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Inducing Systemic Resistance against Root-knot Nematodes in Eggplant by Chemical and Organic Fertilizers and Antioxidant Substances

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ABSTRACT

One of the most significant pests of vegetable crops is known as root-knot nematode (*Meloidogyne* spp.). Although, using synthetic nematicides to manage nematodes are a tried-and-true successful method, this tactic raises concerns due to its high costs and negative environmental impacts. In order to control root-knot nematode infection and infestation on eggplant cv. Black Beauty seedlings in an environmentally friendly manner, experiments were carried out using a chemical fertilizer (NPK), an organic fertilizer (humic acid), and some antioxidant substances (selenium, vitamin C, and E) individually and in combination under greenhouse and field conditions. The obtained results of these investigations, revealed that all tested treatments have the ability to reduce nematode populations, which would raise yield. Vitamins C and E combined with NPK fertilizer significantly increased shoot and root fresh and shoot dry weight over control in greenhouse conditions. They also significantly decreased total final nematode population with reproduction factor (Rf =0.44) less than control. Additionally, simultaneous application of humic acid, selenium, and NPK boosting plant conditions and greatly reduced J_2s, gall formation, and number of egg masses. Plant height, fresh and dry weight, yield per plant, N, P, and K contents, as well as fruit quality (total carbohydrates, vitamin A, and C) were all estimated during field trials. As for, parameters of eggplant as affected by application of humic acid and/or antioxidants concomitantly with NPK fertilization were significantly increased. Furthermore, reducing activity in total nematode population in soil and root was achieved by all tested treatments. It could be observed that humic acid and different antioxidant when combined with NPK induced systemic which consequently caused suppression in eggplant against root knot nematode.

Keywords: *Meloidogyne* spp, eggplant, humic acid organic fertilizer, NPK mineral fertilizer, Vitamin E, Vitamin C, selenium and systemic resistant.

INTRODUCTION

One of the most well-liked and important vegetable crops in Egypt is eggplant *(Solanum melongena* L.), which is also considered a national food in many other tropical and subtropical nations. Huge amounts of proteins, carbohydrates, and several minerals are included in eggplant fruits (Mahmoud, 2000). It is well known that eggplant fruits are low in calories and have a favorable mineral composition for human health. In addition, fruits are a good source of calcium, potassium, magnesium, and iron (Zenia and Halina, 2008). According to the FAO in 2012, only the five countries (China, India, Iran, Egypt, and Turkey) produce 90% of the world's eggplant yield (Ismael et al., 2017).

Eggplant is plagued by a wide variety of pests, but the root-knot nematode stands out because it causes serious root damage that results in stunting, chlorosis, and a sharp decline in yield. In Egypt, (Plant Parasitic Nematodes) PPNs damage caused yield losses of 20% of the total yearly crop, or as much as 298,410.5 metric tons, according to Abd-Elgawad (2014). Actually, nematode population density, the degree of host compatibility, and the application of control measures are all associated to reduced eggplant growth and yield (Abd-Elgawad, 2021).

Nematicides, as synthetic pesticides, are the most effective way to control the pests and diseases. These synthetic chemicals are extremely expensive, highly harmful to humans, and do not ensure environmental safety. Scientists are looking for alternative methods of controlling nematode pests of commercial food crops because chemical nematicides are scarce, expensive, environmentally hazardous, and subject to international import restrictions (Anonymous, 2004). As a result, novel methods for combating plant-parasitic nematodes have been aggressively looked for in recent years. Natural nematicides, organic and inorganic amendments, biological control, and induced resistance have all been the subject of research (Oka et al., 2000). The purpose of resistance inducers or elicitors is to activate the plant's defense mechanisms through the use of certain chemicals or biotic agents (Baysal et al., 2003; Silva et al., 2004; Bonaldo et al., 2005, Dias-Arieira et al., 2012, and Anter et al., 2014). Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two known means of developing plant resistance to pests and diseases. The use of mineral fertilizers is one of the cultural practices (which also have nutritional value). These fertilizers could be used in nematode management programs because they have long been known to have an impact on populations of plant parasitic nematodes (Coyne et al., 2004, Al-Hazmi & Dawabah 2013 and Saeedizadeh et al., 2020).

In agriculture, humic acid has been used to improve crop growth and conditions of the soil. Humic acid is known to have toxic and antagonistic effects against many plant parasitic nematodes, including *Meloidogyne* spp., *Rotylenchulus reniformis, Radopholus similis, Helicotylenchus multicinctus.* The required dose for getting significant nematode control ranged from 0.04 to 2.0% concentration (Nagachandrabose and Baidoo 2021). Humic acid caused intermediate effect against *M. incognita* and *Tylenchulus semipenetrans* on infected olive (Hammad et al., 2021).

There is evidence that selenium (Se) functions in anti-oxidative mechanisms and is a component of glutathione peroxidase in plants (Lin et al., 2005, Cabral Gouveia et al., 2020). Since the soil fauna spends its whole life cycle in the soil and is directly impacted by the chemicals in the soil, excessive Se flux into the soil as a result of Se biofortification may have detrimental effects on fauna survival (Xu et al., 2022).

Normally, plants produce vitamin C (Ascorbic acid) on their own through their mitochondria in response to abiotic challenges such salt, drought, high temperatures, and diseases that would impair the biochemical and physiological performance of plants (Wang et al., 2013 and Kaya, 2017). Vitamin E (α-tocopherol) is a lipophilic antioxidant with a low molecular weight that guards against oxidative damage to chloroplast membranes (Hess, 1993 and El Bassiouny et al., 2005).

From the previous review of research, this study was conducted under greenhouse and field conditions to investigate the effects of NPK and organic humic acid fertilizers and some antioxidants as inducers of resistance of eggplant infected with root-knot nematode (*Meloidogyne* spp.) in Egypt.

MATERIALS AND METHODS

1.Pure Nematode Culture

Using sodium hypochlorite solution according to Hussey and Barker (1973), nematode eggs of *Meloidogyne incognita* were recovered from infected tomato (cv. Castle Rock) roots. Nematode juveniles (J2s) in their 2nd stage were daily collected from the emerged eggs and kept at 15 °C. Less than five days old juveniles were utilized in the tests.

