The Effect of High Salt Stress on The Severity of Root-Knot Nematode in Tomato

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ABSTRACT: Soil and irrigation water salinity has emerged as a major abiotic stress limiting agricultural productivity, particularly in countries facing freshwater scarcity. Therefore, the aim of this study was to investigate the effect of increased salt stress on the severity of infection with root-knot nematodes (*Meloidogyne. incognita*) in tomato cultivar"023: and its effect on growth factors of tomatoes. Increased soil salinity concentration by irrigation with different salt concentrations with nematode infection led to increased nematode infection severity, higher number of galls and number of egg masses in infected and salinized tomato plants. It also led to decreased growth parameters in tomatoes, such as fresh and dry weight of root and shoot, and decreased chlorophyll pigments, including chlorophyll A, chlorophyll B and carotene. Treatment with salinity with nematode infection also led to increased activity of oxidative enzymes such as POD and PPO, especially at high salt concentrations, as well as the level of gene expression of the POD, PPO and PR2 gene in tomato roots increased significantly compared to the control at all salinity concentrations used and at all times compared to the healthy control and the nontreated-infected control. Therefore, the combination of saline irrigation and root knot nematode infection imposes whammy' on tomato: it increases the severity of infection with pathogens, especially nematodes, drains biomass, decrease photosynthetic pigments and scrambles defense signaling.

Keywords: *Tomato. Salinity, abiotic factors, galls, egg masses.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) ranks among the most important vegetable crops globally.but its productivity is increasingly threatened by both abiotic and biotic stresses. Among the most significant abiotic challenges is soil salinity, which affects more than 20% of irrigated agricultural land globally (Munns & Tester, 2008). Salinity stress can reduce plant growth, alter nutrient uptake, and can cause oxidative damage by allowing reactive oxygen species (ROS) to accumulate (Krishna *et al.,* 2022). In parallel, root-knot nematodes (*Meloidogyne* spp.) constitute a significant biotic stress, causing significant yield losses in tomato crops worldwide (Sasser & Freckman, 1987; Williamson & Hussey, 1996).

In Egypt, reliance on groundwater for irrigation in newly reclaimed desert sandy soils has exacerbated the problem, leading to a gradual increase in soil salinity year after year (Abdel-Shafy and Kamel, 2016). This rise in soil salinity has negatively impacted the growth of both field and fruit crops. In the sandy soil, nematodes also cause crops losses and intensifying the problem which may threaten sustainable agriculture. A positive correlation was found between citrus nematode populations and the chloride concentration in soil water (Youssef *et al.,* 1989 and Mashela *et al.,* 1992). Various mineral or salt concentrations have been found to either increase or decrease nematode hatching, development, and reproduction across different nematode species. (Khan and Khan, 1990 and Sweelam, 1994).

Several studies have examined the individual effects of salinity and nematode infection on tomato plants, but relatively few have explored their interaction. Some researchers have suggested that increased salinity may suppress nematode reproduction due to osmotic stress on the nematode eggs or juveniles (Edongali & Ferris, 1982). However, others have found that salt stress weakens plant defenses, for example, making tomatoes more susceptible to nematode infection and increasing damage severity (Noling & Becker, 1994). These conflicting results highlight the complexity of plant responses to combined abiotic and biotic stresses.

At the physiological level, salinity induces oxidative stress, resulting in enhanced activity of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) (Mittler, 2002). Salinity stress can weaken plant defenses by affecting the expression of defense genes, antioxidant activity, and defense signaling proteins. This can lead to increased

susceptibility to nematode infections and other plant diseases (Mustapha *et al.,* 2023).The physiological effects of salinity, such as nutrient deficiency and oxidative stress, can exacerbate the severity and incidence of plant diseases, making salinity management crucial for plant health (Mustapha *et al.,* 2023). While salinity can hinder nematode development, it also poses a significant threat to plant health by weakening plant defenses and increasing susceptibility to infections. Understanding the dual role of salinity is essential for developing effective strategies to manage plant health in saline environments. Nematode infection also stimulates these enzymes as part of the plant's defense response (Melillo *et al.,* 2006). When both stresses occur simultaneously, the plant's antioxidant defense system may become overwhelmed, leading to higher levels of oxidative damage and more severe symptoms. Moreover, the balance of phytohormones such as jasmonic acid, salicylic acid, and abscisic acid—critical in regulating defense and stress responses—is often disrupted under salt stress, potentially altering the plant's susceptibility to pathogens (Fujita *et al.,* 2006).

Recent research has aimed to quantify how varying salt concentrations in irrigation water might influence nematode infection and plant health. For example, Fatemy and Evans (1986) found that moderate salinity (4 dS/m) reduced nematode reproduction in tomato, while higher concentrations (above 6 dS/m) caused significant reductions in plant growth, even in the absence of nematodes. These findings suggest a threshold beyond which the negative impact of salinity outweighs any potential nematode suppression. The present study investigates the extent to which increased salt stress influences the severity of *M. incognita* nematode infection, growth parameters, chlorophyll pigment, antioxidant enzyme activity, and gene expression levels of tomato plant defense under varying salinity levels.

MATERIAL AND METHODS

1.Root-knot nematode culture and inoculum

M. incognita nematode cultures belonged to the pure stock cultures of Plant Pathology Department, Faculty of Agriculture. Damanhour University.

