Inducing resistance in eggplant against *Meloidogyne incognita* by organic and inorganic fertilizers, plant growth regulators and amino acids

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Abstract

Organic and inorganic fertilizers, commercial products containing organic and amino acids, vitamins as well as plant growth regulators were tested for their ability to induce nematode resistance in eggplant. Results indicated that organic fertilizers are significantly better than inorganic ones in reducing all the root-knot nematode counts with superiority to compost, Hyper K® and Union zinc® were the best inorganic fertilizers, although, they all could not reach the effect of vydate 10% G. All the tested commercial products containing organic and/or amino acids as well as plant growth regulators reduced significantly *M. incognita* counts. Indole acetic acid and indole butyric acid preceded all the tested materials in enhancing the resistance of eggplant against the root-knot nematode followed by ascorbic acid, amino zinc and citric acid. Results also proved that organic fertilizers (especially compost) were the best in improving plant growth. Other organic and inorganic materials significantly improved eggplant growth. Indole acetic acid, indole butyric acid, ascorbic acid, citric acid and amino zinc performed the best results.

Oxidant lipid peroxidase (MDA) was at the lowest values in roots of healthy plants and at the highest ones in the infected roots of untreated plants. Application of all the tested materials could preclude the formation of MDA. Only indole acetic acid, compost and indole butyric acid, in that order, brought the levels of MDA in infected roots to be near to that of the healthy plants without significant differences. The antioxidant enzymes SOD and APX increased in infected plants as a feed back to the increase in MDA. Also indole acetic acid, indole butyric acid and compost encouraged plants to produce levels of SOD and APX significantly higher than those of other treatments including healthy plants. The other tested materials heighten the levels of antioxidant enzymes to levels measured up the degree of nematode control they accomplished.

Key words: Resistance, *Meloidogyne*, Oxidant and antioxidant enzymes.

Introduction

Solanaceous plants are the most known susceptible plants to the root-knot nematodes, *Meloidogyne* spp. of which eggplant is the most common damaged by

such nematodes. The root-knot nematode depend upon their development in their hosts on the formation of feeding sites e.g. giant cells. The success of nematode reproduction on compatible hosts depend on the successful formation of such feeding sites which rely on the availability of certain concentrations of some chemicals and enzymes **(Baldacci-crespe*t al*., 2012)** to be available in host tissues. Consequently, any changes in such chemicals and/or enzymes due to pathogens or different stresses on the cultivated plants may affect the formation of such feeding sites.

In plants attacked by nematodes, selective changes occur in the metabolism either as consequence of the establishment of a susceptible host-pathogen interaction or as a result of resistance between host and parasite. Several models for resistance/susceptibility have been developed based on biochemical changes **(Giebel, 1982 and Zacheo*et al.*, 1987).** There are many reports of enhanced peroxidases, polyphenoloxidase, and ascorbic acid oxidase following the interaction of nematodes with their hosts especially the resistant ones and this has led to the hypothesis that these enzymes may be important in the defense mechanism of the host **(Saeed, 2005; Siahpoush*et al*., 2011, Aryal*et al.*, 2011 and El-Belatagi*et al.*, 2012).**

Generally, incompatibility to nematodes expressed after infection and active mechanisms involved compounds produced post-infection rather than performed constitutive plant products **(Kaplan and Keen, 1980).** Accordingly, plants develop defense mechanisms right away after nematode invasion. Most of these defense mechanisms are incompatible resistant interactions between plants and pathogens of which the formation of reactive oxygen species (ROS) are common **(Montes *et al*., 2004 and Bakker *et al*., 2006).** Such reactive oxygen species induced lipid peroxidation accounting for cell death after pathogen invasion. Infected plants exhibit both enzymatic and non enzymatic antioxidant defense systems to frustrate ROS upon nematode infection. The accumulation of such materials in root tissues enhanced resistance in plants against invasion with new nematode larvae **(El-Beltagi*et al*., 2012),**of these antioxidants GSH, SOD, catalase and ascorbate oxidase. Such processexpress the so called systemic acquired resistance (SAR).

Systemic acquired resistance is the ability of plants to become resistant after prior infection by pathogens, exposure to stress, or application of chemical inducers **(Stichere*t al*., 1997).** An initial recognition event leads to the production of signals translocated endogenously to plant parts that are remote from the initial site of infection. The mechanism may have been the result of biochemical substances that were elicited in one side of the root system where incompatible reaction occurred and then expressed systematically **(Chinnasri*et al*., 2006).**

Many reports in literature illustrating the role of different materials and chemicals as systemic acquired resistance inducers in plants against nematode

infection and reproduction. Of these, organic matter e.g. compost, organic manures of animal or plant origin **(Farahat, *et al*., 2010),** organic acids, e.g. salicylic, ascorbic, buteric, humic, folvic, citric **(Saeed,2005 &Kesba and El-Beltagi, 2012),** amino acids **(AmadulHoque*et al*., 2013)** and plant growth regulators **(Farahat, 1989)** are the most effective.

Materials and methods

1. Stock cultures

Pure stock culture of the root-knot nematode, *Meloidogyne incognita* originally obtained from galled eggplant roots was established. Single egg-masses from previously identified females **(Taylor *et al*., 1955)** were used to inoculate healthy eggplants grown in 20 cm clay pots filled with sterilized loamy sand soil. Two months after inoculation, plants were examined for nematode infection and reproduction. The culture was maintained on eggplant using infected roots with enough egg-masses for massive pure subcultures.

2. Test plants

Eggplant (*Solanummelongena*) hyb. Oneta F1 was used in the present study.

3. Materials and doses

Chicken manure, neem and eucalyptus leaves were collected from the Farm of the Faculty of Agriculture, Cairo University. Materials were air dried, ground and used at the rates of 5.0 or 10.0g /plant. Commercial forms of the following materials were purchased from the Egyptian market and applied at the doses illustrated in Table(1).

4.Green house experiment:

Seedlings of eggplant hyb. Oneta F1 were inoculated with 4000 J_2 of *M. incognita*. One week after nematode inoculation, the infected plants were treated with the organic, inorganic fertilizers, commercial formulations of amino and organic acids, vitamins and plant growth regulators with doses as illustrated in table (2). Each treatment was replicated 8 times and 8 inoculated plants were left without treatment as well as another 8 un-inoculated healthy plants to serve as check treatments. Pots were arranged in a complete randomized design on a clean bench in a greenhouse of 30°C ± 2 and horticulturally treated the same. Six weeks after nematode inoculation, plants were taken off and nematode counts in soil and on roots were enumerated. Plant growth criteria were recorded in four replicates. The plants of the other four replicates and both check treatments were sent to the laboratory for determination of the oxidant and antioxidant substances and enzyme activities.

Table (1): Materials, doses/ concentrations and methods of application.