2.Greenhouse experiments

a. Effect of some antioxidants (vitamin C, vitamin E) and NPK fertilizer singly or in combination on *M. incognita* infected eggplant under greenhouse conditions.

To investigate the effects of vitamin C, vitamin E, and NPK fertilizer alone or in combination against root-knot nematode, eggplants, *Solanum melongena* were grown in plastic pots. Seedlings of Eggplants (cv. Black Beauty) that were one-month-old were planted in 20 cm diameter plastic pots with a soil mixture of 1:1 sterilized clay/sand. One week after planting, 36 pots were inoculated with 1,000 J2s for each pot. Six days later, 28 pots were either individually or collectively drenched with 50 ml of sterilized water per plant with (Vitamin C) ascorbic acid (20 mM), (Vitamin E) α-Tocopherol (50 mmol), or NPK fertilizer (19-19-19 @5 g/liter), and combinations among them. Chemical nematicide, oxamyl (10%G) [Methyl N N-dimethyl-N [(methyl) carbamyloxy]-1-thioxamidate] was administered at the rate of 0.3g / pot in four replicates (pots). Four pots inoculated with nematodes (J_2s) only and four pots untreated and uninoculated were served as check. Treatments of antioxidants (vitamin C, vitamin E) and NPK fertilizer singly or in combination on *M. incognita* infected eggplant under greenhouse conditions were as follow:

1- Vitamin E
2- Vitamin C
3- NPK
4- Vitamin E + NPK
5- Vitamin C + NPK
6- Vitamin E + Vitamin C
7- Vitamin E + Vitamin C + NPK
8- Oxamyl
9- Untreated inoculated check
10- Untreated uninoculated plant (check)

b. Effect of selenium, humic acid and NPK fertilizer singly or in combination on the reproduction of *M. incognita* infected eggplant under greenhouse conditions.

Another experiment evaluated the influence of selenium, humic acid, and NPK fertilizer alone or in combination on *M. incognita* reproduction in a greenhouse environment. Seedlings of eggplants (cv. Black Beauty) one month old were planted in 20 cm diameter plastic pots with a soil mixture of 1:1 sterilized clay/sand. One week after planting, 36 pots were inoculated with 1,000 (J2s) for each pot. Six days later, 28 pots were soaked with either NPK fertilizer (19-19-19@5 g/liter), 1 ml/plant humic acid, or 50 ml/plant of sterile water containing 0.62 mM selenium (SE), and combination among them. Four repetitions of each treatment were given. Oxamyl (10%G) [Methyl N N-dimethyl-N [(methyl) carbamyloxy]-1-thioxamidate] was administered at the rate of 0.3g / pot in four pots. However, four pots inoculated with nematodes only and four more pots untreated and uninoculated were served as check. Treatments of selenium, humic acid and NPK fertilizer singly or in combination on the reproduction of *M. incognita* infected eggplant under greenhouse conditions were as follow:

1- Humic acid
2- Selenium
3- NPK
4- Humic acid + NPK
5- Selenium + NPK
6- Humic acid + Selenium
7- Humic acid + Selenium + NPK
8- Oxamyl
9- Untreated inoculated check
10- Untreated uninoculated check

As for two previous experiments, pots were placed at random complete block design and watered as necessary. Plants were collected 45 days of nematode inoculation, which marked the end of the experiment. Fresh shoot and root lengths, fresh shoot and root weights, and shoot dry weight were measured as indicators of plant growth. To count the number of developmental stages, females, galls, and egg masses on plants, 0.01 acid fuchsin in lactic acid was used to stain the roots of the plants (Byrd et al., 1983). According to Taylor and Sasser (1978) root galling and egg masses were graded on a scale of 0 to 5, with 0 denoting no galls or egg masses, 1 denoting 1-2, 2 denoting 3–10, 3–11–30, 4 denoting 31–100, and 5 denoting more than 100 galls or egg masses per root system. Using the Goodey (1957) method, vermiform 2^{nd} stages juveniles of *M. incognita* were collected from the soil. The reproduction factor (Rf) for each treatment was determined by dividing the final population (Pf) by the initial population (Pi).

3.Field experiment

The best treatments for reducing nematode numbers and improve plant growth under greenhouse conditions through the two previous studies, were investigated under field conditions. In order to determine the effects of application by selenium (Se), vitamin C and E, humic acid, and NPK fertilizers on plant growth characteristics of eggplant (*Solanum melongena,* L.) cv. Black Beauty and, consequently, their effect on root knot nematode population, a field experiment was carried out at a Farm located in Qalabsho, Dakahlia Governorate, Egypt, during the summer season of 2021. Before planting, soil samples from the experimental site's vertical spots (30 cm deep) were taken for root knot nematode counts. Initial population of *Meloidogyne* spp. showed that 870 J_2/250 g of soil. The trial area was divided into plots, each of which contained rows of plants spaced 50 cm apart and 6 m long. Ten treatments and four replicates (each containing ten plants) for each treatment comprised the experiment's entirely randomized block design. In the summer, two eggplant seedlings of cv. Black Beauty were sown in each hole during the last week of April. Later, the number of plants was thinned to one seedling per hole. After seven days, tested treatments were applied to the plants by hand-applying 200 ml of NPK fertilizer (19-19-19), 200 ml of vitamin C, 200 ml of vitamin E and 200 ml of selenium with previous concentrations. However, 20 ml of humic acid per plant was applied. Combinations among treatments were also applied. Three times of each treatment were given, each at 20 days intervals. A week after planting, the chemical nematicide oxamyl (24%L) was added twice at the tested rate, one month intervals. Treatments of fertilizers, antioxidants, selenium and humic acid against *Meloidogyne* spp. infected eggplant under field conditions were as follow:

1- Humic acid + NPK
2- Selenium + NPK
3- Vitamin E + NPK
4- Vitamin C + NPK
5- Humic acid+ Selenium
6- Vitamin E + Vitamin C
7- Humic acid + Selenium + NPK
8- Vitamin E + Vitamin C + NPK
9- Oxamyl
10- Control

After 90 days from the date of transplantation, a random sample of 5 plants from each replicate was taken to measure the growth characteristics, which included plant height, fresh weight, shoot dry weight, and yield per plant. A composite soil sample (250g) from each plot was prepared for nematode J_2s extraction using the modified Baermann technique and sieving (Goodey, 1957). According to Byrd et al. (1983), one gram of root from each plant was stained to count the nematode population in root, galls, and egg masses.

