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**ORIGINAL PAPER**

# Systemic Resistance in Tomato Elicited by Some Chemical Inducers Against Root-Knot Nematode, *Meloidogyne incognita*

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## ABSTRACT

Root-knot nematodes are globally considered one of the most common phytonematodes infecting tomato crops. In this current study, treatments were designed to enhancing the tomato plant resistance against *Meloidogyne incognita*, this investigation was conducted by different treatments to reduce root-knot harmful effect on tomato plants. Tomato plants were treated with antioxidants *i.e.*, Selenium (Se) at the concentration of 25 ppm, Vitamin C (VC) at the concentration of 50 ppm and Vitamin E (VE) at the concentration of 50 ppm by irrigation and treated with nematicide Oxineem 24% SL which was added at the rate of 4 L/Fed application method. Generally, the results indicated that treatments of antioxidants showed a significant response in suppression of root-knot nematode. The optimum antioxidant treatment was when irrigating tomato plants with Selenium at 25 ppm 8, 15, 30 and 45 days after inoculation, which decreased all parameters of root-knot nematode compared with control. This positively reflected on the plant health through induction of defense related components. The nematicide Oxineem 24% SL gave the highest reduction in nematode population compared with control. All of treatments were significantly effective in increasing enzyme activities *i.e.*, Catalase, Peroxidase and Polyphenoloxidase which are responsible for defense mechanisms of infected tomato plants. Antioxidative treatments with the used concentrations have a great influencing effect on all growth parameters of tomato plants. Histological modifications in tomato plant roots infected with *M. incognita* treated with different chemical inducers poorly formed giant cells in the central cylinder were detected in the stellar region with limited hypertrophy or hyperplasia contained less or free of cytoplasm, fewer numbers of nuclei, vacuolated in most instances, and were smaller than those of untreated infected roots.

**Keywords:** Tomato, *Solanum lycopersicum*, Root-knot nematode, *Meloidogyne incognita*, Antioxidants, Systemic Resistance, Selenium, Vitamin C, Vitamin E.

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## INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop in the world produced on 4.85 million ha, producing approximately 182.3 million tons (Anon., 2019). Egypt is the world's fifth-largest producer of tomatoes, during the four seasons 2015/16 – 2018/19 in 475.514 thousand feddan cultivated area, produced approximately 7.94 million tons (Hassan *et al*., 2020).

*Meloidogyne incognita* infects roots of tomato plants is considered one of the most important vegetable crops. Root-knot nematodes are the most aggressive phytopathogens infecting tomato plants. Systematic nematicides are usually used to control diseases but their toxicity causes environmental pollutions.

Among the several pests and diseases that affect tomato plants, the plant-parasitic nematodes pose a major threat, resulting in an estimated annual monitoring loss of about 80 billion $ (Nicol et al, 2011). The most prevalent and destructive among the Phyto-nematodes of tomato are *Meloidogyne* spp., which cause major economic losses on vegetables, especially in tropical and subtropical areas (Castagnone-Sereno *et al*., 2013; Anes and Gupta, 2014). In Egypt, tomatoes are known to be highly susceptible to root-knot nematodes, *Meloidogyne* spp., causing annual losses of 12% (Abd-El gawad 2014).

The penetration and development of the root-knot nematode in the resistant species was reduced in comparison with that occurring in susceptible plants. Proite *et al*. (2008) observed cytoplasm and altered organelle structure in the central cylinder of *Arachis stenosperma*, indicating a hypersensitive-like response (HR) of infested host cells.

Endoparasites induce permanent feeding sites that are comprised of 'giant cells' and are subject to extensive changes in vascularization, resulting in the giant cells being encaged within a network of *de novo* formed xylem and phloem cells (Bartlem *et al*., 2013) despite being considered critical to the function of the feeding site, the mechanisms underlying this

vascularization have received surprisingly little attention when compared with the amount of research on giant cell development and function they found defense enzymes polyphenol oxidase and peroxidase secreted by the host responsibility were evaluated for possible enhancement of the systemic acquired resistance.

Therefore, this study aimed to evaluate some antioxidants *i.e*., Selenium, Vitamin C and Vitamin E on root-knot nematode, *M. incognita* on tomato plants and histopathological changes in root-knot nematode (RKN) infected tomato plants as affected by different antioxidant application.

## MATERIALS AND METHODS

The present investigation was carried out at the Experimental Farm under greenhouse condition at Fac. Agric., Menoufia Univ., Shebin El-kom City, Egypt

**2.1. Preparation of *M. incognita* inocula**:

Culture of root-knot nematode, *M. incognita* was established from single egg mass of adult females and identified by the morphological characteristics of the female perianal patterns.

Root-knot nematode eggs were extracted from infected tomato roots using 0.5% Sodium hypochlorite (NaOCl) technique as described by Riekert (1995).

Roots were cut into small pieces and then macerated for two periods of 10 seconds each at high speed by using a waring blender. This method released the highest number of nematodes from roots. The macerated root suspension was then placed into one-liter conical flask containing sodium hypochlorite (NaOCl).

Water was added to adjust the final concentration of NaOCl to 0.5 % as described by Riekert (1995). The suspension in the conical flask was vigorously shaken for three minutes to release the eggs from the gelatin matrix as NaOCl removes the gelatin matrix of egg masses. The obtained suspension was poured through different size sieves to remove the root tissue. Eggs were collected on the 20 micrometer (µm) (635 mesh) sieve and washed several times with tap water to remove the residual of NaOCl. Eggs were transferred to a flask containing tap water then counted under light microscope.

Root-Knot nematode inocula were applied by pipetting aqueous suspension of approximately 3000 eggs around the root zone.