Trade name	Company	Contents	Dose Plant	Method of application
Mega power	Union of Agricultural Development (UAD)	Humic acid 19%, folvic acid 2%,free amino acids 5%,chelated Zinc 0.5%, chelated Fe 0.025%, chelated Mn 0.05% and potassium 2%	1 ml, 2ml/L	Twice with 2 weeks interval, as foliar spray
Nile compost	Egyptian Company for Solid Waste Utilization (ECARU)	41-48% organic matter,1.5-2% nitrogen, 0.8-1.6%potassium, 24-28% carbon, 0.4-0.6% phosphorus, 100 ppm Fe, 25-50 ppm Zn, 100-200 ppm Mn, 20-25% moisture, pH 7.5-8.5, Ec 3-4, C/N ratio 1-14:16.	5g, 10g /pot	One week after infection, as soil drench
NAFK	Union of Agricultural Development (UAD)	Nitrogen 19%, Phosphorus 19%, Potassium 19% + Mg,sulpher, Fe,Zn,Mn, Cu and Bo	1.25,2.50 g/L	Twice with 2 weeks interval, as foliar spray
Union Fer	Union of Agricultural Development (UAD)	6 % chelated iron by organic and amino acids.	2g, 4 g/L	One week after infection, as soil drench
Union Manganese	Union of Agricultural Development (UAD)	13 % chelated manganese by organic and amino acids	2 g, 4 g/L	One week after infection, as soil drench
Union Zinc	Union of Agricultural Development (UAD)	12 % chelated Zinc by organic and amino acids	2 g, 4 g/L	One week after infection, as soil drench
Calsio-X	Union of Agricultural Development UAD	Calcium 9.8%, nitrogen 12%, magnesium 3.4% + active humic and amino acids	3 g, 6 g/L	Twice with 2 weeks interval, as foliar spray
Hyper K	Union of Agricultural Development (UAD)	60 % potassium oxide	2 g, 4 g/L	Twice with 2 weeks interval, as foliar spray
NPK	Grow Tech. for industrial production	19/19/19 NPK	1 g, 2 g/L	One week after infection, as soil drench
Amino Power	Union of Agricultural Development (UAD)	free amino acids 19%, citric acid 3%, potassium 3.5%, 1500 ppm chelated Fe,500 ppm chelated zinc, 500 ppm chelated manganese	0.5 , 1 g/L	One week after infection, as foliar spray
Amino green	Dishnr for Chemicals - commerce – Egypt	15 % organic and amino acids, 2.9% Fe, 1.4% Zinc, 0.7% manganese	1 ml, 2ml/L	One week after infection, as foliar spray

Table (1): Cont'd.

Trade name	Company	Contents	Dose Plant	Method of application
Amino Zinc	Dishnr for Chemicals commerce – Egypt	20 % organic and amino acids, 10% zinc	1 ml, 2 ml/L	One week after infection, as foliar spray
Amino manganese	Dishnr for Chemicals commerce – Egypt	20 % organic and amino acids, 10% manganese	1ml, 2 ml/L	One week after infection, as foliar spray
Amino Iron	Dishnr for Chemicals commerce – Egypt	20 % organic and amino acids, 8% iron	1 ml, 2 ml/L	One week after infection, as foliar spray
Glutamic acid	ROTH Bestellen Sie Zum Nulltarif Germany	aminoglutamic acid 99% [$C_5H_9No_4$]	0.5 g, 1 g/L	One week after infection, as foliar spray
Riboflavin	ROTH Bestellen Sie Zum Nulltarif Germany	lactoflavin, Vitamin B2, Vitamin C [$C_{17}H_{20}N_4O_6$]	o.5g, 1g/L	One week after infection, as foliar spray
Citric acid	ROTH Bestellen Sie Zum Nulltarif Germany	Vitamin C 99% [$C_6H_8O_6$]	0.5g, 1g/L	One week after infection, as foliar spray
UniBor	Union of Agricultural Development (UAD)	boron 6%, gibberellins 0.05%, vitamin B1 [thiamine, riboflavin, nicotinic acid, pyridoxine, pyridoxal, pyrodoxamine, biotin]	0.75g, 1.5g/L	One week after infection, as foliar spray
Gibberellic acid 20 %	ROTH Bestellen Sie Zum Nulltarif Germany	$C_{19}H_{22}O_6$	50, 100 ppm	One week after infection, as foliar spray
Indole-3- acetic acid 98 %	ROTH Bestellen Sie Zum Nulltarif Germany	$C_{10}H_9NO_2$	50,100 ppm	One week after infection, as foliar spray
Indole-3-butyric acid 98 %	ROTH Bestellen Sie Zum Nulltarif Germany	$C_{12}H_{13}NO_2$	50,100 ppm	One week after infection, as foliar spray

Table (2): Treatments and doses / concentrations.

Treatment	Dose/conc.	Treatment	Dose/conc.
Mega power (Humic&falvic)	1 ml/Liter 2ml/Liter	Amino power	0.5ml/Liter 1.0ml/Liter
Compost	5.0 g/pot 10.0 g/pot	Amino green	1.0ml/Liter 2.0ml/Liter
Poultry manure	5.0 g/pot 10.0/pot	Amino Zinc	1.0ml/Liter 2.0ml/Liter
Eucalyptus dry leaves	5.0/pot 10.0 g/pot	Amino manganese	1.0ml/Liter 2.0ml/Liter
Neem dry leaves	5.0g/pot 10.0 g/pot	Amino iron	1.0ml/Liter 2.0ml/Liter
Union Fer	2.0 g/Liter 4.0 g/Liter	Glutamic acid	0.5ml/Liter 1.0ml/Liter
Union manganese	2.0g/Liter 4.0 g/Liter	Citric acid	0.5ml/Liter 1.0ml/Liter
Union Zinc	2.0 g/Liter 4.0 g/Liter	Riboflavin	0.5ml/Liter 1.0ml/Liter
Calsio-X	1ml/Liter 2ml/Liter	Ascorbic acid	0.5ml/Liter 1.0ml/Liter
Hyper-K	3.0 g/Liter 6.0 g/Liter	UniBor	0.75ml/Liter 1.5ml/Liter
NPK	2.0 g/Liter 4.0 g/Liter	Gibberellic acid	50 ppm 100 ppm
Ammonium nitrate	1.0 g /Liter 2.0 g/Liter	Indoleacetic acid	50 ppm 100 ppm
Vydate 10%G	0.2 g/pot -	Indole butyric acid	50 ppm 100ppm

5. Nematode assay

a. Soil population

Upon harvest, each pot was soaked in plastic bucket filled with water until the root system could be easily separated. Each root system was gently dried using soft clean tissue paper, weighed and stored in 5% formaldehyde in plastic jars. The soil suspension was quite stirred, then poured through a series of 60, 200 and 325 mesh screens followed by Baermann set and collected after 48h. Hawksley counting slide was used to calculate the number of juveniles in one milliliter of suspension and then referred to the whole volume.

b. Root population

Roots were stained using acid fuchsine method **(Goody, 1957).** Five grams of the stain were added to one liter of distilled water, stirred and heated to boiling for about one minute. The root was then immersed in the stain for one minute, then removed and soaked in tap water to get rid of the excess stain. Developmental stages, mature females and egg-masses were counted under a stereo-microscope using two fine dissecting needles.

c. Eggs per egg-mass

Ten egg-masses of uniform size were separated from the root, placed into a vial containing 20 ml of sodium hypochlorite (NaOCL, 0.5%) and strongly shacked for 3 minutes. The suspension was then poured through a 500 mesh sieve, and the released eggs were gently washed with slow water stream of tap water to rinse off the residual NaOCL. Eggs were then collected into 250 ml beaker. An amount of 1 ml was withdrawn after the suspension was stirred well and dispensed onto a Hawksley counting slide, and examined under a compound microscope. The counted number was then referred to eggs per single egg-mass.