Determination of resistance related enzymes

In order to homogenize plant tissue (g), 0.2 M Tris-HCl buffer (pH 7.8) with 14 mM b-mercaptoethanol was added at a rate of 1/3 w/v. For 15 minutes, the homogenate was centrifuged at 3000 rpm. Using spectrophotometry (Shimadzu UV–visible Recording Spectrophotometer uv-240), the supernatant was utilized to calculate the enzyme activity (Tuzun et al. 1989). Peroxidase (POX) was measured using the Lee-described technique (1973). The amount of enzyme that, under typical assay circumstances, alters the absorbance at 470 nm per minute at 25 °C is considered one unit of enzyme activity. The increase in absorbance at 470 nm/g fresh weight/min was used to measure POX activity. In accordance with the procedure outlined by Bashan et al. (1985), Polyphenol oxidase (PPO) was assessed. The rise in absorbance at 475 nm/g of fresh weight/min was used to measure enzyme activity.

Determination of phenol compounds

Fresh eggplant leaf samples (1g) from each treatment were promptly chopped into small pieces and macerated in 95% boiling ethanol for 10 minutes. The macerated were put into soxhlet units with extraction solvent made of 75% ethanol. Twelve hours later, the extracting procedure was resumed. Extracts of ethanol were filtered and evaporated until all ethanol was gone. Isopropanol 50% was used to dissolve the dry residue in 5ml. The measurement of total phenols was done in accordance with Simons and Ross' instructions (1971). In a test tube, 0.2 milliliter of the sample extract was combined with 0.25 milliliter of concentrated hydrochloric acid. After that, the mixture was boiled for around 10 minutes. After cooling, 5 ml of sodium carbonate solution (20%) and 1 ml of the Folin reagent were added, and 10 ml of distilled water was used to dilute the mixture. After 30 minutes, the density of the produced blue color was measured at 520 nm with a spectrophotometer while using chatichole as a standard and a blank.

Biochemical analysis:

Using a 70 °C oven to dry the leaf samples, they were then wet digested after being finely powdered. The amounts of N, P, and K in leaves were measured using the techniques described by Mertens (2005a & b) and AGRILASA (2002), respectively. Five additional samples were chosen at random from each treatment to be tested for total carbohydrates % according to (Shumaila and Safdar, 2009), vitamin A according to Parrish (1977), and vitamin C according to Mazumdar and Majumder's technique (2003).

Data analysis:

According to Gomez and Gomez (1984), CoSTATE computer software program was utilized to do statistical analysis on the obtained data. Means were compared according to Duncan's multiple range test at $p \leq 0.05$.

RESULTS

1. Greenhouse experiments:

1.1. *Influence of vitamin C, vitamin E and NPK fertilizer singly or in combination on eggplant growth parameters as well as root knot nematode galling*.

The results of the analysis of variance showed that the treatments by vitamin E concomitant with vitamin C or/and NPK fertilizer recorded the best treatments in increasing shoot length of eggplant (Table 1). Additionally, compared to nematode inoculated check, all treatments increased plant root length. On the observation of the total fresh shoot and root weights and shoot dry weight of eggplant, treatments using vitamin E or vitamin C whether with one, or both of them, with NPK fertilizer, had a very substantial impact.

Table 1: Effect of vitamin E, vitamin C and NPK singly or in combination on plant growth parameters of eggplants infected with *Meloidogyne incognita* under greenhouse conditions.

Treatments	Length/plant (cm)		Fresh weight/ plant (g)		Total fresh weight	Total fresh weight Inc. %	Shoot dry weight (g)
	Shoot	Root	Shoot	Root			
Vitamin E	20.8[bc]	16.3[ab]	18.1[cd]	2.1[cd]	20.2[c]	3.06	2.6[de]
Vitamin C	20.5[bc]	14.8[a-c]	18.4[b-d]	1.2[e]	19.6[c]	0.00	2.8[c-e]
NPK	21.5[bc]	11.8[b-d]	18.3[b-d]	2.1[cd]	20.4[c]	4.08	3.3[b-e]
Vitamin E + NPK	23.3[ab]	12.8[a-d]	20.5 [a-c]	1.6[de]	22.1[a-c]	12.75	4.1[ab]
Vitamin C + NPK	20.3[bc]	13.5[a-d]	20.3[a-d]	2.3[bc]	22.6[a-c]	15.20	3.8[a-c]
Vitamin E + Vitamin C	22.5[a-c]	17.5[a]	22.7[a]	2.3[bc]	25.0[a]	27.55	3.9[a-c]
Vitamin E + Vitamin C + NPK	25.8[a]	16.5[ab]	21.6[ab]	3.0[a]	24.6[ab]	25.51	4.8a
Oxamyl	20.3[bc]	11.8[b-d]	19.3[a-d]	1.9[cd]	21.2[bc]	8.16	3.7[a-d]
Untreated inoculated check	16.0[d]	9[d]	16.9[d]	2.7[ab]	19.6[c]	0.00	2.33e
Untreated uninoculated check	18.8[cd]	10[cd]	21.1[a-c]	1.3[e]	22.4[a-c]	14.28	3.8[a-c]
LSD at 0.05	4.2	5.1	3.4	0.5	3.6		1.15

The mean of four replicates is used for each value.
According to Duncan's multiple range test, the means in each column that are preceded by the same letter(s) are not statistically different (P<0.05).