**2.2. Preparation of Treatments:**

1. Selenium was added as sodium selenite obtained from Sigma Chemical Company, it was applied at the rate of 50 mg/ L.
2. Vitamin E (Tocopherol) was also obtained from Sigma Chemical Company, and was applied at the rate of 50 mg/ L.
3. Vitamin C (Ascorbic acid) was obtained from Multi-Apex Pharma Company, and was applied at the rate of 50 mg/ L.
4. Nematicide Oxineem 24% SL was applied at the rate of 25 mg/ L.

**2.3. The nematicide Oxineem El-Nasr 24% SL:**

Common name: Oxamyl.

Chemical name: $C_7H_{13}N_3O_3S$., Dose per Feddan, 3 L.

**2.4. Pot experiment**

Three hundred and sixty tomato seedlings [*Solanum lycopersicum*] three weeks old were transplanted into 15 cm diam. plastic pots filled with 1 kg per pot with autoclaved sand clay soil (1:2, v/v). Each pot was inoculated with about 3000 eggs of *M. incognita*, one tomato seedling was transplanted in each pot; all the pots were set on the bench in a greenhouse.

The experimental treatments were (1) untreated control inoculated with *M. incognita*; (2) Selenium 25 ppm (3) vitamin C 50 ppm (4) vitamin E 50 ppm (5) nematicide Oxineem 24% SL. Treatments 2–5 and control were all inoculated with *M. incognita*. by pouring the nematode suspension into holes made to a depth of 2-4 cm below the soil surface around the base of the plants. Fifty days after inoculation, tomato plants were uprooted, washed free of adhering soil. Number of the second-stage juveniles (J2), stage juveniles per 250 g soil were extracted from the soil by the decanting and sieving technique and counted. Third stage juveniles, fourth and females in soil, the roots were stained after 8, 15, 30, and 45 days from treatments by acid fuchsin in acetic acid according to Bybd *et al*. (1983) to examine and counting the number of developmental stages, and females/root.

Pots were assigned to two groups and treatments were added as follows:

The first group (sixty pots) each, planted with three seedlings untreated control inoculated with *M. incognita*, antioxidants were added as irrigation treatment and nematicide as spraying, as follows:

1- treatment with 25 ppm of selenium at eighth day after inoculation,
2- treatment with 25 ppm of selenium at fifteenth day after inoculation,

3- treatment with 25 ppm of selenium at the thirtieth day after inoculation,
4- treatment with 25 ppm of Selenium at forty fifth day after inoculation,
5- treatment with 50 ppm of Vitamin C at eighth day after inoculation,
6- treatment with 50 ppm of Vitamin C fifteenth day after inoculation,
7- treatment with 50 ppm of Vitamin C thirtieth day after inoculation,
8- treatment with 50 ppm of Vitamin C forty fifth day after inoculation,
9- treatment with 50 ppm of Vitamin E at eighth day after inoculation,
10- treatment with 50 ppm of Vitamin E fifteenth day after inoculation,
11- treatment with 50 ppm of Vitamin E thirtieth day after inoculation,
12- treatment with 50 ppm of Vitamin E forty fifth day after inoculation,
13- spraying treatments with Oxineem 24% SL at eighth day after inoculation,
14- spraying treatments with Oxineem 24% SL fifteenth day after inoculation,
15- spraying treatments with Oxineem 24% SL thirtieth day after inoculation,
16- spraying treatments with Oxineem 24% SL forty fifth day after inoculation,
17- Untreated control seedlings transplanted in pots inoculated with *M. incognita* at the eighth day after inoculation,
18- Untreated seedlings transplanted in pots inoculated with *M. incognita* at the fifteenth day after inoculation,
19- Untreated control seedlings transplanted in pots inoculated with *M. incognita* at the thirtieth day after inoculation and
20- Untreated control seedlings transplanted in pots inoculated with *M. incognita* at the forty fifth day after inoculation.

Before reading the disease severity, plant height of tomato plant was measured, meanwhile, fresh, and dry weights of shoots and roots were measured, as well plants were placed at 60°C for 3 days in moisture dryer chamber to determine the dry weight. The parameters means were calculated for all treatments and compared with healthy and infected control.

**2.5. Impact of tomato plant treatments with different inducers on the activity of oxidative enzymes:**

Catalase (CAT), Peroxidase (PO) and polyphenoloxidase (PPO) were determined in tissue extracts of surviving tomato plants collected from the following treatments: (1) Selenium; (2) Vitamin C; (3) Vitamin E; (4) Nematicide Oxineem (5) Control (infested soil) and (6) Control (non-infested soil). All treatments were grown in sterilized soil infested with *M. incognita*. Samples of tomato seedlings (shoot) of each treatment were collected 12 days after transplanting with the tested *M. incognita* and infected seedlings were used as control treatments. One gram of plant tissue was homogenized in 50 mM potassium phosphate buffer (pH 6.8) in 10 mL of ice-cold containing, 10 mM β-mercaptoethanol, 1 mM EDTA, 1% polyvinylpyrrolidone and 1M NaCl (Biles and Martyn, 1993). The filtrated homogenates through cheesecloth were centrifuged for 25 min at 8,000 rpm at 4°C. The supernatants (crude enzyme extract) were immediately used for the determination of CAT, PO and PPO activities according to Soltis and Soltis (1990) or were stored at -20°C. Three replicates (three plants/replicate) were used for each treatment, and using a Milton Roy 1201 Spectrophotometer (PEMEDR, Denver, CO, USA) two spectrophotometric readings were taken per replicate, for the determination of enzyme activities.

