	5293.75n	1.06n
Check	-	291.75a	11345.5a	248.25a	147163a	158756.75a	31.75a

Cattle Manure=Manure-1, Chicken Manure=Manure-2; R= Reproduction rate

Table 4. Efficacy of certain mineral salts, organic acids, amino acids, growth regulators, organic matters, mineral fertilizers and a nematicide on some chemical components of okra plants.

Treatment	Dose/ pot (g)	Total Phenols (mg/g)	Total Protein (mg/g)	Total sugars (mg/g)	Reduced sugars (mg/g)
Salicylic acid	0.5	0.60cde	16.89cd	14.70cde	7.37de
Citric acid	0.5	0.69a	18.08b	17.89b	10.05b
GA*	0.5	0.63bc	16.52de	16.31bc	8.38cd
IBA	0.5	0.56de	15.61fg	14.38def	6.51e
Glycine	0.5	o.58de	17.63bc	15.39cd	9.58bc
Cysteine	0.5	0.58de	15.78ef	13.34efg	6.63e
DKO	0.5	0.65ab	15.56fg	15.08cd	8.76bc
DSO	0.5	0.58de	14.84ghi	13.20efg	6.48e
KS	1	0.55ef	15.34fgh	12.88fg	6.31e
Urea	1.1	0.52f	15.03fgh	11.75gh	5.95e
SP	4	0.47g	14.66hi	10.66hi	4.64f
Cattle manure	50	0.47g	11.70j	9.74i	4.39f
Chicken manure	50	0.55ef	14.09i	11.67gh	6.27e
Fosthiazate	0.01	0.60cd	17.73b	16.12cd	9.56bc
Untreated check	-	0.42h	10.41k	7.86j	3.09g
Uninoculated plants	-	0.36i	24.13a	21.79a	13.99a

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05

Table 5. Effect of certain mineral salts, organic acids, amino acids and growth regulators, organic matters, mineral fertilizers and a nematicide on growth indices of okra plants infected with *M. incognita*.

Treatment	Dose/pot (g)	Shoot dry weight (g)	*Increase (%)	Shoot length (cm)	Increase (%)	Root dry weight (g)	Increase (%)	Root length (cm)	Increase (%)
Salicylic acid	0.5	3.60cde	313.79	47.50cd	32.87	1.28bcd	161.22	24.25def	56.45
Citric acid	0.5	3.75bcd	331.03	49.00bc	37.06	1.39b	183.67	26.00bcd	67.74
GA	0.5	4.22b	385.06	51.00b	42.66	1.397b	179.59	28.00b	80.65
IBA	0.5	4.01bc	360.92	50.25bc	40.56	1.30bc	165.31	27.25bc	75.81
Glycine	0.5	3.51cde	303.45	45.75de	27.97	1.28bcd	161.22	22.75efg	46.77
Cysteine	0.5	3.16ef	263.22	44.25e	23.78	1.18cde	140.82	22.00fg	41.94
DKO	0.5	3.86bc	343.68	49.00bc	37.06	1.30bc	165.31	25.00cde	61.29
DSO	0.5	2.98f	242.53	43.75e	22.38	1.20cde	144.90	22.75efg	46.77
KS	1	3.26def	274.71	44.75de	25.17	1.21cde	146.94	23.25efg	50.00
Urea	1.1	2.89f	232.18	43.00e	20.28	1.13de	130.61	22.50efg	45.16
SP	4	2.82f	224.14	42.75e	19.58	1.09e	122.45	22.00fg	41.94
Cattle manure	50	1.48g	70.11	39.50f	10.49	0.69g	40.82	18.50h	19.35
Chicken manure	50	2.88f	231.03	42.50e	18.88	0.92f	87.76	21.00g	35.48
Fosthiazate	0.01	3.86bc	343.68	49.25bc	37.76	1.38b	181.63	26.25bcd	69.35
Untreated check	-------	0.87h		35.75g		0.49h		15.50i	
Uninoculated plants	------	5.44a	525.29	56.00a	56.64	1.73a	253.06	34.00a	119.35

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05; * Increase (%) $= \left(\frac{\text{Treatment}-\text{control}}{\text{control}}\right) \times 100$

of 9.74 mg/g. The remaining treatments recorded values between 10.66 to 16.31 mg/g. The efficacy of applied treatments on reduced sugars was estimated and the values ranged from 10.05 to 4.39 mg/g. The obtained results showed that citric acid occupied the first rank with value of 10.05 mg/g. While plants treated with SP (4.64 mg/g) and cattle manure (4.39 mg/g) came in the last rank with the least values of reduced sugars.

Regarding the effect of the tested compounds and fosthiazate on okra growth characteristics depicted in Table 5 all treatments exhibited significant differences ($p \leq 0.05$) in increasing the okra shoot dry weight ranging from 224.14 to 525.29%, except for cattle manure that recorded the least increasing by 70.11% in comparable to control treatment. Also, all of the evaluated treatments significantly ($p \leq 0.05$) increased the shoot length ranging from 10.49 to 56.64%. Cattle and chicken manures were recorded the least increase in root dry weight by 40.82 and 87.76%, respectively. Rest of the treatments significantly enhanced the root dry weight ranging from 122.45 to 253.06% over inoculated control treatment. All tested treatments recorded augmentation in root length significantly ranged from 41.94 to 119.35%, except the treatments of cattle and chicken manures which gave the less increase by 19.35 and 35.48%, respectively.

It was confirmed that use of chemical fertilizers as nitrogen, phosphorus, iron and zinc caused a significant increase in the plant shoot length, shoot fresh and dry weight of cucumber (Charehgani *et al*., 2010). Furthermore, Zinov'eva *et al*., (2011) reported that salicylic acid stimulated the growth and development of tomato plants. Pankaj & Sharma (2003) found that plant growth of okra significantly increased at 50 and 100 µg salicylic acid/ml whether applied as seed soak, drench and spray and was at par with carbofuran treatment. However, there was a decrease in plant growth with higher SA concentrations. The authors suggested that SA might have induced some resistance in okra against *M. incognita*. El-Sherif *et al*., (2015) documented that organic acids enhanced the fresh and dry weight in addition to lengths of tomato plants significantly over control.

These findings are in agreement with the results of Ibrahim *et al*., (2010) who found that oxamyl and fosthiazate significantly increased the length and weight of tomato shoot system. While Saad *et al*., (2011) found that fosthiazate enhanced tomato growth indices. Our results are at par with the data obtained by several scientists; Radwan *et al*., (2012), Raddy *et al*., (2013) and Mostafa *et al*., (2015). They indicated that some of non-fumigant nematicides enhanced plant growth criteria.

It could be concluded that citric acid recorded suitable effect against the root-knot nematode (*M. incognita*) significantly, as well as improving the plant growth. Also, citric acid induced the chemical responses as total phenols, total protein, total sugars and reduced sugars significantly. Citric acid can be considered a tool in integrated pest management (IPM) program against plant parasitic nematodes.

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