The findings also suggested that, to varying degrees, all previous treatments have nematicidal capabilities against nematode population, whether in the soil or on roots (Table 2). With the exception of oxamyl, which achieved the highest reduction in the final nematode population (RF=0.07) among all treatments, it was clear that concurrent treatment with three components produced superior effects (RF=0.44) than did single component. Combining vitamin E and vitamin C with NPK led to a dramatic reduction in total final nematode population, with reproduction factors of 0.69, 0.79, and 0.89 less than inoculated check, respectively. Since oxamyl achieved the highest decrease when root galls and egg masses indices were 2, similar index was obtained for the production of root galls. The number of galls and egg masses in the other treatments, whether individual or aggregated, did not differ significantly.

1.2 *Influence of humic acid, selenium and NPK fertilizer singly or in combination on eggplant growth parameters as well as root knot nematode galling.*

According to data in Table (3) all tested compounds significantly increased plant growth characteristics. In the majority of treatments, plants receiving two or three components performed better than those receiving just one. The combination of humic acid and selenium as well as NPK integration were the most successful treatments for lengthening plants. The percentages increase in total plant fresh weight above the inoculated check were 21.46, 21.73, and 54.34%, showing a discernible improvement when humic acid was combined with NPK or/and selenium, respectively.

Table 2: Influence of vitamin E, vitamin C and NPK fertilizer alone or in combination on *Meloidogyne incognita* population infecting eggplant under greenhouse conditions.

Treatments	Nematode population in			Total final nematode population (Pf)	*Rf	Galls	** RGI	Egg masses	*** EI
	Soil (250 g)	Root							
		Developed stages	Females						
Vitamin E	1180 bc	24.5 b	33.0 cd	1237.5 c	1.24	49.0 bc	4.0	29.8 cd	3.0
Vitamin C	1480 b	19.8 c	39.0 bc	1538.8 b	1.54	55.8 bc	4.0	35.0 bc	4.0
NPK	1120 cd	18.0 c	34.3 cd	1172.3 c	1.17	49.0.5 bc	4.0	32.5 b-d	4.0
Vitamin E + NPK	720 ef	24.0 b	47.0 b	791.0 e	0.79	63.5 b	4.0	40.3 b	4.0
Vitamin C + NPK	840 de	19.8 c	34.5 cd	894.3 d	0.89	49.5 bc	4.0	31.3 b-d	4.0
Vitamin E + Vitamin C	640 ef	20.0 c	29.5 cd	689.5 e	0.69	45.3 bc	4.0	27.0 cd	3.0
Vitamin E + Vitamin C + NPK	400 f	19.0 c	25.3 d	444.3 f	0.44	38.8 c	4.0	23.5 d	3.0
Oxamyl	60 g	4.8 d	6.3 e	71.1 g	0.07	7.8 d	2.0	5.5 e	2.0
Untreated inoculated check	2050 a	78.0 a	108.0 a	2236.0 a	2.24	170.3 a	5.0	75.0 a	4.0
LSD at 0.05	338.8	3.5	9.7	102.6		20.1		9.3	

* Reproduction factor (Rf) = Final population / Initial population. ** Root gall index (RGI) or egg masses index (***EI) scale: 0= no egg masses, 1= 1-2 egg masses, 2= 3-10, 3= 11-30, 4= 31-100 and 5= more than 100 egg masses / root system. According to Duncan's multiple range test, the means in each column that are preceded by the same letter(s) are not statistically different (P<0.05).

Similar patterns were observed with dry weight of eggplant, where humic acid treatment along with selenium and NPK produced the best results.

Table 3: Effect of humic acid, selenium and NPK singly or in combination on plant growth parameters of eggplants infected with *Meloidogyne incognita* under greenhouse conditions.

Treatments	Length/plant (cm)		Fresh weight/plant (g)		Total fresh weight (g)	Total fresh weight Inc. or Dec. %	Shoot dry weight (g)
	Shoot	Root	Shoot	Root			
Humic acid	21.0^{c}	14.3^{bc}	17.2^{b-e}	2.13^{bcd}	19.33^{de}	5.05	4.9^{bc}
Selenium	16.3^{de}	14.5^{bc}	15.5^{de}	1.82^{cd}	17.32^{f}	-5.86	3.3^{de}
NPK	18.8^{cd}	15.0^{bc}	15.9^{c-e}	1.70^{d}	17.60^{f}	-4.43	3.4^{de}
Humic acid + NPK	26.0^{b}	14.8^{bc}	19.6^{bc}	2.75^{b}	22.35^{b}	21.46	5.1^{a-c}
Selenium+ NPK	16.3^{de}	15.0^{bc}	17.9^{b-e}	2.63^{bc}	20.35^{cd}	10.59	4.2^{cd}
Humic acid+ Selenium	31.8^{a}	20.0^{a}	20.2^{ab}	2.2^{b-d}	22.4^{b}	21.73	5.6^{ab}
Humic acid + Selenium + NPK	32.5^{a}	17.3^{ab}	23.9^{a}	4.5^{a}	28.4^{a}	54.34	6.4^{a}
Oxamyl	26.0^{b}	18.3^{ab}	18.6^{b-d}	2.08^{b-d}	20.68^{cd}	13.36	5.5^{ab}
Untreated inoculated check	14.8^{e}	11.8^{c}	14.5^{e}	3.9^{a}	18.40^{ef}	--	2.2^{e}
Untreated uninoculated check	32.5^{a}	15.0^{bc}	18.8^{b-d}	2.13^{b-d}	20.93^{bc}	13.75	5.1^{abc}
LSD at 0.05	2.8	4.4	3.8	0.87	1.49		1.3

The mean of four replicates is used for each value.
According to Duncan's multiple range test, the means in each column that are preceded by the same letter(s) are not statistically different (P<0.05).

When compared to untreated (inoculated) plants, Table (4) clearly showed that the number of final nematode populations in the soil and roots of eggplants infected with *M. incognita* was dramatically suppressed. The nematode reproduction factor (Rf) for different treatments varied from 0.79 to 1.79 regardless of the chemical nematicide used. The best treatment was humic acid combined with selenium and NPK, which was 0.97, subsequently followed by treatment with selenium and NPK (1.38). Since RGI ranged from 3.0 for oxamyl and treatment (humic acid + selenium + NPK) to 4.0 for the remaining treatments, a similar pattern was recorded with the number of egg masses. The integration of humic acid with selenium and NPK fertilizer was shown to be more effective treatment in lowering the number of egg masses (11.5 egg masses / 1g root), but less than treatment with oxamyl (7.8 egg masses / 1g root) compared to inoculated check (62.8 egg masses/1g root).