6. Determination of oxidants and antioxidants:

a. Lipid peroxidation (MDA contents)

Thiobarbituric acid reaction (TBA) as descrided by **Heath and Packer (1968).** The MDA equivalent was derived from the absorbance according to **Hodges *et al*. (1999).**

b. Assay of SOD activity (SOD; EC 1.15.1.1)

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of NBT using the method of **Beauchamp and Fridovich (1971).**

c. Assay of ascorbate peroxidase (APX) activity (APOX; E.C. 1.11. 1. 11)

Ascorbate peroxidase activity was estimated according to the method of **Nakano and Asada (1981).** Enzyme activity was determined by the decrease in absorbance of ascorbate at 290 nm.

Results

Concerning the influence of organic and inorganic commercial fertilizers, data in table (3) indicate that organic materials are better than inorganic fertilizers in reducing all nematode counts in soil and on roots of eggplant (Hyb. Oneta F1) and varied significantly with those of the check and all the tested inorganic fertilizers. Compost achieved the best results in reducing the number of galls, developmental stages, egg-masses, females fecundity and, in consequence, the soil population, followed by neem dry leaves, poultry droppings and then eucalyptus dry leaves. Mega power (humic and folvic acids) was the least organic materials in reducing nematode counts. Hyper K followed by Union zinc were the best and varied significantly with the other tested inorganic fertilizers. NAFK, Union Fer, Union manganese, Calsio X, NPK and ammonium nitrate came statistically in the second category. Non of the tested materials could stand with Vydate 10% G in reducing nematode counts. Except some cases, no significant differences were observed between the two doses tested in each treatment.

Table (3): Reproduction of *M. incognita* on eggplant as influenced by some organic and inorganic fertilizers.

Treatment	Dose	Nematode counts						
		Root Population				Soil population	Final population	Eggs/ eggmass
		Galls	D. stages	Eggmasses	Total			
Mega power® (Humic & falvic)	1ml/l	474 d-h	142 bc	426 de	568	1750 de	2318	244 hi
	2ml/l	411 e-i	123 efg	389 ef	512	1680 def	2192	261 fg
Compost[w]	5g	227 kl	91 ij	145 i	236	875 hi	1111	156 n
	10g	190 l	57 k	153 i	210	820 i	1030	143 o
Poultry droppings	5g	316 h-l	126 def	286 fgh	412	1255 g	1667	194 m
	10g	293 jkl	86 j	244 hi	330	1195 gh	1525	189 m
Eucalyptus dry leaves	5g	386 f-k	137 bcd	293 fgh	430	1425 efg	1855	206 l
	10g	329 g-l	96 ij	253 hi	349	1380 fg	1729	196 lm
Neem dry leaves	5g	308 i-l	94 ij	268 gh	362	1230 gh	1592	186 m
	10g	286 jkl	88 ij	217 hi	305	1175 gh	1480	184 m
NAFK®	1.25g	657 bc	132 cde	622 b	754	2560 bc	3314	278 de
	2.5g	629 bcd	126 def	565 bc	691	2385 c	3076	283 d
Union Fer®	2g/l	569 b-e	119 efg	521 bcd	640	2565 bc	3205	267 ef
	4g/l	519 cde	101 hi	486 cde	587	2230 c	2817	244 hi
Union Manganese®	2g/l	563 b-e	123 efg	528 bcd	651	2430 bc	3081	268 ef
	4g/l	516 cde	112 gh	476 cde	588	1985 d	2573	251 gh
Union Zinc®	2g/l	491 d-g	94 ij	487 cde	581	1855 d	2436	243 hi
	4g/l	483 d-g	95 ij	386 ef	481	1820 d	2301	220 k
Calsio X®	3g/l	689 b	138 bcd	545 bcd	545	2465 bc	3010	295 c
	6g/l	564 b-e	113 fgh	532 bcd	645	2335 c	2980	268 ef
Hyper K®	2g/l	467 d-h	86 j	429 de	515	1875 d	2390	236 ij
	4g/l	421 e-i	83 j	376 efg	495	1620 def	2115	244 hi
NPK®	1g/l	689 b	146 b	623 b	769	2770 b	3539	326 b
	2g/l	683 b	128 de	585 bc	713	2585 bc	3298	284 cd
Ammonium nitrate	1g/l	494 d-g	148 b	534 bcd	682	1955 d	2637	253 d
	2g/l	485 d-g	123 efg	493 cde	616	1765 de	2381	231 de
Vydate[w] 10%G	0.2g	47 m	53 k	21 j	74	460 j	534	88 p
Check (infected)		1266 a	546 a	1034 a	1580	4750 a	6330	424 a

Regarding the organic, amino acids commercial products and plant growth regulators, data in table (4) signified that all the tested materials significantly reduced the nematode counts in soil and on eggplant roots. The plant growth regulators, indole acetic and indole butyric acids preceded all the tested materials in enhancing resistance in eggplant against the root-knot nematode performing the lowest numbers of all nematode counts. Ascorbic acid, amino zinc and citric acid were statistically ranked in the second category.

Respecting the growth response of eggplant to the tested materials, data in table (5) disclose that compost at both doses was the best among organic and inorganic materials in meliorating the growth of infected eggplant. Thus, it achieved the highest significant values of growth criteria and the highest rates of increase in plant length, plant fresh weight and shoot dry weight followed by poultry droppings and neem dry leaves without significant differences with those of the untreated healthy plants. Other organic and inorganic materials significantly improved the growth of eggplant over the infected untreated plants but failed to improve the growth of plants to stand with the untreated healthy ones. However, the plant growth regulators, indole acetic acid and butyric acid (Table 6) surpassed all the resistance inducing materials accomplishing the best results in improving plant growth criteria followed by ascorbic acid, citric acid and amino zinc.

On the subject of the response of eggplant to nematode infection and application of the tested materials, data in Figs (1 and 2) show that the activity of oxidant lipid peroxidase (MDA) was at the lowest value in the healthy plants and at the highest values in plants infected with the root-knot nematode and untreated with any of the tested materials followed by those treated with the nematicide without significant differences. All treatments, due to their action against the root-knot nematode, could preclude the formation of MDA in roots depending on the degree of nematode control. Only indole acetic acid, compost and indole butyric acid, in that order, brought the levels of MDA in infected roots nearly similar to that of the healthy plants without significant differences. The antioxidant enzymes, superoxide dismutase (SOD) and ascorbate peroxidase (APX) were increased in infected plants as a feed back to the increase in MDA (Figs.3-6). Materials that enhance nematode resistance like indole acetic acid, indole butyric acid and compost encouraged plants to produce levels of SOD and APX significantly higher than those of other treatments including healthy and infected untreated plants. The other tested materials heighten the levels of antioxidant enzymes to levels measured up the degree of nematode control they accomplished. The nematicide, Vydate, in spite it reduced nematode counts to the lowest significant levels, it was not of course of these materials that arose the antioxidant enzymes to high levels.

Table (4): Reproduction of *M. incognita* on eggplant as influenced by some resistance inducers and plant growth regulators.