**2-5-1. Peroxidase (PO) assay:**

PO activity was measured directly by Hammerschmidt *et al*. (1982) spectro-photometrical method using guaiacol as a common substrate. The reaction mixture consisted of a 1.40 mL solution containing sodium phosphate buffer (0.2 mL 1% guaiacol+0.2 mL 1% $H_2O_2$ +1 mL 10 mM potassium phosphate buffer), hydrogen peroxide ($H_2O_2$), and guaiacol and 0.2 mL crude enzyme extract. The mixture was incubated for 5 min at 25°C and in absorbance over 1 min at 470 nm. the initial rate of increase was measured, the activity was expressed as units of PO/mg protein (Urbanek *et al*., 1991).

**2-5-2. Polyphenoloxidase (PPO) assay:**

The activity of PPO was determined according to Gauillard *et al*. (1993). The increase of absorbance at 410 nm at 30°C during 10 min was measured, by adding crude extract (50 µL) to 3 mL of a solution containing 25mM pyrocatechol and 100 mM potassium phosphate buffer, pH 6.5. the activity was expressed as units of PPO/mg protein.

**2-5-3. Catalase (CAT) assay:**

Catalase activity was determined using the method of Dhindsa *et al*. (1981) by measuring $H_2O_2$ consumption. A mixture 3 mL reaction contained 15 mM $H_2O_2$, 50 mM potassium phosphate buffer pH 7.0 and 50 ml enzyme extract. By adding the $H_2O_2$ the reaction was initiated. Spectrophotometrically for 3 min. at 240 nm ($\Sigma$= 39.4 mM-1 cm-1) the consumption

of $H_2O_2$ was measured. The CAT activity was expressed as CAT units/mg protein/min.

**2.6. Histopathological studies:**

Two random samples were obtained from every treatment at 8, 15, 30 and 45 days after transplanting to study the histological response (host-parasite relationship). The plants were uprooted and carried immediately to the laboratory and the roots were separated and cut into 0.5 mm in length. Pieces representing each treatment were immersed in Formalin Acetic Acid (FAA) solution as described by Johansen, (1940) and Southey (1986).

Microtome sections were prepared at approximately 4-10 µm thickness with a Leica Ultracut UCT ultramicrotome. Thin sections were stained with toluidine blue (1X) then sections were examined by camera Leica ICC50 HD (Finley, 1981).

**2-7. Statistical Analysis:**

All obtained data using the MSTATC software program version 4 were subjected to the analysis of variance procedures and treatment means using the L.S.D. at P≤0.05 of confidence as described by Gomez and Gomez (1984) were compared.

## RESULTS

**Effect of applications time of different treatments on different stages of *M. incognita* in tomato plants infected with *M. incognita*:**

Tomato seedlings were transplanted in soil infested with *M. incognita*. Different treatments were conducted to reduce the harmful effect of root knot nematode at eight days, fifteen days, thirty days and forty-five days after transplanting with different treatments, Selenium, 25 ppm, V.C 50 ppm and V.E 50 ppm by irrigating. Nematicide was used by spraying. Histological changes were observed in plant roots due to nematode infection (Fig. 1). The 2nd and 3rd stages were counted at eight days, 3rd stage (Fig. 2) was counted at thirty days after planting in the infested soil under different treatments. 4th stage (Fig. 3) was counted at thirty days after soil infestation under different treatments. Females (Fig. 4) were counted forty-five days after soil infestation under different treatments compared with the counts of the same stages in other treatments and control.

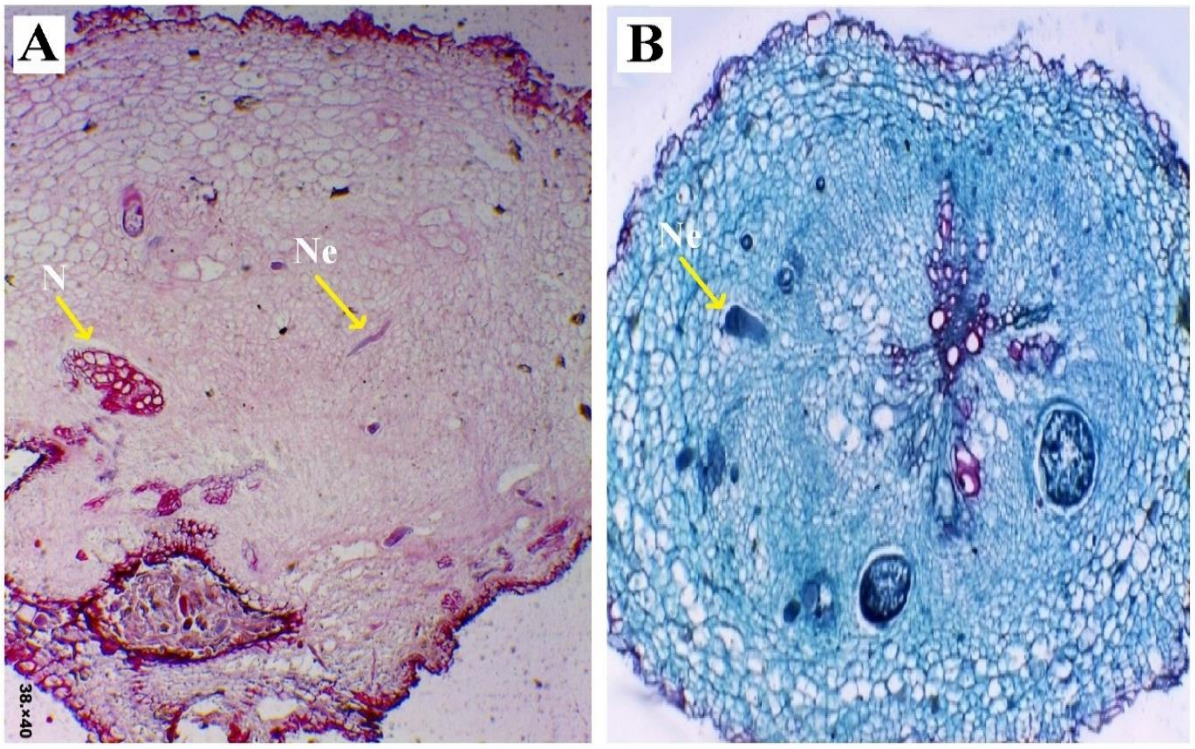


**Figure (1): Cross sections in tomato root: A. control ,8 days after planting in infested soil. B. section in root treated with vitamin E 50 ppm showing 2nd juvenile stage 8 days after inoculation (×100).**