2.Field experiment:

Data in Table (5) demonstrated the effects of humic acid, NPK fertilizer, and certain antioxidants on the plant development parameters of eggplant infected with *Meloidogyne* spp. in a field setting. It was clear that, as compared to the untreated check, all treatments significantly increased plant growth parameters. The best treatments for increasing plant length were applications of humic acid + selenium +

Table 4: Influence of humic acid, selenium and NPK fertilizer alone or in combination on *Meloidogyne incognita* population infecting eggplants under greenhouse conditions.

Treatments	Nematode population in			Final population	*Rf	Galls	**RGI	Egg masses	***EI
	Soil (250 g)	Root							
		Developed stages	Females						
Humic acid	1560^{bc}	26.5^{b}	37.5^{c}	1624.0^{cd}	1.62	42.8^{c}	4.0	28.8^{c}	3.0
Selenium	1640^{b}	20.8^{c}	37.8^{c}	1680.6^{bc}	1.68	42.0^{c}	4.0	31.8^{c}	4.0
NPK	1720^{b}	24.0^{bc}	49.3^{b}	1793.3^{b}	1.79	58.5^{b}	4.0	40.5^{b}	4.0
Humic acid + NPK	1480^{bc}	10.3^{d}	32.0^{c}	1522.3^{d}	1.52	38.8^{c}	4.0	27.5^{c}	3.0
Selenium+ NPK	1320^{c}	22.8^{bc}	34.3^{c}	1377.1^{e}	1.38	40.8^{c}	4.0	27.8^{c}	3.0
Humic acid+ Selenium	1320^{c}	21.0^{c}	49.0^{b}	1390.0^{e}	1.39	55.5^{b}	4.0	41.5^{b}	4.0
Humic acid + Selenium + NPK	940^{d}	11.0^{d}	18.8^{d}	969.8^{f}	0.97	25.0^{d}	3.0	11.5^{d}	3.0
Oxamyl	240^{e}	9.0^{d}	14.8^{d}	263.8^{g}	0.26	22.0^{d}	3.0	7.8^{d}	2.0
Untreated inoculated check	2400^{a}	36.5^{a}	72.0^{a}	2508.5^{a}	2.51	83.8^{a}	4.0	62.8^{a}	4.0
LSD at 0.05	286.1	3.9	5.9	127.9		7.7		5.4	

* Reproduction factor (Rf) = Final population / Initial population ** Root gall index (RGI) or egg masses index (***EI) scale: 0= no galls or egg masses, 1= 1-2 galls or egg masses, 2= 3-10 galls or egg masses, 3= 11-30 galls or egg masses, 4= 31-100 galls or egg masses and 5= more than 100 galls or egg masses / root system.
Means in each column followed by the same letter(s) are not significantly different (P<0.05) by Duncan's multiple range test.

Table 5: Influence of some fertilizers and antioxidants concomitantly on plant growth parameters of eggplant infested with *Meloidogyne* spp. under field conditions

Treatments	Length (cm)	Inc. %	Plant weight (g)	Inc.%	Dry weight (g)	Inc. %	Yield g/ plant	Inc, %
Humic acid + NPK	83.75^{b}	60.28	330.5^{bc}	70.09	53.2^{d}	87.32	486.8^{f}	68.85
Selenium+ NPK	71.00^{c}	35.88	269.0^{f}	38.44	42.6^{ef}	50.0	538.3^{d}	86.71
Vitamin E + NPK	63.5^{d}	21.53	212.8^{g}	9.52	37.2^{g}	30.98	385.0^{h}	33.54
Vitamin C + NPK	74.25^{c}	42.1	290.3^{e}	4904	46.1^{e}	62.32	446.3^{g}	54.80
Humic acid+ Selenium	92.5^{a}	77.03	325.8^{cd}	67067	57.9^{c}	103.87	623.3^{b}	116.19
Vitamin E + Vitamin C	63.5^{d}	21.53	253.3^{f}	30.36	39.6^{fg}	39.43	326.8^{i}	13.35
Humic acid + Selenium + NPK	96.75^{a}	85.16	407.5^{a}	109.72	73.9^{a}	160.21	731.3^{a}	153.65
Vitamin E + Vitamin C + NPK	95.00^{a}	81.81	345.5^{b}	77.81	65.8^{b}	131.69	576.3^{c}	99.89
Oxamyl	82.25^{b}	57.41	312.5^{d}	60.83	55.1^{cd}	94.1	513.8^{e}	78.21
Untreated check	52.25^{e}		194.3 ^{h}		28.4^{h}		288.3^{j}	
LSD at 0.05	5.327		16.98		3.64		13.42	

According to Duncan's multiple range test, the means in each column that are preceded by the same letter(s) are not statistically different (P<0.05).

NPK (85.16%), vitamin E + vitamin C + NPK (81.81%), and humic acid + selenium (77.03%). Humic acid and selenium as single (109.27, 160.21%), as well as vitamin E and vitamin C as single (77.81, 131.69%), when used in conjunction with NPK, significantly (P≤ 0.05) increased the fresh and dry weight of eggplants that had been infected with *Meloidogyne* spp. The weight of the yield per plant increased by 153.65% with treatment by humic acid + selenium + NPK as well. Humic acid combined with selenium was the second most successful treatment, greatly increased the yield of eggplants (116.19%) in soil naturally contaminated with *Meloidogyne* spp.

The findings also suggested that, to varying degrees, all previous treatments had nematicidal effects on the population of root-knot nematode (Table 6). With the exception of oxamyl, which sustained the highest nematode population suppression (61.12%), concurrent treatments using three components clearly outperformed dual ones. In comparison to untreated plants, treatment using humic acid with selenium and Vitamin E and Vitamin C combined with NPK fertilizer performed the best and dramatically reduced overall nematode population. The second best treatment included a combination of humic acid and selenium with a decrease percentage of (48.94%). Additionally, the number of galls and egg masses were significantly (P≤0.05) suppressed in concurrent treatments using three components (humic acid + selenium + NPK) and (vitamin E + vitamin C + NPK) since the numbers were 57.0 & 74.0 galls/ 1g root and 56.5 & 39.8 egg masses/ 1g root), respectively.

