



RESEARCH

Elicitation of sunflower resistance to *Meloidogyne incognita* by ascorbic and glutamic acids: integrating direct nematicidal activity and defense enzyme induction

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Abstract

Root-knot nematode (*Meloidogyne incognita*) causes a significant reduction in the production of sunflower (*Helianthus annuus*) globally. The paper assessed the nematicidal activity of ascorbic acid and glutamic acid and their ability to trigger systemic resistance in sunflower within controlled conditions. Laboratory evaluations proved that both compounds significantly suppressed egg hatch to 29.5% (from 92.3% in control) and elevated juvenile mortality to 62.1% (from 6.0% in control) in a concentration-dependent manner, with ascorbic acid (2 mg/ml) showing the strongest effects. Experiments in greenhouses found that pre- and post-inoculation foliar application of these acids suppressed nematode root galling by up to 59% and population density by up to 57%, while boosting shoot fresh weight by 21.1% and root fresh weight by 24.8% compared to inoculated controls. Treated plants also showed increased the activities of major defense enzymes, such as peroxidase (POX), phenylalanine ammonia-lyase, (PAL), superoxide dismutase (SOD), and ascorbate peroxidase (APX). Pre-inoculation treatments were more efficient, which demonstrates the role of priming in the activation of defense. Our results indicate that ascorbic and glutamic acid have a combination of direct nematicidal activity with induction of host defense systems, which is promising as a safe means of managing nematode infestation in sunflower farming.

Keywords Root galling · Resistance elicitors · Foliar application · Oxidative stress enzymes · Plant-nematode interaction · Systemic acquired resistance

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1 Introduction

Root-knot nematodes (*Meloidogyne* spp.) are some of the most destructive plant-parasitic nematodes throughout the world and they result in significant economic losses in various crops (Onkendi et al. 2014). Such nematodes cause root galling, interfere with the uptake of water and nutrients, and eventually decrease the vigor and yield of plants (Habteweld et al. 2024). *Meloidogyne incognita* is especially perilous in the tropics and subtropics, resulting in negative economic effects on high-value crops that are under the protection of cultivation, which explains why its control is of high urgency to local producers (Prajapati et al. 2025).

Although chemical nematicides have historically been the primarily control method for the root-knot nematodes, growing concerns over environmental pollution, health hazards, and regulatory restrictions have intensified the search for sustainable alternatives (Forghani and Hajihassani 2020; Maleita et al. 2023). Within these alternatives, plant-derived

compounds and resistance-inducing agents have demonstrated high potential in reducing the negative impact of the environment, and at the same time have proven to be effective against nematodes (Barbosa et al. 2024).

Due to its role in plant antioxidants and defense activation, ascorbic acid (vitamin C) has gained attention (Akram et al. 2017; Al-Sayed and Montasser 1986). It plays an important role in the detoxification of reactive oxygen species produced during nematode infection, the regulation of the expression of defense-related genes, and results in an increase in systemic resistance (Singh et al. 2021). Ascorbate oxidation triggers other metabolic pathways, such as the phenylpropanoid cascade, which elevates the level of defense enzymes, including peroxidase and phenylalanine ammonia-lyase, which is vital in the defense of nematodes (Singh et al. 2020).

In a similar manner, glutamic acid serves significant functions in the plant stress responses through its influence on antioxidant enzyme functions and also in the redox balance of the cell (Guo et al. 2017). It positively influences growth and photosynthesis during stress and triggers defense mechanisms that lead to a decrease in nematode penetration and reproduction (Hamza et al. 2013). Glutamic acid is also used as a precursor in the production of glutathione, a strong antioxidant that is utilized in cellular detoxification (Noctor et al. 2014).

The use of ascorbic acid and glutamic acid in nematode management programs is a sustainable solution that tries to integrate the direct nematicidal effect of the product with the development of host resistance mechanisms (Abd-Elgawad 2025). This double action is likely to decrease nematode populations and increase plant resistance, which makes the use of BCAs a safer and more effective approach for RKN management (Singh et al. 2021).

While previous studies have reported the individual effects of both acids on plant stress responses, their dual role as both direct nematicidal agents and inducers of systematic resistance has not been comprehensively investigated in sunflower (*Helianthus annuus*)—*M. incognita* pathosystem. Furthermore, a direct comparative analysis of their efficacy and the optimal timing of application (pre- vs. post-inoculation) remains unexplored. Therefore, this study aimed to: (1) evaluate the direct nematicidal activity of ascorbic and glutamic acids on *M. incognita* egg hatching, and juvenile mortality *in-vitro*; (2) assess the effects of foliar application (pre- and post-inoculation) on nematode infection parameters and plant growth in sunflower under greenhouse conditions; and (3) elucidate the biochemical basis of induce the resistance by measuring the activities of key defense-related enzymes (POX, PAL, SOD, APX) in treated plants. This integrated approach provides a holistic evaluation of the dual modes of action of these compounds and their potential for sustainable nematode management.