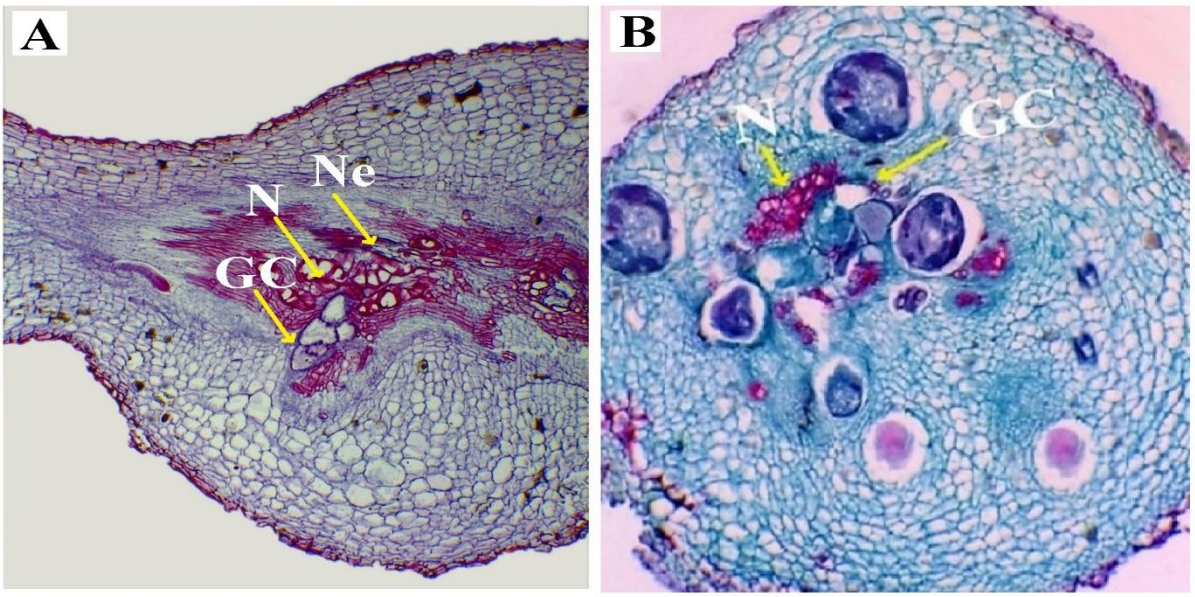


**Figure (2): Cross sections in tomato roots: A. control 15 days after planting in infested soil. B. treated with selenium 25 ppm showing 3rd stage 15 days after planting in infested soil, Ne: Nematode, N: Necrosis was decreased due to the Selenium treatment, GC: decreased giant cell due to the Selenium treatment (×100).**

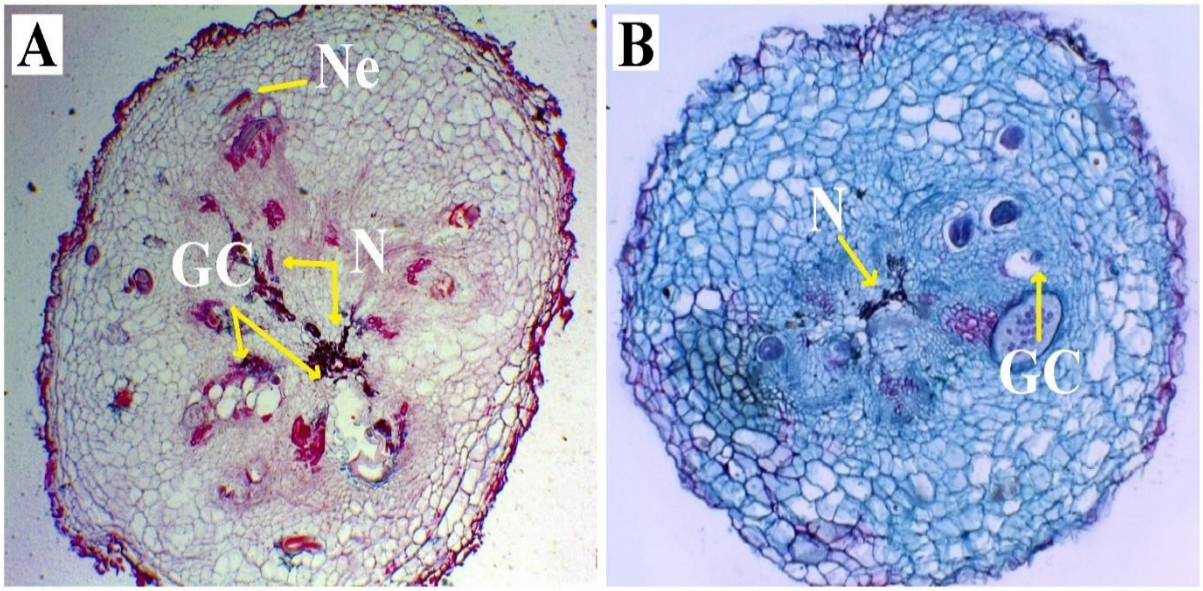


**Figure (3): cross sections in tomato roots: A. control 30 days after planting in infested soil. B. treated with vitamin C 50 ppm showing 4th stage and giant cells 30 days after planting in infested soil, Ne: due to Nematode, N: decreased necrosis area due to vitamin C treatment, GC: decreased giant cell due to vitamin C treatment (×100).**

**First and second sample of infected tomato plants with root knot nematode (*M. incognita*) treated with Selenium 25 ppm, V.C 50 ppm, V.E 50 ppm and nematicide Oxineem 24% SL at eight days, fifteen days, thirty days and forty-five days:**

Results illustrated in Table 1 show that tomato root treated with Selenium 25 ppm by irrigation showed the least counts of second and third stages at eight days after planting compared to other treatments and the control with 25% and 88% reduction for decrease in the 2nd stage count for the 3rd stage counts, respectively.