The experiments were planted and conducted in 2024 in the greenhouses of the Faculty of Agriculture, Damanhour University, and the experiment was repeated twice. Tomato seeds were obtained fromnAl-Fayrouz Nurseries for Seeds and Agricultural Seedlings.

2. Greenhouse experiments

Tomato seeds (023 cultivar) that had been surface sterilized were planted in seedling trays with sterilized peat moss and given four weeks to grow in the greenhouse. Four-week-old healthy and uniform seedlings that were healthy and uniform were moved into 20 cm-diameter plastic pots, one per pot, and filled with a 2.5-kilogram mixture of autoclaved sand and clay (3:1, v:v). Until the end of the experiment, plants were maintained in a greenhouse at 27±5 ºC–32 degrees Celsius with natural daylight length. They Pots were watered every two days and fertilized once a week using commercial fertilizer that, at a rate of 2 g/l of water, comprised N (20%), P (20%), K (20%), and S (1.26%) (Vascon 20-20-20, Farmers for Agriculture Development, Egypt).

The experiment was conducted using five sodium chloride (NaCl) concentrations (0, 500, 1000, 2000, and 3000 ppm). These solutions were used to the tomato cultivar "025" grown in 15 cm diameter clay pots filled with loamy sand soil (1:1 v/v, sand to loam).

Fifteen days after cultivation, Pots were separated into three groups and trimmed to one plant per pot. Pots of the first group were inoculated with 2000 J2 of the root-knot nematode, M. incognita. Those of the second group were inoculated with 2000 J2 of the root-knot nematode, M. incognita and plants were irrigated every other day with 200 ml of the salt prepared solutions (which one 500, 1000, 2000, or 3000 ppm). Pots of the third group was left uninoculated to serve as the control (healthy plants), with all control treatments irrigated using the same volume of tap water until the experiment concluded.

For every salt concentration, ten replicates were employed, the untreated inoculated plant and uninoculated healthy plants. chlorophyll A, chlorophyll B, carotenoids, enzyme activities of peroxidase (POD) and , polyphenol oxidase (PPO), and gene expression of peroxidase (POD), PPO, and β-1,3-glucanases genes (PR2) were measured after 1, 4, 6, and 24 d post-inoculations. At the end of the experiment, plants were harvested, growth parameters were recorded, and both soil and nematode populations were assessed..

3.Determination of photosynthetic pigments:

The following is the determination of chlorophyll A, B, and β-carotene based on Wintermans and Mats (1965): About 15 ml of 85°C acetone and 0.5 g of calcium carbonate were used to extract half a gram of fresh leaves. The mixture was then filtered through a glass funnel, and the residue was rinsed with a tiny amount of acetone until it reached 25 ml. The wavelengths used to measure the optical density (O.D.) of a constant volume of filtrate were 662 nm for chlorophyll A, 644 nm for chlorophyll B, and 440 nm for carotene.

The following formulas were used:-

The value of Chl.A= 9.784 E.662 - 0.99 E.644 = mg/l.

The value of Chl.B= 21.426 E.644 - 4.65 E662 = mg/l.

The value of carotene= 4.695 E.440 - 0.268 (Chl.A- Chl.B) = mg/l.

Where, E. = Optical density at the wavelength indicated.

4.Determination of growth parameters:
After 60 days of growth in under regular conditions, after being pulled up, the tomato were cleaned under running water. The fresh weights (g) of the shoot and root were measured. We counted the number of *M. incognita* galls and egg masses for each tomato root. J2 was extracted using Coolen's technique (Coolen, 1979) from 250 g of soil in each pot, and the quantity number was counted. Henderson and Tilton's equation was used to determine the percentages of reduction in galls, egg masses, and J2. Following a 15-minute staining period in an aqueous Phloxine B dye solution (0.15 g/l water) (Holbrook *et al.,* 1983), the roots were carefully rinsed with tap water. Growth factors of plants (increased shoot fresh weights and shoot dry weights and root fresh weight) were recorded

5.The activity of antioxidant enzymes:
Tomato cultivar ("023") was utilized to investigate the impact of varying salinity levels on the defense-related enzymes' activity, specifically polyphenol oxidase (PPO) and peroxidase (POD) by infected with nematode. Root samples were collected, demineralized water was used to rinse, and kept at -80°C for subsequent analysis. Enzymatic activities of POD and PPO were assessed in root tissues at 1, 6, 12, and 24 days post-inoculation. Each treatment included 12 replicates.

a. Estimation of POD activity
Peroxidase activity was measured following the method described by Hammerschmidt and Kuc (1982). To extract the enzyme, 1 g of root tissue was homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) using a pre-chilled mortar and pestle at 4 °C. The resultant homogenate was centrifuged for 15 minutes at 4°C at 10,000 rpm, and the enzyme source was the supernatant. 1.5 mL of 0.05 M pyrogallol, 0.5 mL of enzyme extract, and 0.5 mL of 1% hydrogen peroxide ($H2O_2$) made up the reaction mixture. A spectrophotometer was used to set the initial absorbance at 420 nm to zero while the mixture was incubated at 28 ± 2 °C. Absorbance readings were then recorded every 20 seconds for a total of three minutes. A boiled enzyme extract served as the control. The change in absorbance per minute per gram of fresh tissue was used to express peroxidase activity (El-Argawy and Adss, 2016; Ghareeb *et al.,* 2019).

b. Estimation of PPO activity
Two milliliters of 0.1 M sodium phosphate buffer (pH 6.5) were used to homogenize one gram of the sample using a pre-cooled mortar and pestle. After that, the homogenate was centrifuged for 15 minutes at 4°C at 10,000 rpm, and the resulting supernatant served as the enzyme source. PPO activity was determined following the method of Mayer et al. (1965). 200 µl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) made up the reaction mixture. The reaction was initiated by adding 200 µl of 0.01 M catechol. The absorbance at 495 nm was initially set to zero, and the reaction mixture was incubated at room temperature. Absorbance readings were taken every 30 seconds for two minutes. PPO activity was expressed as the change in absorbance per minute per gram of fresh tissue (Nassar and Adss, 2016; El-Argawy *et al.,* 2017).