2 Materials and methods

2.1 Maintenance of root-knot nematode (*M. incognita*)

A pure culture of *M. incognita* was maintained on susceptible plants of sunflower (*Helianthus annuus* cv. Sunspot) that were planted in sterile sandy loam soils of and under greenhouse conditions at 28–32 °C. The nematode species identity was confirmed by examining the perineal pattern of mature females under a light microscope (Hartman and Sasser 1985); ensuring the purity of the culture used in all experiments. Standard methods were used to collect egg masses from the infected roots, according to the procedure by Hartman and Sasser (1985). Freshly laid egg masses were allowed to hatch in aerated distilled water at room temperature, and second-stage Juveniles (J2) were collected for use in further studies.

2.2 Laboratory studies (Survival and Mortality of *M. incognita* J2)

Bioassays involving the assessment of the impact of ascorbic acid and glutamic acid on *M. incognita* second-stage juveniles (J2) survival and mortality were conducted following protocols developed by Neeraj et al. (2017). Freshly hatched J2s were produced by incubating egg masses collected on infected sunflower roots by extracting them with 0.5% sodium hypochlorite, in aerated distilled water at 28 °C. Approximately 100 newly hatched J2s were released in each well of 24-well plates containing aqueous solutions of ascorbic acid or glutamic acid concentrations of 1 mg/ml and 2 mg/ml. These concentrations were selected based on preliminary dose–response trails (0.5–4.0 mg/ml) and relevant literature (Al-Sayed and Montasser 1986; Singh et al. 2021), as they provided clear dose-dependent effects without phytotoxicity. Each treatment was replicated four times. The plates were maintained in the dark at 25 ± 2 °C for 12 days. To maintain consistent concentrations, test solutions were renewed every 3 days. Juvenile mortality was assessed microscopically at 3, 6, 9, and 12 days; nematodes were considered dead if they did not respond to probing with a fine needle. Mortality was corrected using Abbott's formula (Abbott, 1925) to account for natural mortality in the control: Corrected Mortality (%) = $\frac{((\text{Treatment Mortality (\%)} - \text{Control Mortality (\%)}))}{(100 - \text{Control Mortality (\%)})} \times 100$. The percentage of egg hatch was calculated using the formula: Egg Hatch (%) = $\frac{(\text{Number of hatched J2s} / \text{Initial number of eggs}) \times 100}{100}$. The percentages of egg hatching and juvenile mortality were obtained by dividing the

number of juveniles hatched and the number of juveniles killed by the initial counts, respectively, and the percentages were used to give quantitative data on the nematicidal effects of the treatments.

2.3 Greenhouse studies

The seeds of sunflower (cv. Sunspot) were surface sterilized in 1% sodium hypochlorite solution for 5 min and rinsed in distilled water, and then sown individually in plastic pots (15 cm diameter) filled with 2.5 kg of steam-sterilized loamy sand soil per pot. Following three weeks of growth under controlled conditions, uniform seedlings were chosen and transplanted in separate pots.

The study design was a randomized complete block design of four replications per treatment. Treatments involved foliar application of ascorbic acid or glutamic acid at two concentrations (1 mg/ml and 2 mg/ml). For pre-inoculation treatments, plants received foliar application of the tested solution one week before nematode inoculation. Foliar sprays were applied uniformly to runoff using a hand-held sprays, covering all leaves. Seven days after treatment, each plant was inoculated by pipetting 5 ml of suspension containing 1000 freshly hatched J2s into three shallow holes (2–3 cm deep) made around the root zone.

For post-treatments, plants were first inoculated with 1000 freshly hatched J2s per pot using the same method described above. One week after nematode inoculation, plants received foliar application of ascorbic acid or glutamic acid at 1 mg/ml or 2 mg/ml. The control plants were inoculated and not treated.

Following the completion of all treatments and inoculation, the plants were kept in a greenhouse under temperature control and day temperatures of between 28–32 °C, relative humidity was maintained at a constant level, and the plants were irrigated and fertilized regularly to provide optimal growth conditions.

Forty-five days after inoculation, plants were carefully uprooted and kept assessing the parameters of nematode infection, the measures of plant growth, and the biochemical analyses.

2.4 Growth and nematode assessments

After uprooting, plants were handled with utmost care and roots were gently washed free of soil. The number of root galls was scored using a 0–10 scale. The reproduction of nematodes was measured by counting egg masses and the extraction of the nematodes from the soil and roots using modified Baermann funnels (Hooper et al. 1983). Shoot and root fresh weights were measured. These parameters

were applied to measure the effects of the treatment on plant growth and the effects on nematode infestation.