Also, with Selenium treatment 25 ppm at fifteen days after planting the third and the fourth stages of *M. incognita* showed the least counts compared to other treatments and the control with 58.18 % decrease in the 3rd stage count and mean percentage 66.67% decrease in the 4th stage count compared with control.

Tomato roots treated with Selenium 25 ppm by irrigation showed the least counts of the third, and the fourth stages and females with

mean percentages 84.62, 66 and 56% reduction in the counts, respectively at thirty days after planting in infested soil compared to the control.

Tomato roots treated with Selenium 25 ppm by irrigation and soaked has the least counts of females at forty-five days after planting with 68.89% reduction in females count compared to other treatments and the control.

Infected tomato plants treated with Vitamin C at 50 ppm came at the second place after Selenium 25 ppm treatment in the counts of 2nd and third stages at eight days after planting in infested soil, the counts of third and fourth stages at fifteen days after planting, the counts of third, fourth and females at thirty days after planting, the count of females at forty-five days after planting.

Infected tomato plants treated with Vitamin E at 50 ppm came the third after Selenium 25 ppm treatment and vitamin C 50 ppm treatment in the counts of 2nd and third stages at eight days after inoculation, the counts of third and fourth stages at fifteen days after planting, the counts of the third, fourth and females at thirty days after planting, the count of females at forty-five days after planting.

**Influence of different chemical inducers on histopathological modifications induced by Root-Knot nematode in tomato plant tissues:**

The root-knot nematode, *M. incognita* induced pronounced alterations in cells of cortical and stellar regions in roots of tomato plants. Infected roots of tomato plants had well-defined and rounded or oval giant cells in the stele regions with a number ranging from 2 to 4 cells. Also, the existence of giant cells and cell wall shows a specific, clear and core spindle and ranges from 3-9 in a single cell, the cytoplasm dense and dark, the presence of a large food site, showing clearly the female inside the cell, as well as showing clearly the death of cells. There may be in some giant cell cytoplasm focused on the cell wall, we note that some giant cells, which have the female nematodes, are free from the cytoplasm. Hypertrophied nuclei were scattered or aggregated in the cytoplasm with numbers ranging from 4-8. In some instances, dense cytoplasm was found to encounter small vacuoles. As for the histological modifications in tomato plant roots infected with *M. incognita* and treated with different chemical inducers, poorly formed giant cells in the central cylinder ranged from 2-5 were detected in the stellar region with limited hypertrophy or hyperplasia. Giant cells contained less or free of cytoplasm, fewer numbers of nuclei, vacuolated in most instances, and were smaller than those of untreated infected roots (Figs. 2 and 3).

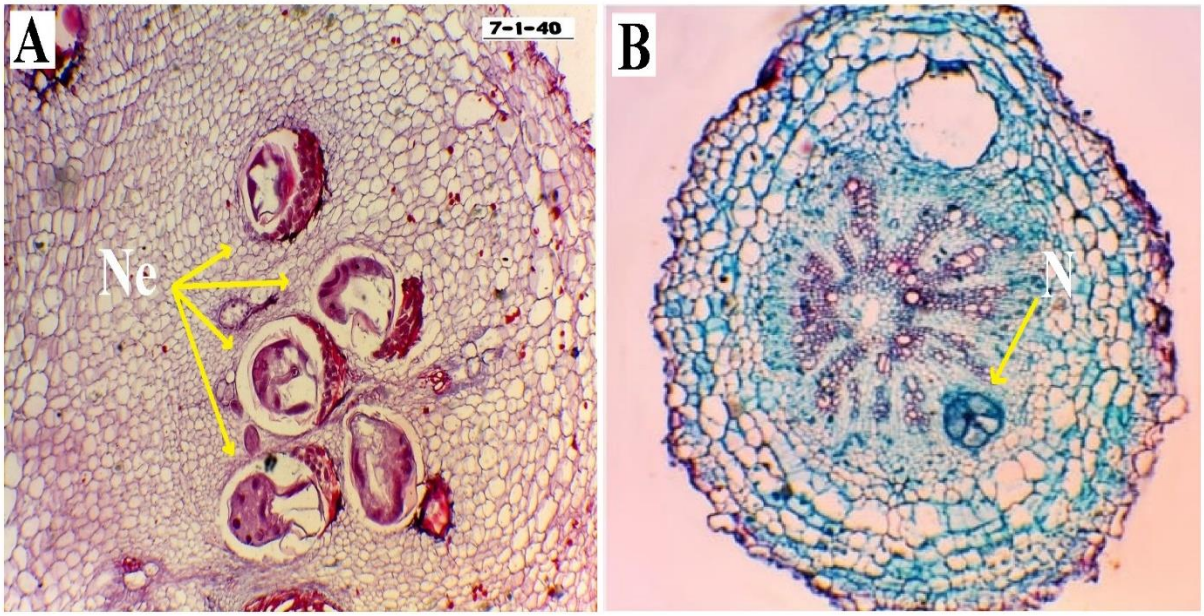


**Figure (4): Cross sections in tomato roots: A. control (infested soil), 45 days after planting. B. treated with Selenium 25 ppm 45 days after planting showing Female *M. incognita*, Ne: Nematode multiple females and egg masses in the infected control without treatment (×100).**

All these results are summarized in Figures (5 and 6) in which the total population of *M. incognita* in four stages at different times 8, 15, 30 and 45 days without treatments and also the reduction in the total population of *M. incognita*.