6.Expression analysis of the peroxidase (POD), polyphenol oxidase (PPO) and pathogenesis-related protein PR2 using Real Time-q PCR
RNA was extracted using the BioTeke Corporation RNA Isolation Kit I based on the guanidine isothiocyanate method, following the manufacturer's instructions (Maxim Biotech Inc., USA). Using an oligo(dT) primer, dNTPs, and M-MLV reverse transcriptase (Fermentas, USA), cDNA was produced from total RNA, following the standard protocol. Quantitative real-time PCR (qRT-PCR) was performed to analyze the expression of PR2, POD, and PPO genes. An internal reference (housekeeping gene) was the tomato 18S rRNA gene (Jayanna and Umesha, 2017; Nassar and Adss, 2016).

The RT-PCR reactions were performed using specific primers in table 1. 12.5 µl of QuantiTect SYBR® Green RT Mix (2×) (Fermentas, USA), Each 25 µl RT-PCR reaction mixture contained 1 µl of each primer (10 pmol/µl), 1 µl of template cDNA (50 ng), and 9.5 µl of RNase-free water. According to Chin et al. (2000), the PCR process consisting of an initial denaturation at 95°C for 10 minutes, 40 cycles of denaturation at 95°C for 15 seconds, annealing (According to the temperature of each primer) for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 7 2°C for 10 minutes. Using a Rotor-Gene 6000 (Qiagen, USA), The reaction was conducted, and relative gene expression was quantified using the

following calculations: ΔCq = Cq of the target gene – Cq of the reference gene; ΔΔCq = ΔCq of the sample – ΔCq of the control. Relative expression levels were then determined using the 2^(-ΔΔCq) method, as described by Livak and Schmittgen (2001). For normalization, the expression of the target genes was compared to the 18S rRNA reference gene, with the expression level of untreated control plants at each time point set as the baseline (value = 1).

Table 1: primers were used in real-time q PCR

Primer	Sequence		Reference
POD	Forward	5′-GCTTTGTCAGGGGTTGTGAT-3′	Jogaiah *et al.,* 2013
	Reverse	5′-TGCATCTCTAGCAACCAAC-3′	
PPO	Forward	5′- CATGCTCTTGATGAGGCGTA-3′,	Goel *et al.,* 2017
	Reverse	5′-CCATCTATGGA CGGGAAGA-3′;	
PR2	Forward	5′-CTCGACATCGGTAACGACCAG-3′	Amaral *et al.,* 2012
	Reverse	5′-GCGGCGATGTACTTGATGTTC-3′	
18S rRNA	Forward	5′-GTGCATGGCCGTTCTTAGTTG-3′	Jayanna and Umesha, 2017
	Reverse	5′-CAGGCTGAGGTCTCGTTCGT-3′	

7.Statistical analysis

Analyzing the data with SAS software (SAS Institute Inc., Cary, NC, USA) Version 9.4 obtained from the pot experiments, considering both treatment and time as factors. Tukey's Honest Significant Difference (HSD) test was used to identify statistically significant differences among means at a probability level of ≤ 0.05.

RESULTS

The results in Table 2 showed that the effect of salinity treatment on the 023 tomato cultivar, in measuring the plants' sensitivity to nematode infection at specific salt concentrations, clearly indicate that higher soil salt levels lead to greater infection rates, and vice versa, compared to the untreated infected control. There were Significant in the number of galls as salt concentration increased compared to the untreated control. Specifically, the number of galls was 100.3 at 500 ppm, 108.8 at 1000 ppm, and 130.7 at 2000 ppm. At 3000 ppm, gall numbers increased further to 169, in contrast to the untreated infected control, which had 72.6 galls. Additionally, the number of egg masses increased with rising salinity levels: 72.9 at 500 ppm, 83.7 at 1000 ppm, and 97.3 at 2000 ppm. At 3000 ppm, egg masses rose significantly to 124.5, compared to 56.3 in the untreated infected control.

Table 2: Effects of NaCl concentrations on the root-knot nematode, Meloidogyne incognita and their effect on pathological parameters on tomato cultivar "023" in greenhouse

Salt (ppm)	Number of galls/ plant	Number of egg masses/ plant
0	72.6^{d}	56.3^{e}
500	100.3^{c}	72.9^{d}
1000	108.8^{c}	83.7^{c}
2000	130.7^{b}	97.3^{b}
3000	169^{a}	124.5^{a}
L.S.D	9.5	5.8

Means with the same letters(s), in each column, are not significantly different at according to Tukey's post-hoc test at P=0.05,

The data in Table 3 illustrate the effect of increasing soil salinity concentrations on tomato growth parameters, such as the fresh and dry weight of the stem and root. We find that the data show that increasing salinity concentrations led to a decrease in the fresh and dry weight of the vegetative system compared to the untreated infected control and the healthy control. There were significant differences between the results at various salinity levels compared to the control. Regarding the fresh weight of the shoot system, the 3000 ppm concentration was more affected than the fresh shoot weight, which was 112.75. There were no significant differences between the 2000 ppm and 1000 ppm concentrations, which were 126.12 and 130.24, respectively. At the 500 ppm concentration, the fresh weight was 143.63, compared to the healthy control (176.24) and the untreated infected control (143).