Treatment	Dose	Nematode counts						
		Root Population				Soil	Final	Eggs/
		Galls	D. stages	Eggmasses	Total	Population	population	eggmass
Amino power ®	0.5 ml/l	392 e-i	78 kl	397 o-f	475	1935 g-k	2410	213 h-k
	1 ml/l	359 e-j	79 kl	372 o-i	451	1560 h-k	2011	189 i-l
Amino green ®	1 ml/l	498 d-g	100 hij	455 od	555	2160 e-h	2715	256 d-g
	2ml/l	468 d-h	52 pq	432 ode	484	1835 g-k	2319	233 gh
Amino zinc ®	1ml/l	343 f-g	73 lm	313 d-i	386	1335 i-l	1721	194 i-l
	2ml/l	312 g-j	59 op	296 d-i	355	107o i-l	1625	188 i-l
Amino manganese ®	1ml/l	571 d	134 e	486 od	620	2850 def	3470	264 c-f
	2ml/l	487 d-g	97 ij	436 ij	533	2620 d-g	3153	221 g-i
Amino iron ®	1ml/l	536 de	107 gh	483 od	590	2540 d-g	3130	264 c-f
	2ml/l	472 d-h	94 j	431 ode	525	2325 d-h	2850	237 fgh
Glutamic acid	0.5 /l	586 d	117 f	541 c	658	2965 ode	3623	289 od
	1 g /l	539 de	108 g	483 od	591	2675 d-g	3266	276 ode
Citric acid	0.5 /l	375 e-j	95 j	380 o-f	475	1865 g-k	2340	238 fgh
	1 g /l	329 g-j	66 mno	365 o-i	431	1550 h-k	1981	220 g-j
Riboflavin	0.5 /l	759 c	152 d	828 b	980	3150 bod	4130	296 bc
	1 g /l	487 d-g	77 kl	467 od	544	2320 d-h	2864	203 ijk
Ascorbic acid	0.5 /l	336 g-j	68 mn	295 d-i	363	1195 j-m	1558	186 jkl
	1 g /l	311 g-i	62 no	262 e-i	324	1060 klm	1384	182 kl
Uni boron ®	0.75 ml/l	434 d-i	104 ghi	411 ode	515	1985 f-i	2500	263 c-f
	1.5 ml/l	387 e-i	72 lm	395 o-f	467	1625 h-k	2092	245 e-h
Gibberellic acid	50 ppm	989 b	232 c	876 ab	1108	3750 bc	4858	322 b
	100 ppm	1014 b	246 b	893 a	1139	3955 b	5094	395 a
Indole acetic acid	50 ppm	203 jkl	46 q	190 ijk	236	790 lm	1026	140 m
	100 ppm	182 kl	36 r	156 jk	192	765 lm	957	107 n
Indol e butyric acid	50 ppm	276 h-k	83 k	211	294	875 lm	1169	172 lm
	100 ppm	242 ijk	48 q	190 ijk	238	855 lm	1093	156 lm
Vydate® 10%G	0.2 g	47 l	53 pq	21 k	74	460 m	534	88 n
Check (infected)		1266 a	546 a	1034 a	1580	4750 a	6330	424 a

*Values followed by the same letter(s) are not significantly different (p=0.5).

Table (5): Growth of eggplant infected with *M. incognita* as influenced by organic and inorganic fertilizers.

Treatment	Dose	Growth criteria									
		Fresh weight (g)				Length (cm)				Shoot dry weight	
		Shoot	Root	Total	% change	Shoot	Root	total	% change	Weight	% change
Mega power® (Humic & falvic)	1ml/l	5.0 ij	2.9 g-j	7.9	54.9	23.5 g-j	15.0 a-e	38.5	71.1	0.5 g-j	66.7
	2ml/l	6.2 fgh	3.2 e-i	9.4	84.3	25.8 d-h	16.5 a-d	42.3	88.0	0.8 d-g	166.7
Compost[w]	5g	11.2 b	4.5 ab	15.7	207.8	32.8 ab	18.5 ab	51.3	128.0	1.2 a-d	300.0
	10g	12.4 a	4.8 a	17.2	237.3	35.0 a	19.0 a	54.0	140.0	1.5 a	400.0
Poultry droppings	5g	9.0 d	3.5 o-f	12.5	145.1	29.5 a-f	16.5 a-d	46.0	104.4	1.1 a-e	266.7
	10g	9.5 cd	3.9 b-e	13.4	162.7	31.0 a-d	17.0 a-d	48.0	113.3	1.2 a-d	300.0
Eucalyptus dry leaves	5g	6.3 fg	3.1 e-j	9.4	84.3	26.5 c-g	14.5 a-e	41.0	82.2	0.9 c-f	200.0
	10g	7.3 e	3.5 o-f	10.8	111.8	27.5 b-g	17.0 a-d	44.5	97.8	1.0 b-e	233.3
Neem dry leaves	5g	9.3 cd	3.8 b-e	13.1	156.9	30.0 a-e	17.5 abc	47.5	111.1	1.1 a-e	266.7
	10g	9.9 c	4.2 abc	14.1	176.5	32.0 abc	18.2 abc	50.2	123.1	1.3 abc	333.3
NAFK[w]	1.25g	2.9 mno	2.6 hij	5.5	7.8	18.8 jkl	13.5 a-e	32.3	43.6	0.3 ij	0.0
	2.5g	3.2 l-o	2.7 g-j	5.9	15.7	20.5 i-l	13.8 a-e	34.3	52.4	0.3 ij	0.0
Union Fer[w]	2g/l	4.0 kl	2.9 g-j	6.9	35.3	22.8 h-k	13.3 b-e	36.1	60.4	0.4 hij	33.3
	4g/l	5.3 hij	3.1 e-j	8.4	64.7	24.5 e-i	15.2 a-d	39.7	76.4	0.6 f-i	100.0
Union Manganese[w]	2g/l	4.6 jk	3.0 f-j	7.6	49.0	23.8 g-j	15.2 a-d	39.0	73.3	0.5 g-j	66.7
	4g/l	5.8 ghi	3.1 e-j	8.9	74.5	24.5 e-j	16.6 a-d	41.1	82.7	0.7 e-h	133.3
Union Zinc[w]	2g/l	5.6 ghi	3.2 e-i	8.8	72.5	24.2 f-j	15.5 a-d	39.7	76.4	0.6 f-i	100.0
	4g/l	6.2 fgh	3.4 c-g	9.6	88.2	25.8 d-h	16.6 a-d	42.4	90.7	0.7 e-h	133.3
Calsio X[w]	3g/l	3.5 lmn	2.6 hij	6.1	19.6	22.0 h-k	13.2 cde	35.2	56.4	0.3 ij	0.0
	6g/l	3.8 kl	2.9 g-j	6.7	31.4	22.5 h-k	14.5 a-e	37.0	64.4	0.4 hij	33.3
Hyper K®	2g/l	6.1 fgh	3.5 o-f	9.6	88.2	25.5 d-h	16.2 a-d	38.7	72.0	0.8 d-g	166.7
	4g/l	6.8 ef	3.9 b-e	10.7	109.8	26.7 c-g	16.8 a-d	43.5	93.3	0.9 c-f	200.0
NPK®	1g/l	2.6 o	2.5 ijk	5.1	0.0	17.5 klm	12.2 de	29.7	32.0	0.3 ij	0.0
	2g/l	3.9 kl	2.8 g-j	6.7	31.4	21.5 h-l	13.9 a-e	35.4	57.3	0.4 hij	33.3
Ammonium nitrate	1g/l	5.7 ghi	3.1 e-j	8.8	72.5	24.5 e-i	15.8 a-d	40.3	79.1	0.7 e-h	133.3
	2g/l	6.1 fgh	3.3 d-h	9.4	84.3	25.5 d-h	16.5 a-d	42.0	86.7	0.8 d-g	166.7
Vydate[w] 10%G	0.2g	3.7 lm	2.3 jk	6.0	17.6	16.5 lm	14.0 a-e	30.5	35.6	0.3 ij	0.0
Check(infected)		2.7 no	2.4 k	5.1		12.5 m	10.0 e	22.5		0.3 ij	
Check(Healthy)		10.1 c	4.1 a-d	14.2	178.4	30.5 a-e	18.2 abc	48.7	116.4	1.4 ab	366.7

Table (6): Growth of eggplant infected with *M. incognita* as influenced by some resistance inducers and plant growth regulators.