**Table (1): Developmental stages of *M. incognita* on tomato under greenhouse conditions.**

| Treatments | 8 days | | | | 15 days | | | | 30 days | | | | 45 days | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Second stage | | Third stage | | Third stage | | Fourth stage | | Third stage | | Fourth stage | | Female | | Female | |
| | In. | Rd. % | In. | Rd. % | In. | Rd. % | In. | Rd. % | In. | Rd. % | In. | Rd. % | In. | Rd. % | In. | Rd. % |
| Selenium | 30 | 25 | 3 | 88 | 23 | 58.18 | 10 | 66.67 | 2 | 84.62 | 17 | 66 | 11 | 56 | 28 | 68.89 |
| Vitamin C. | 36 | 10 | 7 | 72 | 26 | 52.73 | 15 | 50 | 3 | 76.92 | 24 | 52 | 11 | 56 | 35 | 61.11 |
| Vitamin E. | 40 | 0 | 24 | 4 | 37 | 32.73 | 23 | 23.33 | 5 | 61.54 | 36 | 28 | 19 | 24 | 52 | 42.22 |
| Oxineem | 25 | 37.5 | - | 100 | 13 | 76.36 | 10 | 66.67 | - | 100 | 13 | 74 | 9 | 64 | 19 | 78.89 |
| Infected control | 40 | - | 25 | - | 55 | - | 30 | - | 13 | - | 50 | - | 25 | - | 90 | - |
| Healthy control | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - | 0 | - |

In. = incidence, Rd. = Reduction to control.

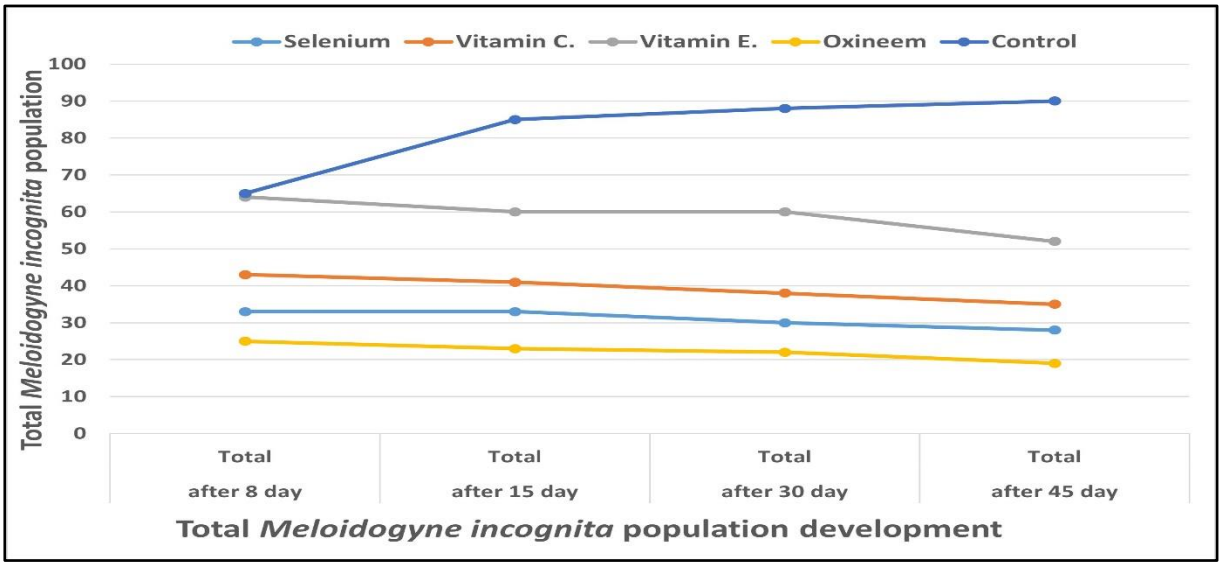


**Fig. (5): Development of the *M. incognita* total population during the fourth stage *i.e.*, after 8, 15, 30, and 45 days.**