The dry weight of the shoot system also decreased with increasing soil salinity concentration, with the lowest score being at the 3000 ppm concentration, which was 12.5. This was followed

by the 1000 and 2000 ppm concentrations, which showed no significant differences. This was followed by the 500 BPM concentration, which was 15.2, compared to the healthy control (25.09) and the untreated infected control (16.03).

As for the root's fresh and dry weight, there were significant differences in the results with the increase in salt concentration compared to the control. The fresh weight of the root at a salt concentration of 3000 was 61.24, at a concentration of 2000 it was 50.12, at a concentration of 1000 it was 43.67, and at a concentration of 500 it was 40.17, compared to the healthy control (34.12) and the untreated infected control (25.23). The dry weight results for the root at a salt concentration of 3000 was 14.34, at a concentration of 2000 it was 12.04, and at a concentration of 1000 it was 14.34. 11.70 and at a concentration of 500 it was 11.66 compared to the healthy control (8.80) as well as the untreated infectious control (9.28).

Table (3): The effect of treatment with different NaCl concentrations on M. incognita infection on and their effect on growth parameters in the 023 tomato cultivar

NaCl (ppm)	Weight of the shoot system (g)		Weight of the root system (g)	
	Fresh	Dry	Fresh	Dry
Control	176.24[a]	25.09[a]	25.23[f]	8.80[e]
0 (infected)	143.67[c]	16.03[b]	34.12[e]	9.28[d]
500	150.12[b]	15.2[c]	40.17[d]	11.66[c]
1000	130.24[d]	13.7[d]	43.67[c]	11.70[c]
2000	126.12[d]	14.09[d]	50.12[b]	12.04[b]
3000	112.73[e]	12.5[e]	61.24[a]	14.36[a]
L.D. S	5.12	0.47	3.08	0.47

Means with the same letters(s), in each column, are not significantly different at according to Tukey's post-hoc test at P=0.05,

Table 4 showed that the effect of soil salt concentration on chlorophyll pigments (chlorophyll A, chlorophyll B, Carotene) when infected with nematodes on the 023 tomato cultivar. The increasing increase of the salt concentration in the soil led to a decrease in the chlorophyll pigments in the plant and yellowing of the plant. There were significant differences between the treatment treatments with different salinity concentrations compared to the control. It was found that the lowest percentage of chlorophyll pigments was at a salinity concentration of 3000 ppm, followed by a concentration of 2000 ppm, then a concentration of 1000 ppm, then a concentration of 500 ppm, compared to the untreated infected control and the healthy control.

Table (4): The effect of treatment with NaCl concentrations on M. incognita infection on and their effect on Chlorophyll pigment in 023 tomato cultivar.

NaCl (ppm)	Chlorophyll A	Chlorophyll B	Carotene
Control	0.990[a]	0.738[a]	1.061[a]
0 (infected)	0.606[b]	0.600[b]	0.765[b]
500	0.367[c]	0.319[c]	0.622[c]
1000	0.315[d]	0.335[d]	0.630[c]
2000	0.293[e]	0.312[e]	0.500[d]
3000	0.283[e]	0.290[f]	0.455[e]
L.D. S	0.20	0.14	0.18

Means with the same letters(s), in each column, are not significantly different at according to Tukey's post-hoc test at P=0.05,

Figure 1 shows the extent to which increasing soil salinity concentrations affected the PPO enzyme activity in the nematode-infected tomato cultivar. The data in figure 1 showed that PPO activity in tomato roots increased significantly compared to the control at all salinity concentrations used and at all times compared to the healthy control and the untreated infected control. The concentration of 3000 ppm gave the highest enzyme activity, followed by 2000 ppm, then 1000ppm and 500 ppm compared to the untreated infected control and the healthy control. While the highest concentration of enzyme activity was found at 6 days, followed by 12 days, as time after infection increased, the enzyme activity decreased.