2.5 Enzyme activity assays

Samples of leaves and roots were collected immediately after harvest, frozen in liquid nitrogen, and stored at –80 °C for enzyme assays. For protein extraction, 0.5 g of frozen tissues was homogenized in 2 ml of ice-cold extraction buffer (50 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 2% (w/v) polyvinylpyrrolidone (PVPP)). The homogenate was centrifuged at 12,000 × g for 20 min at 4 °C, and the supernatant was used for enzyme activity assays and protein quantification. All assays were conducted in triplicate.

Activities of defense-related enzymes including peroxidase (POX), phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD), and ascorbate peroxidase (APX), were measured spectrophotometrically following protocols by Pandey et al. (2018) and Singh et al. (2021). POX activity was measured by monitoring the oxidation of guaiacol at 470 nm. PAL activity was determined by measuring the production of cinnamic acid at 290 nm. SOD activity was assayed by monitoring the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm. APX activity was measured by monitoring the decrease in ascorbate concentration at 290 nm. Soluble protein content was determined by the method of Bradford (1976), using bovine serum albumin (BSA) as a standard. Enzyme activities were expressed per mg of protein.

2.6 Statistical analysis

Data from egg hatch and juvenile mortality tests were analyzed using one-way analysis of variance (ANOVA). Data from greenhouse experiments were analyzed employing two-way ANOVA to assess the main influences and interaction effects of the following two factors: treatment concentration (1 mg/ml, 2 mg/ml) and time of application (pre-inoculation vs. post-inoculation). Prior to ANOVA, assumptions of normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) were tested and confirmed. Where necessary, data transformations (logarithmic or square root), were applied to meet ANOVA assumptions. When significant treatment effects were identified ($p < 0.05$), means were separated using Tukey's Honestly Significant Differences (HSD) test for multiple comparisons. All analyses were performed using SPSS statistical software (version 16). Both laboratory and greenhouse experiments were repeated independently in time, yielding similar results; data presented are from a representative experiment.

3 Results

3.1 Effect of ascorbic and glutamic acids on *M. incognita* egg hatch and J2 mortality

Table 1 presents the effects of ascorbic acid and glutamic acid on the egg hatch percentage and juvenile mortality of *M. incognita* after 12 days of exposure to the treatments. The control group had a high hatching rate of eggs, $92.3 \pm 2.1\%$, which suggests healthy uninhibited juvenile emergence and only $6.0 \pm 1.2\%$ juvenile mortality. These values bring out the base reproductive capability and survival of the nematodes in the absence of chemical interference.

The exposure to ascorbic acid had a significant inhibiting effect on the hatching of eggs in a concentration-dependent manner. With 1 mg/ml, the hatching of the eggs was low at 59.6 ± 3.4 , which was significantly lower than the controls ($p < 0.001$). At 2 mg/ml, the hatching of the eggs was

reduced to $29.5 \pm 2.9\%$, which exhibited pronounced ovicidal activity. Likewise, Juvenile mortality also increased tremendously, i.e., $38.9 \pm 2.8\%$ at a concentration of 1 mg/ml and reached $62.1 \pm 3.0\%$ at a concentration of 2 mg/ml, depicting the direct larvicidal effects ($p < 0.001$).

Inhibitory effects were also exhibited by glutamic acid, though with a moderate lower extent than ascorbic acid at equal concentrations. The hatching rate was reduced to $65.4 \pm 2.8\%$ and $42.7 \pm 3.2\%$ at 1 mg/ml and 2 mg/ml, respectively, which were significantly different from that for the control ($p < 0.001$). Mortality of Juveniles under glutamic acid was $34.4 \pm 2.5\%$ at 1 mg/ml and $49.5 \pm 2.7\%$ at 2 mg/ml, which were once again significantly high in comparison with the control mortality ($p < 0.001$).

Finally, the results indicate that ascorbic acid at 2 mg/ml is the most effective in reducing egg hatch and increasing juvenile mortality of *M. incognita*, followed by glutamic acid, emphasizing their potential utility for nematode management.

3.2 Effect of pre- and post-treatments with ascorbic and glutamic acids on nematode infection parameters

Table 2 summarizes the efficacy of pre- and post-inoculation treatments with ascorbic acid and glutamic acid. Pre-inoculation treatment with ascorbic acid significantly reduced nematode infection severity in a concentration-dependent manner. At 2 mg/ml, this treatment reduced the gall index by 59% (from 7.8 to 3.2) and the nematode population by 57% (from 2150 to 920 eggs + J2/g root) compared to the inoculated control ($p < 0.001$). Glutamic acid applied pre-inoculation also significantly suppressed nematode infection, reducing galling by 50% and nematode numbers by 51% at 2 mg/ml ($p < 0.001$), though it was slightly less effective than ascorbic acid at the same concentration.