Table 6: Influence of some antioxidants integrated with humic acid and/or NPK fertilizer on population density of *Meloidogyne* spp. infesting eggplants under field conditions

Treatments	Nematode population / 250 g soil	*Rf	Red. %	Galls/1 g root	** RGI	Egg masses / 1g root	*** EI
Humic acid + NPK	1827.5^{d}	2.10	45.03	115.8^{e}	5.0	75.3^{d}	4.0
Selenium+ NPK	2062.5^{c}	2.27	37.96	122.8^{de}	5.0	88.3^{c}	4.0
Vitamin E + NPK	2412.5^{b}	2.77	27.44	154.0^{b}	5.0	99.3^{b}	4.0
Vitamin C + NPK	2120.0 ^{c}	2.44	36.24	127.3^{d}	5.0	76.3^{d}	4.0
Humic acid+ Selenium	1697.5^{e}	2.02	48.94	93.3^{f}	4.0	68.3^{d}	4.0
Vitamin E + Vitamin C	2465.0^{b}	2.83	25.86	141.8^{c}	5.0	89.3^{c}	4.0
Humic acid + Selenium + NPK	1546.5^{f}	1.78	53.48	57.0^{h}	4.0	56.5^{e}	4.0
Vitamin E + Vitamin C + NPK	1623.0^{ef}	1.87	51.18	74.0^{g}	4.0	39.8^{f}	4.0
Oxamyl	1292.5^{g}	1.48	61.12	44.3^{i}	4.0	35.8^{f}	4.0
Untreated check	3325.0^{a}	3.82		190.3^{a}	5.0	135.3^{a}	5.0
LSD at 0.05	93.8			8.82		9.3	

Initial population = 870 J2/ 250 g soil * Reproduction factor (Rf) = Final population / Initial population.
** Root gall index (RGI) or egg masses index *** (EI) scale: 0= no galls or egg masses, 1= 1-2 galls or egg masses, 2= 3-10, 3= 11-30, 4= 31-100 and 5= more than 100 galls or egg masses / root system.
According to Duncan's multiple range test, the means in each column that are preceded by the same letter(s) are not statistically different (P≤0.05).

In addition, the current investigation clarified the effects of screened treatments on the peroxidase (PO), polyphenol oxidase (PPO) activities, as well as total phenols in fresh leaves of eggplants infected with *Meloidogyne* spp (Figure 1). The maximum PO and PPO activities were recorded in eggplants treated concurrently with humic acid, selenium + NPK, with percentages of 75.6 and 268.75%, respectively, followed by treatment with vitamin E, vitamin C + NPK, with percentages of 53.88 and 256.25%, respectively. Additionally, data showed that as compared to screened treatments, eggplant grown in soil that was naturally infested with *Meloidogyne* spp. (untreated control) showed a substantial drop in total phenols. On the other hand, oxamyl (132.29%) and concurrent treatments using three components of humic acid + selenium + NPK had the highest percentage of total phenols (109.81%).

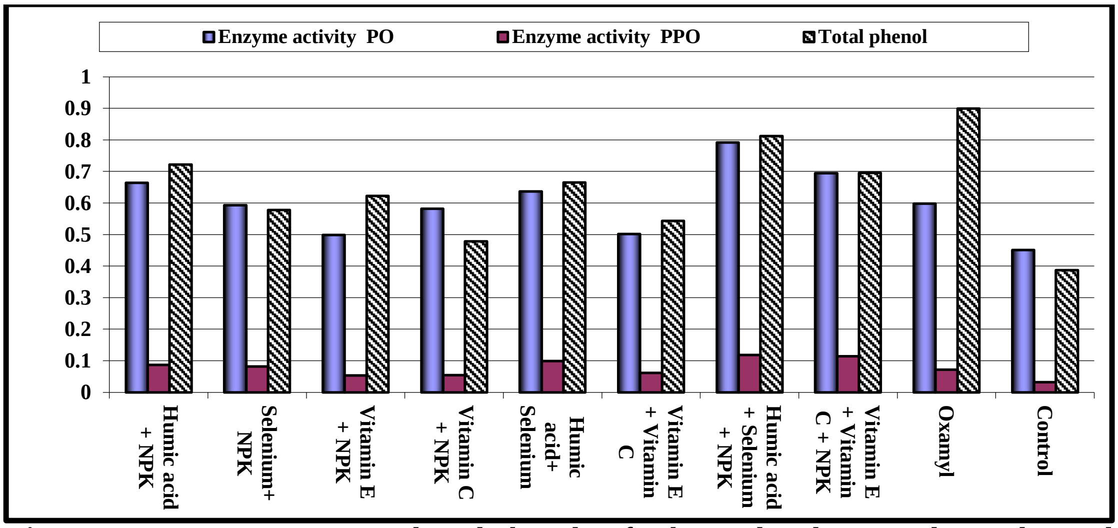

Figure 1: Enzyme activities and total phenol in fresh eggplant leaves cultivated in soil naturally contaminated with *Meloidogyne* spp. and treated with humic acid or other antioxidants combined with NPK fertilizer.

Concerning N, P and K contents% in eggplant leaves, data dipected in Figure (2) illustrated that the interaction between humic acid with selenium and NPK fertilizer treatment had a significant effect on N, P and K contents (%) followed by dual treatment of humic acid and NPK fertilizer.