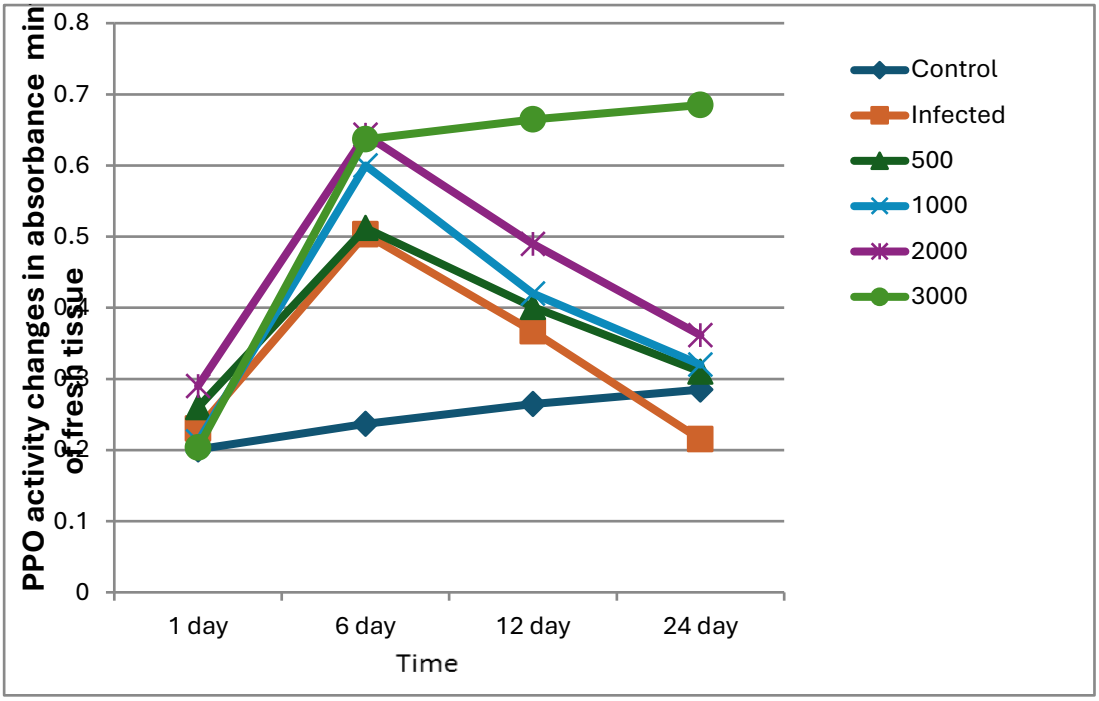

Figure 1: The effect of treatment with different concentrations of salinity on M. incognita infection and their effect on PPO enzyme activity in the 023 tomato cultivar

Figure 2 shows the effect of increasing soil salinity concentrations affected POD enzyme activity in the nematode-infected tomato cultivar. The data in figure 2 showed that POD activity in tomato roots increased significantly compared to the control at all salinity concentrations used and at all times compared to the healthy control and the untreated infected control. The concentration of 3000 ppm gave the highest enzyme activity, followed by the concentration of 2000 ppm. There were no significant differences in POD enzyme activity between the concentration of 1000 ppm and the concentration of 500 ppm compared to the untreated infected control and the healthy control. While it was found that the highest concentration of POD enzyme activity was at 6 days, as time after infection increased, the enzyme activity decreased.

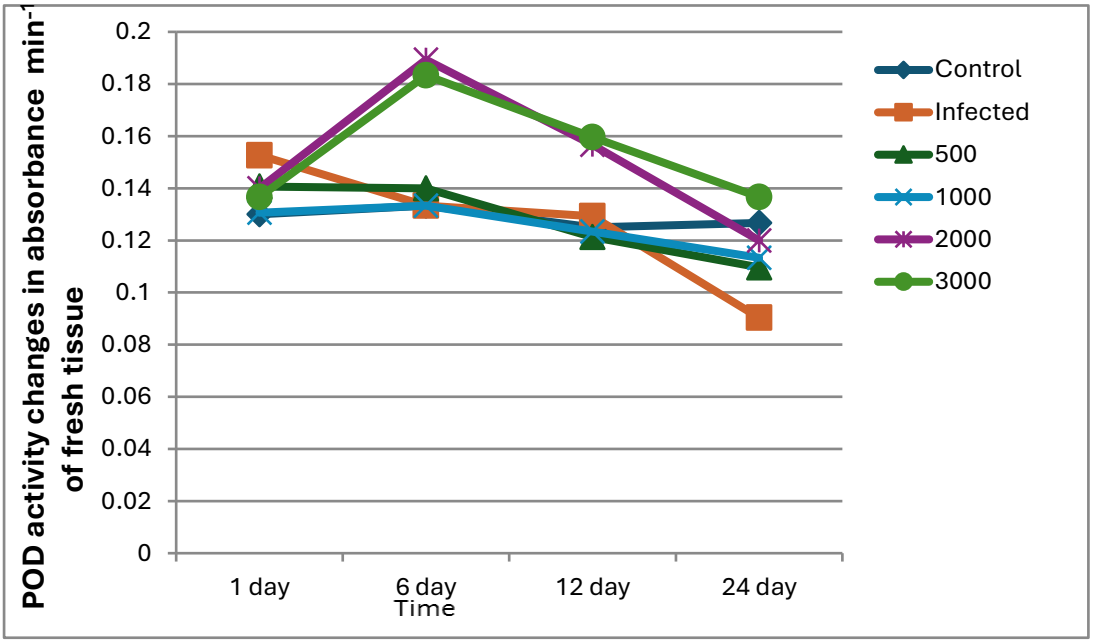

Figure 2: The effect of treatment with different concentrations of salinity on *M. incognita* infection and their effect on POD enzyme activity in 023 tomato cultivar

Figure 3 shows the extent to which increasing soil salinity concentrations affect the POD gene expression in a nematode-infected tomato cultivar. The data in figure (3) showed that the level of POD gene expression in tomato roots increased significantly compared to the control at all salinity concentrations used and at all times compared to the healthy control and the nontreated-infected control. The concentration of 3000 ppm gave the highest level of gene expression, followed by the concentration of 2000 ppm and then the concentration of 1000 ppm, and the lowest was the concentration of 500 ppm compared to the untreated-infected control and the healthy control. and then there is not significant between the concentration of 500 ppm, and untreated infected control . While it was found that the highest level of POD gene expression was at 6 days, followed by 12 days, and then with increasing time after infection, the gene expression of the gene decreased.