Treatment	Dose	Growth criteria									
		Fresh weight (g)				Length (cm)				Shoot dry weight	
		Shoot	Root	Total	% change	Shoot	Root	Total	% change	Weight	% change
Amino power [w]	0.5 ml/l	6.5 hi	3.5 ef	10.0	96.1	27.5 ghi	15.8 i	43.3	92.4	0.8 efg	166.7
	1 ml/l	6.9 gh	3.8 cde	10.7	109.8	27.8 f-i	17.2 fgh	45.0	100.0	1.0 cde	233.3
Amino green [w]	1 ml/l	4.7 lmn	2.8 gh	7.5	47.1	22.5 lmn	13.5 mn	36.0	60.0	0.5 ghi	66.7
	2ml/l	4.9 k-n	2.9 g	7.8	52.9	24.2 jkl	14.3 j-m	38.5	71.1	0.6 f-i	100.0
Amino zinc [w]	1ml/l	7.5 fg	3.9 b-e	11.4	123.5	29.3 d-h	17.0 gh	46.3	105.8	1.0 cde	233.3
	2ml/l	8.9 e	4.1 bcd	13.0	154.9	30.0 d-g	17.5 e-h	47.5	111.1	1.1 b-e	266.7
Amino manganese [w]	1ml/l	4.6 l-o	2.7 ghi	7.3	43.1	22.5 lmn	14.0 k-n	36.5	62.2	0.5 ghi	66.7
	2ml/l	5.1 klm	2.9 g	8.0	56.9	24.2 jkl	14.5 jkl	38.6	71.6	0.6 f-i	100.0
Amino iron [w]	1ml/l	5.0 klm	2.9 g	7.9	54.9	23.4 klm	14.2 klm	37.6	67.1	0.5 ghi	66.7
	2ml/l	5.8 ijk	3.1 fg	8.9	74.5	25.5 ijk	14.9 ijk	40.4	79.6	0.7 e-h	133.3
Glutamic acid	0.5 /l	4.2 m-p	2.7 ghi	6.9	35.3	20.8 mn	13.2 no	34.0	51.1	0.4 hi	33.3
	1 g /l	4.5 m-p	2.8 gh	7.3	43.1	22.6 lmn	13.5 mn	36.1	60.4	0.4 hi	33.3
Citric acid	0.5 /l	7.5 fg	3.8 cde	11.3	121.6	28.0 e-i	17.1 gh	45.1	100.4	1.0 cde	233.3
	1 g /l	8.0 f	3.9 b-e	11.9	133.3	29.3 d-h	17.8 d-g	47.1	109.3	0.9 def	200.0
Riboflavin	0.5 /l	3.6 op	2.4 hi	6.0	17.6	20.0 n	12.5 o	32.5	44.4	0.3 i	00.0
	1 g /l	4.0 op	3.1 fg	7.1	39.2	21.5 mn	13.6 lmn	35.1	56.0	0.4 hi	33.3
Ascorbic acid	0.5 /l	9.8 cd	4.0 bcd	13.8	170.6	30.8 d	17.8 d-g	48.6	116.0	1.2 bcd	300.0
	1 g /l	10.3 c	4.3 b	14.6	186.3	31.1 cd	18.5 bcd	49.6	120.4	1.3 bc	333.3
Uni boron [w]	0.75 ml/l	5.5 jkl	3.5 ef	9.0	76.5	25.8 ijk	15.2 ij	41.0	82.2	0.6 f-i	100.0
	1.5 ml/l	6.3 hij	3.7 de	10.0	96.1	27.2 hi	16.8 h	44.0	95.6	0.8 efg	166.7
Gibberellic acid	50 ppm	5.5 hij	3.8 cde	9.3	82.4	30.2 def	17.5 e-h	47.7	112.0	0.6 f-i	100.0
	100 ppm	6.3 ijkl	3.9 b-e	10.2	100.0	31.5 cd	18.8 bc	50.3	123.6	0.8 efg	166.7
Indole acetic acid	50 ppm	11.6 b	4.9 a	16.5	223.5	33.8 b	19.1 b	52.9	135.1	1.3 bc	333.3
	100 ppm	12.9 a	5.1 a	18.0	252.9	36.5 a	20.5 a	57.0	153.3	1.8 a	500.0
Indole butyric acid	50 ppm	9.2 de	4.0 bcd	13.2	158.8	31.0 cd	18.1 c-f	49.1	118.2	1.1 b-e	266.7
	100 ppm	9.5 cde	4.2 bc	13.7	168.6	33.5 bc	18.8 bc	52.3	132.4	1.2 bcd	300.0
Vydate® 10% G	0.2g	3.7 op	2.3 hi	6.0	17.6	16.5 no	14.0 k-n	30.5	35.6	0.3 i	00.0
Check(infected)		2.7 q	2.4 hi	5.1		12.5 q	10.0 p	22.5		0.3 i	
Check (Healthy)		10.1 c	4.1 bcd	14.2	178.4	30.5 de	18.2 b-	48.7	116.4	1.4 b	366.7

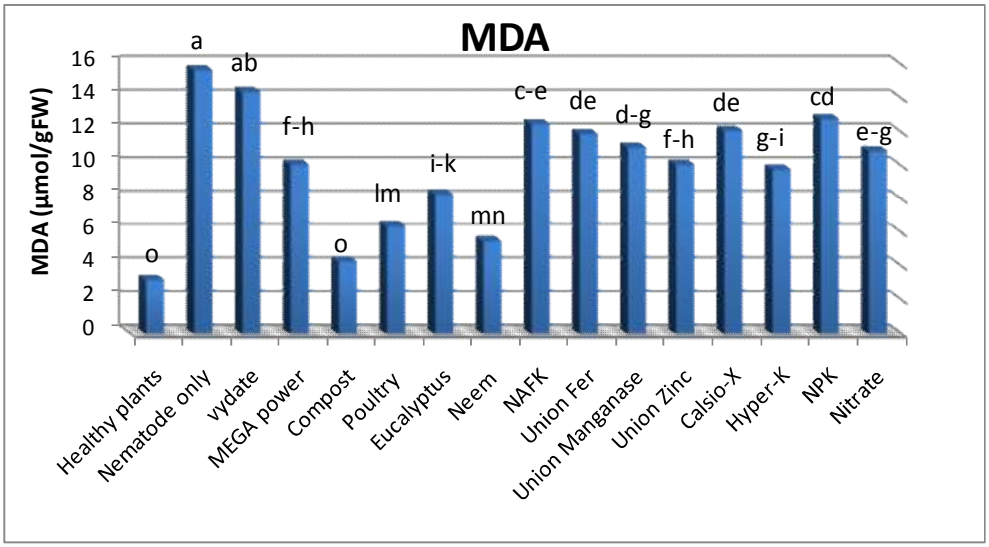
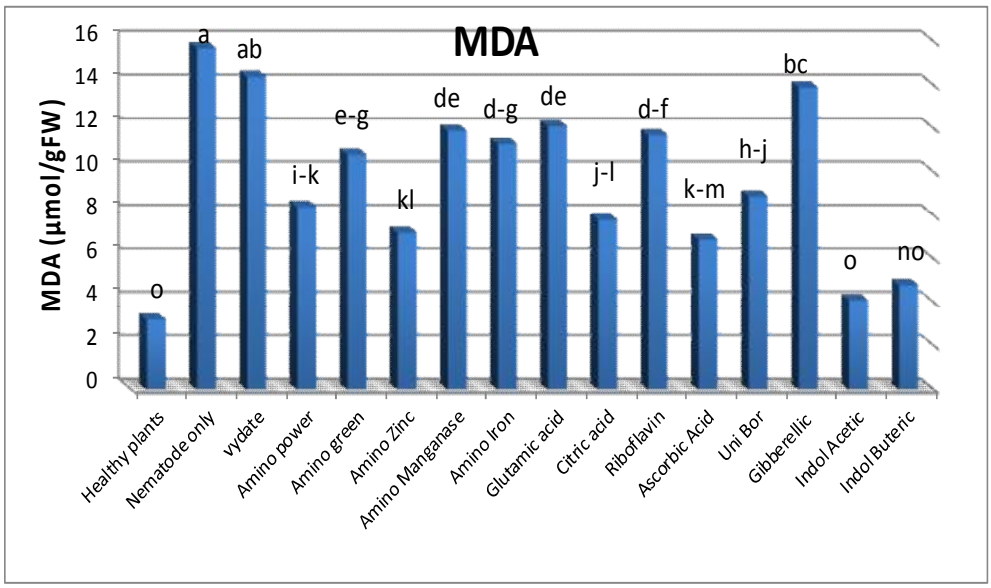