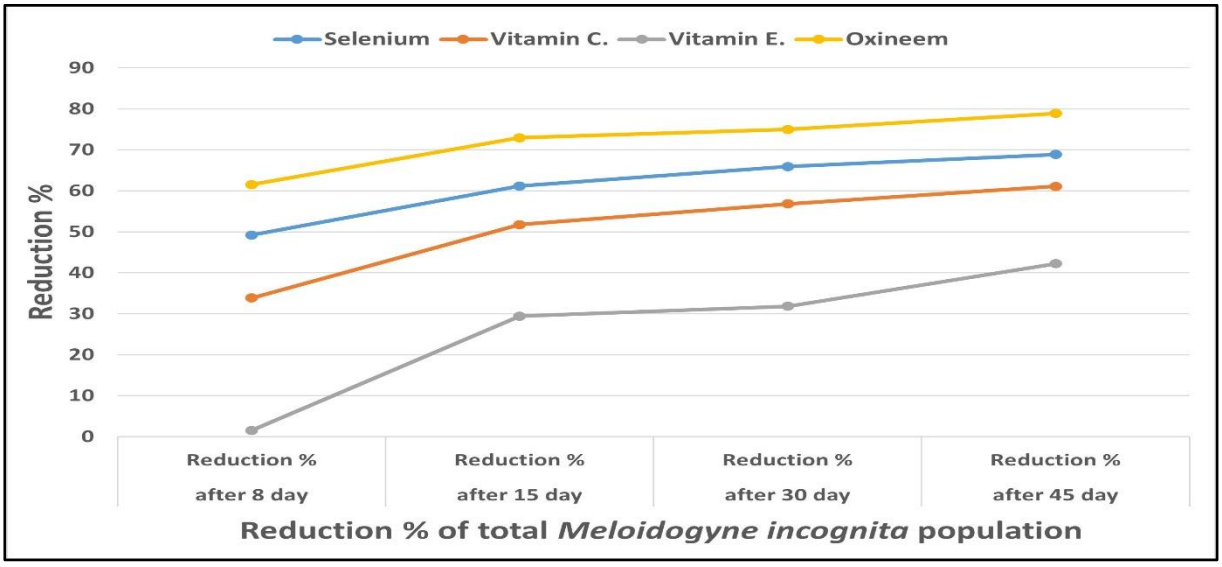


**Fig. (6): Reduction percentage of the *M. incognita* total population during the four stages *i.e.*, after 8, 15, 30, and 45 days.**

**Impact of tomato plant treatments with some chemical inducers on the activity of oxidative enzymes:**

Activities of Polyphenoloxidase (PPO), Peroxidase (PO), and Catalase (CAT) enzymes of tomato plants treated with different chemical inducers were evaluated in the presence of *M. incognita* (Fig. 7, 8 and 9). Results show that all treatments were significantly effective in increasing enzyme activities. The highest increase of CAT, PO and PPO activities as compared to the untreated control was achieved with Vit. C treatment (82.23 for CAT, 46.97 for PO and 54.17 % PPO) over untreated control, in the presence of *Meloidogyne incognita*. Meantime, Selenium, and Vit. E treatments showed a significant considerable increase in the three enzymes (70.35 and 58.88% for CAT; 43.62 and 26.45% for PO, and 44.20 and 34.49% for PPO) to untreated control, as well the least increase of the enzyme's activity, being 49.18 for CAT; 33.17 for PO and 18.29% for PPO over untreated control, was obtained when the nematicide Oxineem was applied.

However, results showed clear higher values of CAT activity than PO and PPO activities in all treatments in the presence of the pathogens. Also, the percentage of increase in enzyme

activity due to inducers treatments presented clear higher values of CAT than PO and PPO in all treatments. Meanwhile, it was noticed that soil infestation with *M. incognita* in the absence of inducers significantly increased the activity of the three enzymes CAT, PO and PPO than that recorded in healthy untreated plants as blank of all treatments.

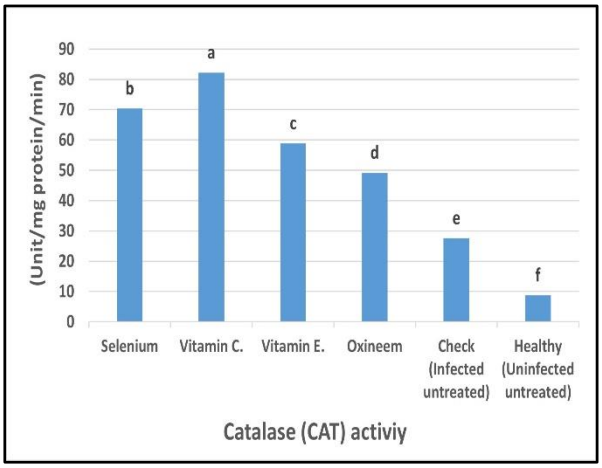


**Fig. (7): Influence of some chemical inducers treatments on Catalase activity in tomato plants grown in artificially infested soil by *M. incognita* under greenhouse conditions, (L.S.D. at 0.05 = 5.142).**

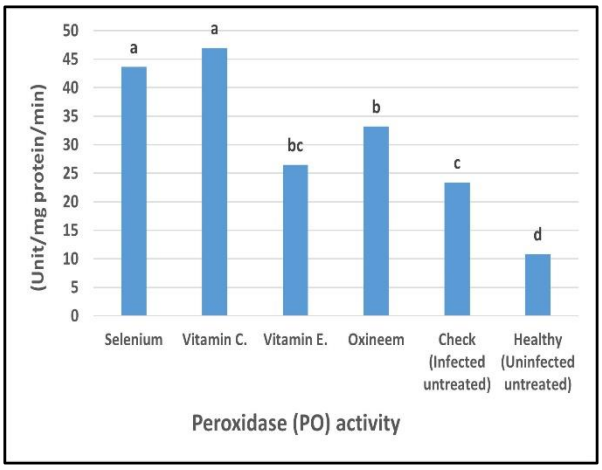


**Fig. (8): Influence of some chemical inducers treatments on the Peroxidase activity in tomato plants grown in artificially infested soil by *M. incognita* under greenhouse conditions, (L.S.D. at 0.05 = 8.197).**

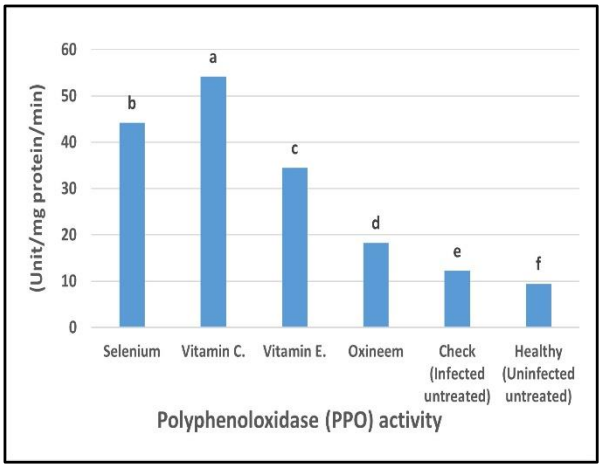


**Fig. (9): Influence of some chemical inducers treatments on the Polyphenoloxidase activity in tomato plants grown in artificially infested soil by *M. incognita* under greenhouse conditions, (L.S.D. at 0.05 = 2.363).**