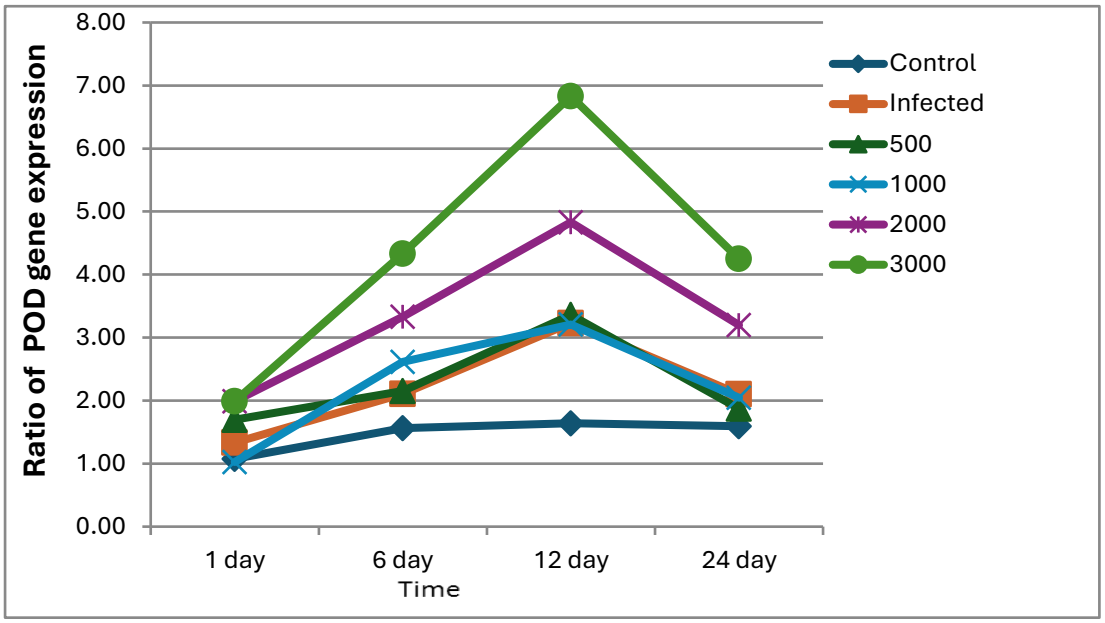

Figure 3: The effect of treatment with different concentrations of salinity on M. incognita infection and their effect on POD gene expression in the 023 tomato cultivar

Figure 4 shows the extent to which increasing soil salinity concentrations affect the PPO gene expression in a nematode-infected tomato cultivar. The data in figure (4) showed that the level of gene expression of the PPO gene in tomato roots increased significantly compared to the control at all salinity concentrations used and at all times compared to the healthy control and the nontreated-infected control. The concentration of 3000 ppm gave the highest level of gene expression, followed by the concentration of 2000 ppm and then the concentration of 1000 ppm, and the lowest was the concentration of 500 ppm compared to the untreated-infected control and the healthy control. While it was found that the highest level of PPO gene expression was at 12 d, followed by 24 d, and then with increasing time after infection, the gene expression of the gene decreased.

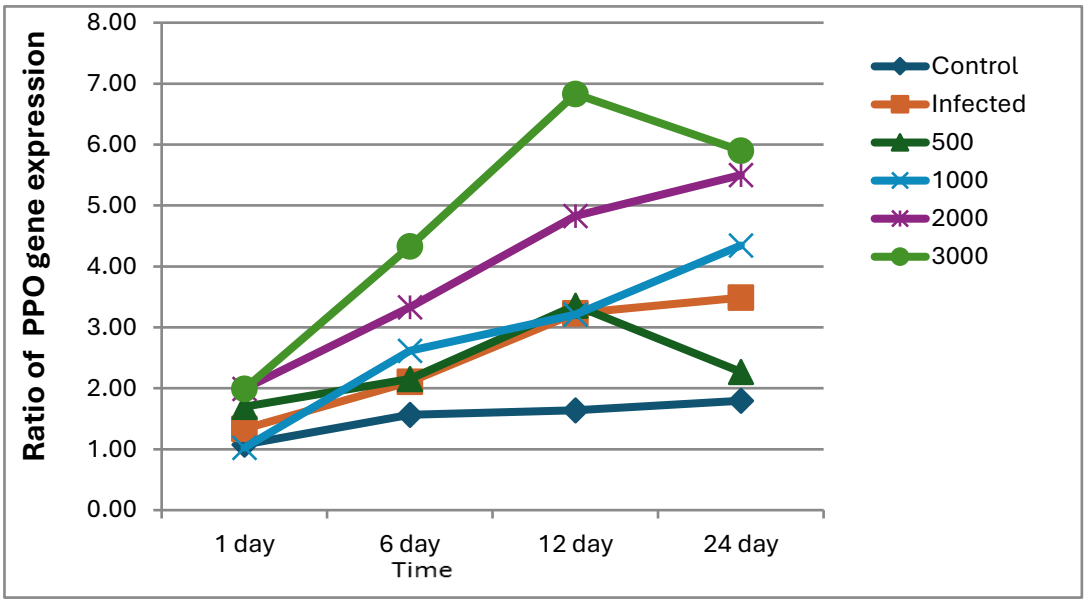

Figure 4: The effect of treatment with different concentrations of salinity on M. incognita infection and their effect on PPO gene expression in the 023 tomato cultivar