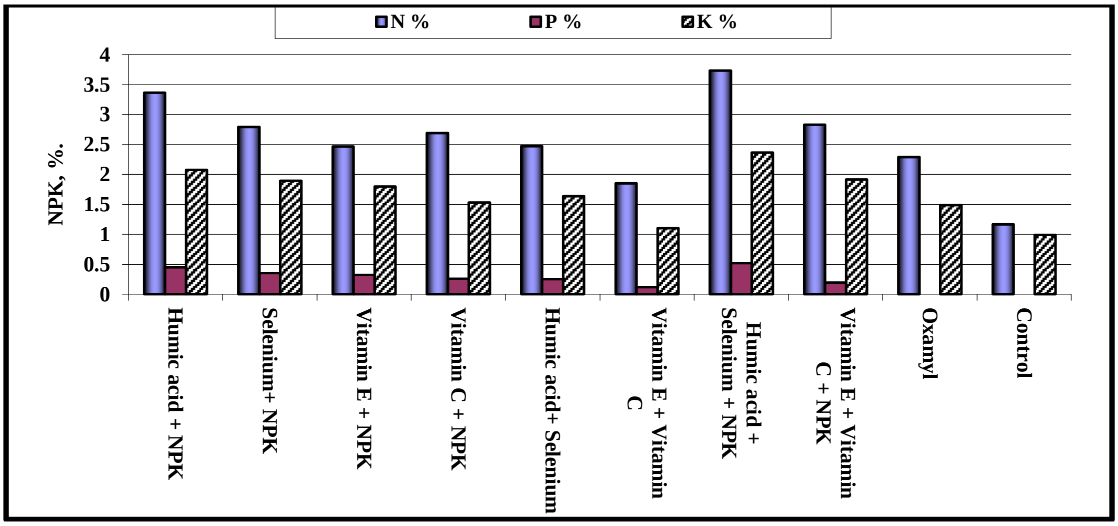

Figure 2: Effect of some antioxidants integrated with humic acid and/or NPK fertilizer on N, P and K percentage in leaves of eggplant infested with *Meloidogyne* spp. under field conditions.

According to the results in Table (7), there were notable favorable impacts on all fruit quality indices in eggplant fruits as a result of the interaction between antioxidant, humic acid, and NPK fertilizer. The combination of humic acid, selenium, and NPK, with a percentage of 30.295%, as the highest value of total carbohydrates, which was followed by treatment of vitamin E + vitamin C + NPK (28.555%). Similar trends were observed for vitamin A and C values, as three-component treatments outperformed dual treatments.

Table 7: Effect of interactions between some antioxidants, humic acid and NPK fertilizer on eggplant fruit quality.

Treatments	Total carbohydrate %	Vitamin A IU	Vitamin C (mg/100g)
Humic acid + NPK	26.605^{c}	31.380^{c}	3.885^{e}
Selenium+ NPK	21.600^{g}	25.485^{f}	3.625^{fg}
Vitamin E + NPK	22.575^{f}	29.625^{d}	3.575^{g}
Vitamin C + NPK	21.745^{g}	23.605^{g}	4.540^{c}
Humic acid+ Selenium	24.420^{d}	31.495^{c}	2.895^{h}
Vitamin E + Vitamin C	23.630^{e}	26.705^{e}	4.235^{d}
Humic acid + Selenium + NPK	30.295^{a}	35.385^{a}	4.900^{b}
Vitamin E + Vitamin C + NPK	28.555^{b}	34.685^{b}	5.625^{a}
Oxamyl	22.660^{f}	29.395^{d}	3.725^{f}
Untreated check	21.535^{g}	22.665^{h}	2.645^{i}
LSD 5%	0.382	0.328	0.128

According to Duncan's multiple range test, the means in each column that are preceded by the same letter(s) are not statistically different ($P \leq 0.05$).

DISCUSSION

The findings of this study indicated that the population of the root knot nematode in soil and roots, under greenhouse and field conditions, significantly decreased after chemical and organic fertilizers and antioxidant substances were applied. Under greenhouse conditions, the plant characteristics of eggplant infected with *M. incognita* significantly improved with percentage increases over control for both vitamin C (ascorbic acid) and vitamin E (α-tocopherol). Ascorbic acid application may have a stimulatory impact on plants, as evidenced by the fact that it significantly increased tomato yields and vegetative development metrics (Abdel-Halim, 1995). Similar findings were made by other researchers on the stimulatory effects of vitamin C on other plants such as on pepper (Shehata et al., 2002), potato (El-Banna et al., 2006), and on pea plants (Helal et al., 2005). Applying vitamin E made the amount of plant criteria much more obvious. One could draw the conclusion that vitamin E was thought to prevent photo oxidation of chloroplast membranes and contribute to the creation of an ideal environment for the photosynthetic apparatus (Bosch, 1995). Similar growth enhancement caused by α-tocopherol was noted in geranium (Ayad et al., 2009), lettuce, Shafeek et. al. (2013), *Hibiscus rosasineses* (El-Aziz et al., 2009), *Vicia faba* (Orabi and Abdelhamid, 2014, Semida et al., 2014), and *Calendula officinalis* L (Soltani et al., 2012) under stress conditions.

Treatment with Vitamin C combined with Vitamin E and NPK fertilizer led to a substantial decrease in the nematode population. This outcome is consistent with that by Farahat et al. (2018) who showed that the commercial inorganic fertilizer (Mega NPK) was significantly successful in lowering the nematode counts. Olowe's (2012) research yielded comparable outcomes. The major substrate in the cycle pathway for the detoxification and neutralization of superoxide radicals and singlet oxygen is the tiny and water-soluble antioxidant molecule ascorbic acid (Noctor and Foyer, 1998). It has been demonstrated that ascorbate plays a variety of roles in plant growth, including those related to cell division, cell wall expansion, and other developmental processes (Pignocchi and Foyer, 2003). To combat the O_2 radicals generated by the Mehler reaction and photorespiration, ascorbate works in conjunction with glutathione and a number of enzymatic antioxidants (Noctor and Foyer, 1998). Ascorbate is also thought to detoxify O_2 and OH (Asada, 1999). Exogenous ascorbate treatment has also been reported to improve tolerance to salt stress and lessen oxidative damage (Shalata and Neumann, 2001).