Fig.(1): Changes in MDA in roots of eggplant infected with *M. incognita* and treated with organic and inorganic fertilizers.



*In both Figs, similar letter(s) means insignificant differences.

Fig. (2): Changes in MDA in roots of eggplant infected with *M.incognita* and treated with some resistance inducers

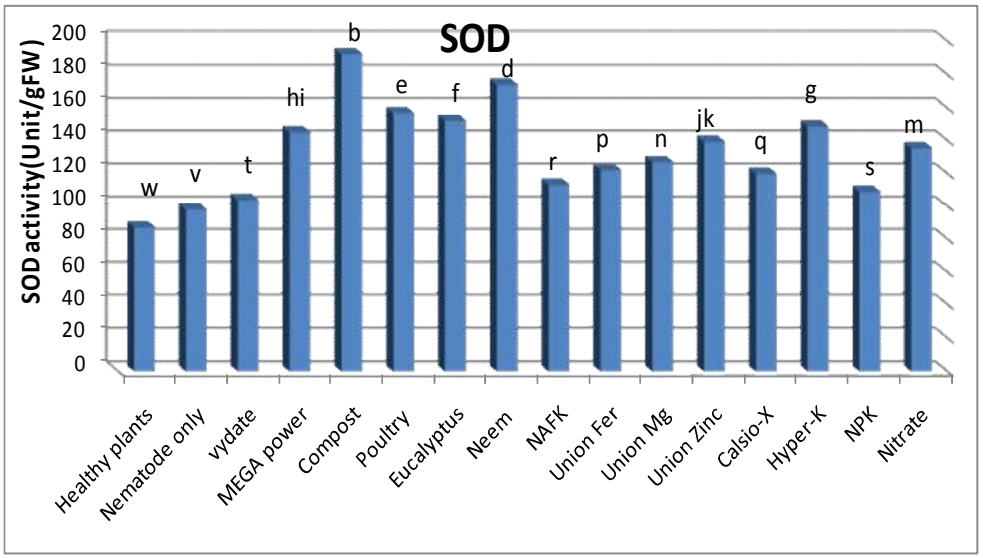
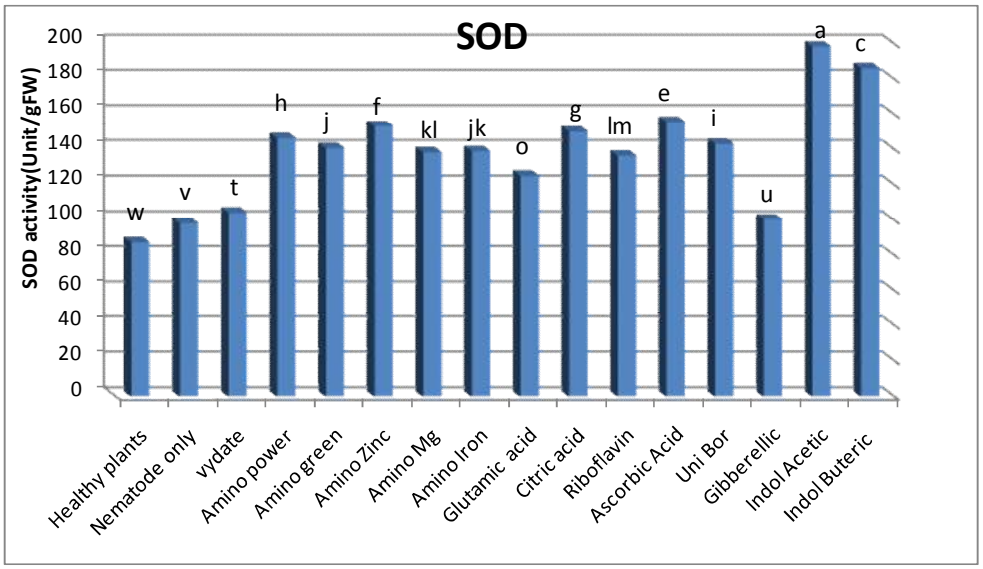


Fig. (3): Changes in the activity of SOD in roots of eggplant infected with *M.incognita* and treated with organic and inorganic fertilizers.



*In both Figs, similar letter(s) means insignificant differences.

Fig.(4): Changes in the activity of SOD in roots of eggplant infected with *M.incognita* and treated with some resistance inducers.