Figure 5 shows the extent to which increasing soil salinity concentrations affect the PR1 gene expression in a nematode-infected tomato cultivar. The data in Figure (5) showed that the level of gene expression of the PR1 gene in tomato roots increased significantly compared to the control at all salinity concentrations used and at all times compared to the healthy control and the untreated-infected control. The concentration of 3000 ppm gave the highest level of gene expression, followed by the concentration of 2000 ppm and then there is no significant difference between the concentration of 1000 ppm, and concentration of 500 ppm compared to the untreated-infected control and the healthy control. While it was found that the highest level of PR1 gene expression was at 12 days, followed by 24 days, and then with increasing time after infection, the gene expression of the gene was decreased.

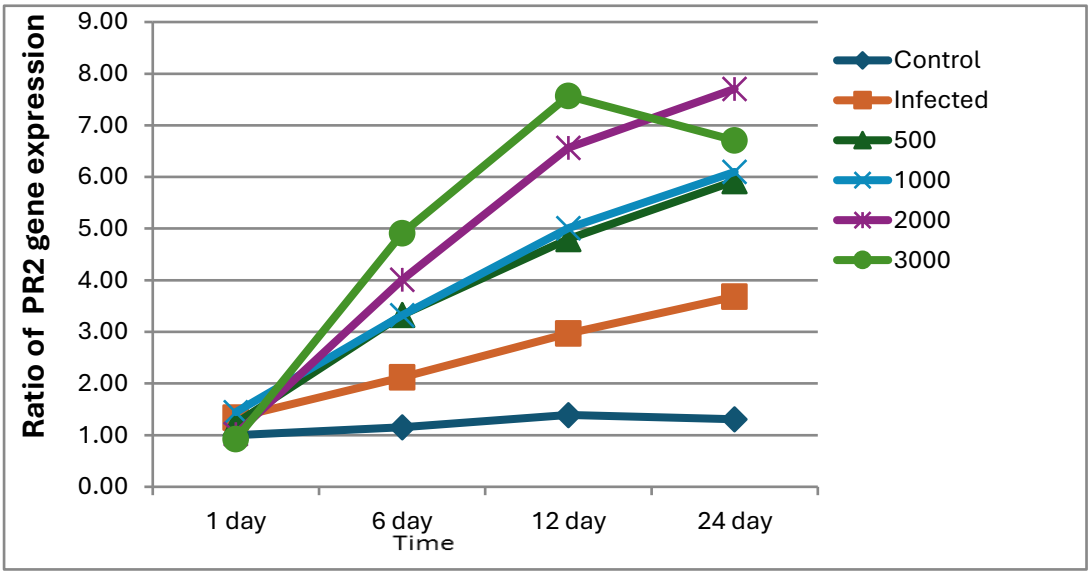

Figure 5: The effect of treatment with different concentrations of salinity on M. incognita infection on and their effect on PR2 gene expression in the 023-tomato cultivar

DISCUSSION

Salinity predisposes tomato roots to more aggressive root-knot nematode development

When NaCl is present in the rhizosphere—even at moderate levels (EC ≈ 3–6 dS m^{-1}) both the frequency of successful Meloidogyne spp. infections and the subsequent development of the nematode rise sharply. Controlled-environment

work has shown a step-wise increase in gall (knot) number and egg production as salinity in the irrigation solution was raised from 0 to 8 dS m^{-1}, with the 8 dS m^{-1} treatment recording almost double the egg masses per plant compared with the non-saline control (Boualem *et al.,* 2018). These data confirm earlier pot studies in which every 1 dS m^{-1} increment above the crop's threshold electrical conductivity produced roughly a 12 % rise in the nematode reproduction factor (Maggenti, & Hardan, 1973). These findings align with the current study's findings, the result data that the higher the salt concentration in the soil, the greater the infection (galls and egg masses) and vice versa compared to the untreated infected control.

Growth suppression is additive under the combined stresses

We find that the data in the current study show that increasing salinity concentrations led to a decrease in the fresh and dry weight of the vegetative system compared to the untreated infected control and the healthy control. There were significant differences between the results with increasing salinity concentration compared to the control. These findings align with the current study's findings such as Al-Sayed *et al.,* (2014) found that the tomato already sacrifices growth to maintain osmotic adjustment under salt stress, the extra carbon cost of feeding sedentary endoparasites leaves even less photo assimilate for vegetative tissues. In dual-stress treatments, shoot and root fresh weight fell 30–40 % relative to the non-infected, non-saline control; dry weight reductions were of similar magnitude (Al-Sayed *et al.,* 2014). Interestingly, salinity or nematode infection alone each reduced biomass, but their combination produced a significantly greater drop than the arithmetic sum of the individual effects, pointing to a true synergism rather than mere additivity.