**Plant growth parameters:**

Results illustrated in Table (2) demonstrate significant increase in the plant height, shoot, and root, fresh and dry weights of tomato plants treated with the three tested inducers *i.e*., selenium, Vitamin C, Vitamin E as well the nematicide Oxineem to the untreated infected control, indicating their efficacy as resistance inducing chemicals. the maximum growth parameters values *i.e*., plant height, shoot and root, fresh and dry weights per plant were recorded with Vitamin C and selenium and significantly differed from the rest of all treatments. Meantime, there were no significant differences among the treatments with Vitamin E and Oxineem.

**Table (2): Impact of different chemicals inducers as well as the nematicide, Oxineem on growth parameters of tomato plants grown in soil infested by *M. incognita* under greenhouse conditions.**

| Treatments | Plant height (cm) | Shoot weight (g) | | Root weight (g) | |
|---|---|---|---|---|---|
| | | Fresh | Dry | Fresh | Dry |
| Selenium | 47.34 b | 26.1 abc | 4.75 b | 20.3 c | 4.45 c |
| Vitamin C. | 47.27 b | 25.4 ab | 4.65 b | 19.55 b | 4.05 b |
| Vitamin E. | 43.01 c | 23.3 c | 4.45c | 18.1 d | 3.9 e |
| Oxineem | 42.18 c | 22.75 bc | 4.2 bc | 17.75 d | 3.7 d |
| Infected control | 25.92 d | 14.25 d | 2.65 d | 11.15 e | 2.25 f |
| Healthy control | 50.30 a | 27.25 a | 5.4 a | 21.3 a | 4.8 a |
| L.S.D. at 0.05 | 2.42 | 3.09 | 0.29 | 0.53 | 0.17 |

## DISCUSSION

The results obtained during this study showed that different antioxidants have suppressive effect as an alternative for chemical nematicide. These findings are consistent with those of Anter *et al*. (1994), Lashein (2002), and Ibrahim *et al*. (2007), who showed that antioxidant treatments by irrigation method improved tomato plant resistance to nematode that decreasing all root knot nematode parameters. When compared to control plants, selenium treatment at a dose of 25 ppm was the most effective in reducing nematode infection. These data are compatible with those of Anter *et al*. (2014) who found that selenium had a significant effect on *M. incognita* population inhibition after the third dose.

On the other hand, treatment by vitamin C (Ascorbic acid) gave a remarkable reduction on developmental stage of *M. incognita* infected tomato plant. Our findings support those of Homayed (2009), who found that watering with ascorbic acid reduced all parameters of *M. incognita*-infected tomato plants when compared to control plants.

When conducting an analysis to observe enzyme activity, it was found that treatments with selenium and vitamin c led to an increasing in peroxidase and polyphenoloxidase activity. According to Saeed, (2005), the use of chemical substances such as salicylic acid and ascorbic acid increased PO and PPO activity. PO and PPO activation is a common response of infected plant tissue, and its leaves have been linked to resistance (Sridhar and Ou, 1974). PO activity, in particular, has been found to be a biochemical marker for resistance and to be linked to systemic resistance (Mosa, 2002 and Nawar and Kuti, 2003). As a result, measuring PO and PPO activities could be a useful tool for screening and quantifying inducer activity. Selenium, vitamin E, and vitamin C may improve scavenging of synthesis $H_2O_2$ by increasing peroxidase and polyphenoloxidase activity, which may be implicated in electrophile detoxification (Rios *et al*., 2009). Plants cultivated without Selenium, vitamin E, and vitamin C sprays produced more oxidants (H2O2 and O2), according to Djanaguiraman *et al.* (2005), these are two types of reactive oxygen species (ROS) that can cause oxidative damage (Shanker *et al*., 2004). Antioxidant enzymes (peroxidase and polyphenoloxidase) are important in preventing oxidative stress. Several studies have found a link between Se concentration and antioxidant enzyme activity (GSH peroxidase) in lettuce and ryegrass (Hartikainen *et al.*, 2000 and Xue *et al*., 2001), tea (Yokota et al., 1988), wheat (Hartikainen *et al*., 2000, (Yao *et al*., 2009) and soybean and rap (Xue *et al*., 1993).

The addition of various antioxidant therapies has a good effect on plant development in general. Our findings are in agreement with those of Midan and Sorial (2011), who found that antioxidant substances (vitamin E, vitamin C and Selenium,) have an antioxidative operation, as evidenced by decreased $H_2O_2$ and superoxide radical production, lipid peroxidation and higher antioxidant enzymes (peroxidase & polyphenoloxidase), and larger chlorophyll content than controls. According to Thomas *et al*. (2001), the increment in chlorophyll content in Selenium treated plants over control plants could be credited to antioxidant enzymes' efficient antioxidant capacity of reactive oxygen species, which would otherwise have devastated the chlorophyll pigments, and Selenium also restricts chlorophyll deterioration and enhances photosynthesis.

Our results indicated that the ability of treatments with resistance inducers to reduce the ability of the root-knot nematode to cause more damage to root tissues, in line with what Pegard *et al*. (2005) found. These changes may be an expression of the plant's response to the tested treatments as a defense against penetration of root-knot nematode as reported by Tordable *et al*. (2010) and Pegard *et al*. (2005).

## CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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