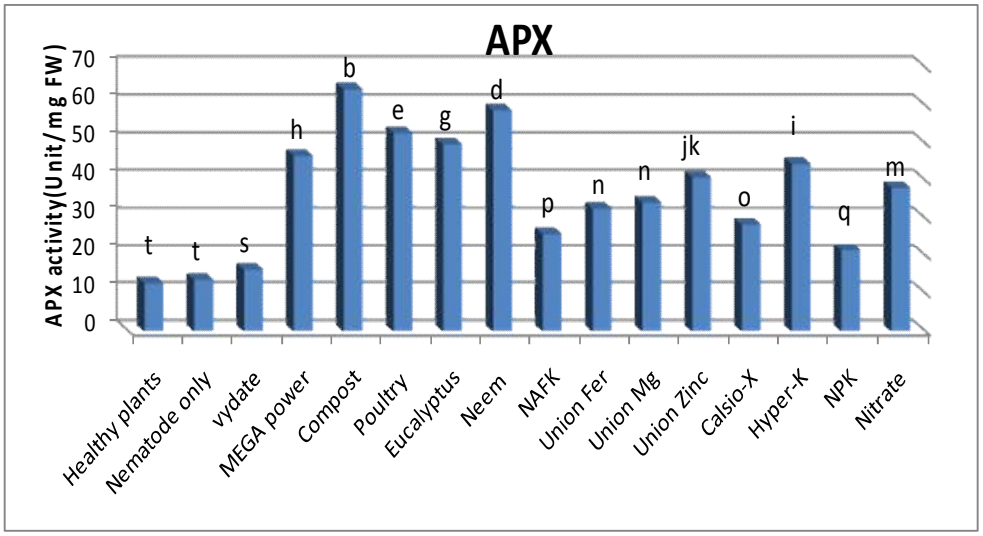
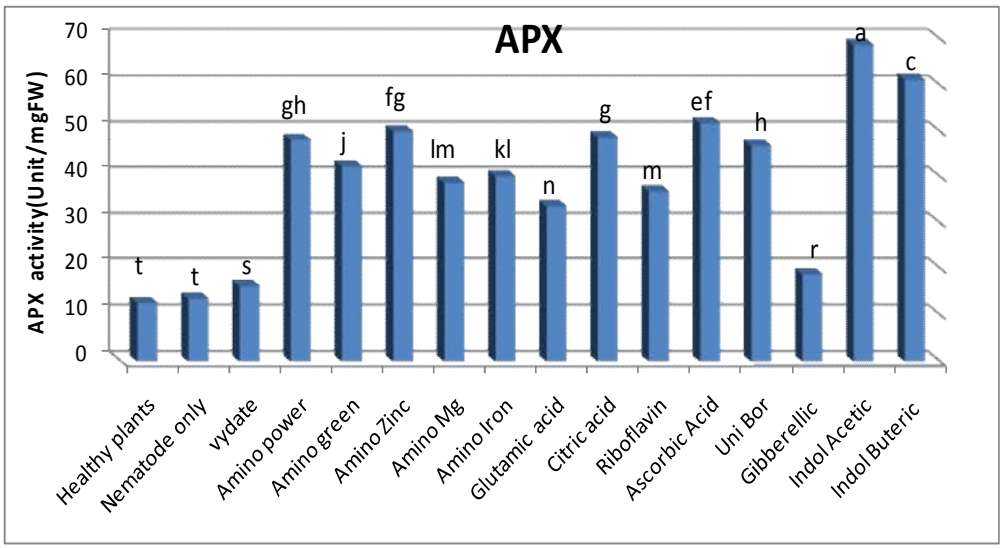


Fig. (5): Changes in the activity of APX in roots of eggplant infected with *M.incognita* and treated with organic and inorganic fertilizers.



*In both figs, similar letter(s) means insignificant differences.

Fig. (6): Changes in the activity of APX in roots of eggplant infected with *M.incognita* and treated with some resistance inducers.

Discussion

Data of the present study show that organic fertilizers are significantly better than commercial inorganic fertilizers in reducing the root-knot nematode counts in soil and on eggplant and improving the growth of treated plants which agreed with the findings of **Siddiqui*et al*. (2001).** Compost achieved the best results followed by neem dry leaves, poultry droppings and then eucalyptus dry leaves. Such efficiency may partially due to direct toxic effect of the substances produced during the degradation of the organic matter; and to the role of these substances in helping the treated plants to acquire some resistance against invading nematodes. Compost surpassed all the tested materials in improving growth of the treated plants infected with the root-knot nematode. Substances produced during the degradation of organic matter include volatile fatty acids and organic acids **(Kesba and Al-Shalaby, 2008 and Abd El-Rahman *et al*., 2008).** Nitrogenous compounds, phenols, hydrogen sulfide are also, generated from organic materials with low C/N ratio in soil **(Riegal and Noe, 2000, and Oka *et al*., 2007).** Furthermore, the indirect effect is related to the role of organic matter in supplying and encouraging microorganisms where many of which exhibits some antagonistic action against nematodes either as direct parasites or by their metabolites produced during their activities **(Mankau, 1963).**

The nematicidal action of neem formulations is not only due to the compounds present within the neem product, namely, nimbidin and thionimone but also due to other byproducts such as ammonia, formaldehyde, phenols and fatty acids produced during decomposition of neem formulations **(Khan *et al*., 1974).** Also, it have been stated that essential oils produced during the degradation of different parts of eucalyptus are responsible for diminishing nematode populations **(Dawar*et al*., 2007 and Moreira, *et al*., 2009).**

Many reports in literature agreed with the findings in the present study and illustrate the role of compost **(Rashad*et al*., 2010 and Zakaria*et al*., 2013),** chicken manure **(Karmani*et al*., 2011 and Abolusoro and Abolusoro, 2012),** neem**(Javed*et al*., 2007 and Farahat*et al*., 2012)** and eucalyptus **(Moreira *et al*., 2009)** in reducing nematode populations in soil and on roots as well as improving the growth of the infected plants.

Concerning the commercial inorganic fertilizers, data in the present study show that, hyper K® followed by union zinc® were significantly the best in reducing the number of galls, root and soil population of *M. incognita* on eggplant. NAFK®, Union Fer®, Union manganese®, Calsio X®, NPK® and ammonium nitrate came statistically in the second category. No significant differences were observed between the two tested doses in the majority of cases.Hyper K® is a commercial product containing 66% potassium. Potassium sulphate (SO_4KNO_3) reduced the population density of *M. incognita* in soil and on roots of cowpea **(Ahmed *et al*., 1991).**

Union zinc® is a commercial product containing 12% chelated zinc by organic and amino acids. Absence of zinc increased nematode density in soil and reduced plant growth **(Haque and Mukhopadhyaya, 1975 and Siddique*et al.*, 2002).**The involvement of minerals especially Fe, Mg, Zn and Ca in the formation of enzymes **(Graham *et al.*, 1988 and Auld, 2001)**, may explain their role in the acquired systemic resistance by increasing the antioxidant enzymes included in the defense mechanisms which resulted in reducing nematode populations.

NPK® NAFK®, Union Fer®, Union manganese®, Calsio X®, ammonium nitrate came after hyper K and union Zn in reducing the root-knot counts in soil and on eggplant roots and improving the growth of treated plants. Many reports in literature are in accordance with our findings concerning NPK **(Coyne *et al.*, 2004; Farahat, *et al.*, 2010 and Al-Hazmi and Dawaba,2014).** The role of Mn, Fe, Ca in hindering nematode reproduction had also been documented by **Coyne *et al.* (2004) and Kheir*et al.* (2009).** Organic and amino acids presented in the tested inorganic fertilizers may affect the reproduction of nematodes on treated host plants and eliminate their biological activities **(Al-Sayed and Thomason, 1988; Oka and Cohen, 2001; Abd El-Rahman*et al.*, 2008 and AmdadulHoque*et al.*, 2013).**

The biocidal mode of action of ammonia is not clear, but several mechanisms are thought to be involved: cell membrane disruption **(Rush and Lyda, 1982),** elimination of protein gradients across membranes **(Docherty and Snider, 1991),** and exhaustion of the chemical energy of the cells removing cytosolic ammonia against a concentration gradient **(Britto*et al.*, 2001).**

Concerning the organic, amino acid-containing commercial products and plant growth regulators, data in the present study signified that all the tested materials significantly reduced the root-knot nematode counts in soil and on roots of eggplant. Indole acetic and indole butyric acids preceded all the tested materials including organic amendments in enhancing the resistance of treated plants and performing the lowest numbers of nematode counts. Ascorbic acid, amino zinc and citric acid were statistically ranked in the second category.