Photosynthetic pigment loss underpins lowered carbon gain

Chlorophyll a, chlorophyll b and carotenoids are all sensitive to ionic and oxidative damage. In a hydroponic trial, chlorophyll a fell by ~25 % when NaCl was increased to 0.5 g kg^{-1} substrate; the decline was accentuated (-35 %) when plants at the same salinity level were inoculated with M. incognita (Roșca *et al.,* 2023)Concomitant drops in SPAD readings mirror these biochemical changes and partly explain the reduced photosynthetic rate recorded in gas-exchange assays. These results are consistent with the results of the current study. The increasing of the salt concentration in the soil led to a decrease increasing pigments in the plant and yellowing of the plant. There were significant differences between the treatment with different salinity concentrations compared to the control

Oxidative enzyme activities show a biphasic pattern

Both stresses trigger an oxidative burst. In gall tissues the activities of class III peroxidase (POX) and polyphenol oxidase (PPO) rose 1.5- to 2-fold compared with healthy roots; salt alone produced a similar but slightly smaller increase. When the stresses were combined, POX and PPO activities reached their highest values, particularly at ≥100 mM NaCl, indicating that the oxidative machinery is being pushed harder under cumulative stress (Ramavath *et al.,* 2024). The transient spike probably represents a front-line attempt to wall off invading juveniles, but it also carries a risk of collateral damage to host membranes and pigments.

These findings align with the current study's findings. The current study showed that the extent to which increasing soil salinity concentrations affected the PPO and POD enzyme activity in the nematode-infected tomato cultivar. that PPO and POD activity in tomato roots increased significantly compared to control at all salinity concentrations used and at all times compared to healthy control and the untreated infected control

Defense-gene expression is attenuated despite high enzyme activity

Surprisingly, qRT-PCR analyses reveal that transcripts of several defense-related genes (two POX isozymes, a PPO homolog and the canonical PR-1 marker) are down-regulated during the early compatible interaction with M. incognita, and the repression is stronger when salinity is superimposed (Guan *et al.,* 2017). The disconnect between high enzyme activity and low gene expression suggests post-transcriptional activation of pre-existing POX/PPO pools or even nematode-mediated manipulation of host enzymes to detoxify reactive oxygen species in the feeding site.

These results are consistent with the results of the current study. The data in the current study showed that the level of gene expression of the POD,PR2, and PPO gene in tomato roots increased significantly compared to control at all salinity concentrations used and at all times compared to the healthy control and the nontreated-infected control.

Salt stress drives abscisic acid (ABA) accumulation; ABA is well-known to antagonize salicylic-acid (SA) signaling and therefore the transcription of SA-responsive defense genes such as PR-1 and PR-5. Studies in tomato lines exposed to 100 mM NaCl confirmed that elevated ABA suppresses SA-dependent transcripts,

rendering plants more susceptible to biotic attack (Pye *et al.,* 2018 and Pye *et al.,* 2013) Thus, the ABA surge triggered by salinity likely underlies the observed repression of PR genes under dual stress, weakening systemic acquired resistance even as local oxidative enzymes are induced.

CONCLUSION, the combination of saline irrigation and root-knot nematode infection creates a "double whammy" for tomato plants. Salinity in irrigation water not only stresses plants but also makes them more vulnerable to pathogens, especially nematodes, which are already destructive pests. Together, these stresses intensify disease severity, reduce plant biomass, degrade or bleach photosynthetic pigments like chlorophyll, and disrupt the plant's defense signaling systems. Such effects weaken the plant's ability to grow, produce energy, and resist further attacks. Because these two factors—salinity and nematodes—interact to magnify damage, any effective management strategy for tomatoes grown in marginal or brackish environments must address both simultaneously. This means controlling soil salinity to reduce physiological stress while also suppressing nematode populations to prevent root damage. Any management programme for tomato in marginal, brackish environments must therefore treat salinity control and nematode suppression as inseparable goals.

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الملخص العربي

تأثير الإجهاد الملحي المرتفع على شدة الإصابة بنيماتودا تعقد الجذور في الطماطم

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أصبحت ملوحة التربة أو مياه الري من أهم العوامل غير الحيوية التي تؤثر على الإنتاج الزراعي، وخاصة في البلدان التي تعاني من نقص موارد المياه العذبة. لذلك، هدفت هذه الدراسة إلى دراسة تأثير زيادة الإجهاد الملحي على شدة الإصابة بنيماتودا تعقد . (M. incognita)في صنف الطماطم 023 وتأثيره على عوامل النمو في الطماطم. أدت زيادة تركيز ملوحة التربة بالري بتركيزات مختلفة من الملح مع الإصابة بالنيماتودا إلى زيادة شدة الإصابة بالنيماتودا، وزيادة عدد العقد وعدد كتل البيض في نباتات الطماطم المصابة والمملحة. كما أدى ذلك إلى انخفاض معايير النمو في الطماطم، مثل الوزن الطازج والجاف للجذور ، وانخفاض أصباغ الكلوروفيل، بما في ذلك الكلوروفيل أ والكلوروفيل ب والكاروتين. كما أدى المعاملة بالملوحة مع إصابة النيماتودا إلى زيادة نشاط الإنزيمات المؤكسدة مثل POD وPPO، وخاصة في تركيزات عالية من الملح، وكذلك مستوى التعبير الجيني لجين POD و PPOو PR2في جذور الطماطم زاد بشكل ملحوظ مقارنة مع الكنترول في جميع تركيزات الملوحة المستخدمة وفي جميع الأوقات مقارنة بالنباتات الصحية والنباتات غير المعالجة المصابة.

الكلمات الدالة: الملوحة ، العوامل غير الحيوية ، العقد ، كتل البيض، التعبير الجيني