Selenium, humic acid, and NPK fertilizer were also tested for their nematicidal abilities against *M. incognita*, infected eggplant. The majority of treatments resulted in significant nematode population suppression in both soil and roots, as well as a notable improvement in plant growth parameters. This outcome was consistent with Song et al. (2022) who observed a negative association between nematode abundance and soil accessible Se as a decrease in nematode abundance caused by selenite. Additionally, Hammad et al. (2021) demonstrated that humic acid had an intermediate impact on the populations of citrus and root knot nematodes infected olive trees.When *Meloidogyne* spp. infected eggplant under field settings, integrated management clarified that most applications with three components performed better than those with only two. All treatments significantly increased plant criteria, especially when NPK fertilizer was present. In this regard, researchers found that increasing the amount of NPK fertilization was effective in raising eggplant yield (Lawal et al., 2015; Muoneke et al., 2016).

Humic acid and selenium treatments combined with NPK significantly decreased final nematode populations, whether they were in the soil or the roots. There are two ways that selenium might reduce the number of nematodes. Firstly, high Se directly disrupts the antioxidant defense system and protein expression in nematode (Lv et al., 2021). Secondly, it is possible that Se indirectly reduced nematode abundance by altering biotic (microorganisms associated with plant roots) and abiotic (soil characteristics) components (Liu et al., 2016). Additionally, Saeedizadeh et al. (2020) found that the NPK and humic acid treatments showed the greatest reduction in nematode parameters (developmental stages, females, egg masses, and the reproduction factor) and galls.

In addition, it became clear that all treatments induced systemic resistance by triggering a number of defense-related enzymes, including peroxidase (PO), polyphenol oxidase (PPO), and total phenols. The host plants' enzymes are a key component of the nematode resistance systems. In a previous study conducted by Osman et al. (2013) they showed that measuring specific enzymatic activities of eggplant infected with *M. incognita* and treated with abiotic inducers (GABA, chitosan and ascorbic acid) revealed a generalized increase in the activity of the enzymes PPO, POX, and chitinase compared to inoculated untreated plants. The peroxidase (POX) and polyphenol oxidase (PPO) in the roots of resistant and susceptible tomato cultivars infected with *M. javanica* were identified by Mohamed and Hammad (2003). They observed that various tomato cultivars had variable levels of enzyme activity stimulation. Their activities were greater in the resistant cultivars than in the susceptible ones.

The chemical elements nitrogen, potassium, and phosphorus in eggplant leaves, as well as total carbohydrates, vitamin A, and vitamin C in fruits, were significantly increased in the tested treatments. These outcomes supported by Baddour’s (2010) research, which showed that applying NPK fertilizer significantly increased the average Vitamin C levels compared to the control treatment. According to the survey, the availability of nutrients led to a rise in the nutritional value of eggplant. The harvest economic products' quality was inevitably improved as a result. This suggested having production with a high nutritional value and appropriate care. Overall, the results are consistent those found by Nafiu et al. (2011) and Lawal et al. (2015).

CONCLUSION

Environmental nutrition factors have a considerable impact on plant tolerance and resistance to diseases, even though these traits are genetically determined. Mineral nutrients can influence a plant's ability to fight or tolerate diseases and pests. Plants that receive enough nourishment are more disease resistant and tolerant. This is due to the fact that stronger plants are better able to compensate for a loss of photosynthates or a reduction in root and leaf surface area brought on by pathogen infection or inadequate nutrition, for example. In this perspective, plant nutrition can be thought as an environmental nutrition factor that is manageable and plays a key role in the prevention of pathological system as well as the management of phytonematodes, which have been linked to losses in numerous significant agricultural crops. Additionally, using antioxidants in the present study induced plant systemic resistance, suppressed nematode population and lessened the negative impacts of root knot nematodes.

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الملخص العربي

استحثاث المقاومة ضد نيماتودا تعقد الجذور على نبات الباذنجان بواسطة سماد كيماوي وعضوي ومواد مضادة للأكسدة

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تعتبر نيماتودا تعقد الجذور (*Meloidogyne* spp.) من اهم آفات المحاصيل الصيفية. وعلى الرغم من أن استخدام مبيدات النيماتودا التركيبية لمكافحتها طريقة ناجحة ومجربة وحقيقية، إلا أن هذه الطريقة تثير مخاوف بسبب ارتفاع تكاليفها وتأثيراتها البيئية السلبية. تم اجراء تجربه باستخدام الاسمدة مثل الأسمدة الكيماوية (NPK) والسماد العضوي (حمض الهيوميك) وبعض المواد المضادة للأكسدة (السيلينيوم وفيتامين سي وفيتامين هـ) منفردة او متجمعة لمكافحة نيماتودا تعقد الجذور على شتلات الباذنجان صنف Black Beauty تحت ظروف الصوبة والحقل. وفقًا لنتائج هذه المعاملات، وجد ان جميع المعاملات لديها القدرة على تقليل أعداد نيماتودا تعقد الجذور، مما يزيد من إنتاج المحصول. أدت الفيتامينات C و E مع سماد NPK إلى زيادة معنوية في وزن النبات الطازج (٢٥,٥١٪) والوزن الجاف تحت ظروف الصوبة الزراعية. كما أنها قللت بشكل كبير من عدد النيماتودا الكلى مع معدل تكاثر أقل (٠,٤٤) بالمقارنة بالنباتات الغير معاملة ومعدية بالنيماتودا. بالإضافة إلى ذلك، أدت المعاملة المركبة الثلاثية لحمض الهيوميك والسيلينيوم وNPK المعززة لنمو النبات إلى انخفاض كبير في أعداد الطور اليرقى الثانى وتكوين العقد وكتل البيض. تم قياس طول النبات، والوزن الطازج والجاف، والمحصول لكل نبات، ومحتويات N، و P ، و K ، وكذلك جودة الثمار (إجمالي الكربوهيدرات ، وفيتامين A ، وفيتامين C) فى التجربة الحقلية. حيث وجد ت زيادة معنوية في النمو الخضري لنبات الباذنجان خاصة عند استخدام حمض الهيوميك و / أو مضادات الأكسدة المصاحبة لسماد NPK. كذلك وجد خفض في أعداد النيماتودا في التربة والجذر خاصة بواسطة معاملات حمض الهيومك ومضادات الاكسدة او عند اتحادها مع سماد NPK المختبرة حيث استحثت المقاومة ومن ثم احدثت تثبيطا ضد نيماتودا تعقد الجذور فى نبات الباذنجان.