Growth regulators play an important role in the mechanism of gall formation in *Meloidogyne* infections. Changes caused by nematode species in cells of a susceptible host are similar to those caused by exogenous indole acetic acid, that is, hypertrophy, hyperplasia, adventitious roots, nuclear division without cell division and break down of the cell wall. Two possible sources of indole compounds in the root galls have been suggested. First, nematodes inject through their saliva the enzymes glycosidase and protease into the host cells and release free auxins from the complexes in the host. The proteases breaking down proteins to amino acids including tryptophan, the IAA precursor, and also acids such as phenylalanine, alanine, histidine and serine which promote auxinsynthysis. The second possibly is that the nematode itself releases auxins during feeding. Perhaps indole compounds

are formed in nematodes as end products of metabolism and are exerted by endo-parasites into plant tissue or by ectoparasites into the root region. IAA have been detected in larvae and egg masses of *Meloidogyne* species **(Decker, 1981).**

Successful host-parasite relationship relies on the formation of feeding sites which depends mainly on the availability of some amino acids and plant auxins at specific concentrations **(Khanna and Yadav, 2004).** Accordingly, any disturbance in such concentrations may restrict the activities of nematode biology which shows sings of resistance. From this point of view, such disturbance may result from the exogenous application of indole IAA or IBA as well as amino acid-containing formulations which ultimately aquire some resistance to the treated eggplant against the root-knot nematode. These findings are in accordance with those of Yu and **Zhena (2007).** They reported that IAA stimulated catalase, peroxidase and polyphenol oxidase activities in pear fruits, indicating that IAA can induce fruit-mediated resistance against pear fruit diseases although it had no direct antifungal activity. Both indoles (IAA and IBA) are considered pesticide derivatives **(Omar and Muneer, 2005)** and IBA is registered by **EPA (1992)** as a biocontrol pesticide with the PC Code 046701. They reported that IBA has been classified as a biocontrol pesticide because it is similar in structure and function to the naturally-occurring plant growth indole-3-acetic acid.

In the present study, ascorbic acid came after IAA, IBA in reducing the root-knot nematode counts in soil and on roots of eggplant. Ascorbic acid, in the present results is considered a very good resistance inducer against this nematode. Our findings agreed with those of **Arrigoni*et al*., 1979** when they found that artificial increase in ascorbic acid concentration transforms susceptible plants into resistant ones. These results also agreed with those of **Hamada *et al*. (2000)** and **Moawad (2005)**. They reported that ascorbic acid was effective in reducing stresses of the root-knot nematodes on their hosts.

Our results proved that all the tested amino acids and organic acids-containing commercial products significantly reduced the counts of *M. incognita*on eggplant with superiority of amino zinc, followed by amino power and amino green. These results agreed with those in literature illustrating the role of amino acids in diminishing nematode populations and inducing resistance in treated plants **(Oka and Cohen, 2001; Kesba, 2003, Saeed, 2005 and AmdabulHoque*et al*., 2013).** The superiority of amino zinc (a commercial product containing organic and amino acids and 10% zinc) may be due to the effect of both amino and organic acids as well as zinc.

The formation of reactive oxygen species (ROS) is the most common defense mechanism in which lipid peroxidation (accounting for cell death after nematode invasion) is induced **(Montes *et al*., 2004 and Bakker, *et al*., 2006).** Hence, increasing the rates of MDA and H_2O_2 in different hosts in response to

infection with *M. incognita*, in the present study as compared to healthy plants accounted for the defense mechanism against nematode invasion. Our results agreed with those of **Davis *et al*. (2000) and Huang *et al*. (2004)** who stated that the initial reaction of the susceptible cultivars is similar to that of resistant hosts and may be resulted from nematode secretions into plant tissues.

Increase in superoxide dismutase (SOD) and peroxidase activity results to be an adaptive response which provides the plant with protection against biotic and abiotic stress **(Guida*et al.*, 1992).** The protective activity of SOD, and catalase (CAT) was enhanced in susceptible plants but decreased in resistant ones **(Zacheo*et al.*, 1983).** Superoxide dismutase prevents the deleterious effect of O_2 radicals in root cells and transform it to H_2O_2 which is then transformed by catalase to harmless O_2+H_2O. Accordingly, in susceptible tomato roots infected with *M. incognita,*SOD activity was considerably increased in comparison to uninfected controls and decreased in resistant cultivars **(Zacheo*et al.*, 1987 and Sgherri*et al.*, 2013).** These findings are in accordance with our results whereas superoxide dismutase (SOD), ascorbate oxidase (APX) were significantly higher in treated plants.

Systemic acquired resistance can be enhanced by applying materials of different sources which, in many cases, suppress nematode populations and improve the growth of treated plants either directly by their effects on nematodes or by enhancing resistance of treated plants.

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إكساب الباذنجان صفة المقاومة لنيماتودا تعقد الجذور باستخدام الأسمدة العضوية وغير العضوية وبعض المنتجات التجارية التي تحتوي على الأحماض الأمينية ومنظمات النمو

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تم في هذا البحث اختبار قدرة بعض الأسمدة العضوية والغير عضوية والعديد من المنتجات التجارية المحتوية على الأحماض العضوية والأمينية وكذلك بعض الفيتامينات ومنظمات النمو على إكساب نباتات الباذنجان صفة المقاومة لنيماتودا تعقد الجذور.أثبتت النتائج أن الأسمدة العضوية تفوقت معنويا على الأسمدة المعدنية في خفض أعداد نيماتودا تعقد الجذور في التربة وعلى جذور نباتات الباذنجان حيث أعطى الكومبوست أفضل النتائج بينما كان هيبر بوتاسيوم ويونيون زنك أفضل الأسمدة غير العضوية. سجلت المنتجات التجارية التي تحتوي على الأحماض العضوية والأمينية ومنظمات النمو خفضًا معنويًّا في أعداد النيماتودا في التربة وعلى جذور الباذنجان حيث تفوق اندول حمض الخليكواندول حمض البيوتيريك على كل المنتجات والمواد المختبرة في زيادة مقاومة الباذنجان للإصابة وتلاهما حمض الأسكربيك ثم أمينو زنك ثم حمض الستريك. أثبتت النتائج أيضا أن الأسمدة العضوية كانت الأفضل في تحسين نمو النباتات المصابة، أما باقي المنتجات المختبرة فقد حسنت النمو معنويًّا بالمقارنة باانباتات المصابة والغير معاملة وحقق اندول حمض الخليكواندول حمض البيوتيريك وحمض الستريك أفضل النتائج.

كان نشاط انزيم البيروكسيداز عند أقل مستوى له في النباتات السليمة بينما بلغ الذروة في النباتات المصابة بالنيماتودا . استطاع كل من اندول حمض الخليكوالكومبوستواندول حمض البيوتيريك على التوالي إعادة نشاط الإنزيم إلى ما كان عليه تقريبًا في النباتات السليمة وبدون فروق معنوية وهي ذات المعاملات التي أعطت أفضل النتائج في مكافحة النيماتودا وتحسين نمو النبات. زاد نشاط الإنزيمات المضادة للأكسدة في النباتات المصابة كرد فعل على زيادة نشاط إنزيم الأكسدة وحقق أيضا اندول حمض الخليكواندول حمض البيوتيريكوالكومبوست أعلى معدل لنشاط هذه الإنزيمات حيث أن تراكم هذه الإنزيمات يزيد من آلية مقاومة النباتات للإصابة بالنيماتودا.