Table 1 Effect of ascorbic and glutamic acids on *M. incognita* egg hatch and juvenile mortality (%) at 12 days post-treatment

Treatment	Concentration (mg/ml)	Egg Hatch (%)	Mortality (%)
Control	0	$92.3 \pm 2.1a$	$6.0 \pm 1.2d$
Ascorbic Acid	1	$59.6 \pm 3.4b$	$38.9 \pm 2.8c$
	2	$29.5 \pm 2.9c$	$62.1 \pm 3.0a$
Glutamic Acid	1	$65.4 \pm 2.8b$	$34.4 \pm 2.5c$
	2	$42.7 \pm 3.2c$	$49.5 \pm 2.7b$
<i>p</i> -value		< 0.001	< 0.001

Values are means $\pm$ SE of four replicates. Egg hatch percentages were calculated based on initial counts of approximately 100 eggs per well. Juvenile mortality was corrected using Abbott's formula. Different letters within columns indicate significant differences at $p < 0.05$ by Tukey HDS test

Table 2 Effect of pre- and post-treatments with ascorbic and glutamic acids on nematode infection parameters in sunflower infected with *M. incognita*

Treatment	Application Time	Concentration (mg/ml)	Root Gall Index (0–10)	Nematode Population (eggs + J2/g root)
Control (Inoculated only)	-	0	$7.8 \pm 0.3a$	$2150 \pm 120a$
Ascorbic Acid	Pre-Inoculation	1	$4.5 \pm 0.4c$	$1280 \pm 90c$
		2	$3.2 \pm 0.3d$	$920 \pm 85d$
Glutamic Acid		1	$5.6 \pm 0.4b$	$1450 \pm 110b$
		2	$3.9 \pm 0.4d$	$1050 \pm 100c$
Ascorbic Acid	Post-Inoculation	1	$6.5 \pm 0.5a$	$1800 \pm 110a$
		2	$5.1 \pm 0.4b$	$1550 \pm 100b$
Glutamic Acid		1	$6.7 \pm 0.5a$	$1850 \pm 120a$
		2	$5.5 \pm 0.3b$	$1600 \pm 110b$
<i>p</i> -value			< 0.001	< 0.001

Values are means $\pm$ SE of four replicates. Different letters within columns indicate significant differences at $p < 0.05$ by Tukey HSD test

Post-inoculation treatments were less effective than pre-inoculation applications but still significantly lower gall formation and nematode densities relative to control ($p < 0.001$). For example, ascorbic acid at 2 mg/ml applied post-inoculation reduced the gall index to 5.1 and the nematode population to 1550/g root.

Pre-inoculation treatment with ascorbic acid significantly reduced nematode infection severity in a concentration-dependent manner. At 1 mg/ml, the gall index decreased by 42% to 4.5 ± 0.4 , and the nematode population declined by 40% to 1280 ± 90 . The higher concentration of 2 mg/ml provided better control, reducing galling by 59% (3.2 ± 0.3) and nematode numbers by 57% (920 ± 85). These reductions were statistically significant compared to the control ($p < 0.001$). Glutamic acid applied pre-inoculation also significantly suppressed nematode infection, albeit slightly less effectively than ascorbic acid. Gall indices were reduced to 5.6 ± 0.4 and 3.9 ± 0.4 at 1 and 2 mg/ml, representing decreases of 28% and 50%, respectively. Corresponding nematode populations were similarly reduced to 1450 ± 110 and 1050 ± 100 at these concentrations ($p < 0.001$).

Post-inoculation treatments were less effective but still significantly lowered gall formation and nematode densities relative to the control. Ascorbic acid at 1 mg/ml post-inoculation reduced the gall index to 6.5 ± 0.5 and nematode population to 1800 ± 110 , while 2 mg/ml reduced them further to 5.1 ± 0.4 and 1550 ± 100 , respectively. Glutamic acid at the same concentrations resulted in gall indices of 6.7 ± 0.5 and 5.5 ± 0.3 and nematode populations of 1850 ± 120 and 1600 ± 110 , respectively ($p < 0.001$).

Finally, pre-inoculation application of ascorbic acid at 2 mg/ml was most effective in minimizing root galling and nematode populations, confirming its potential as a nematocidal agent promoting resistance against *M. incognita* in sunflower cultivation.

3.3 Effect of pre- and post-treatments with ascorbic and glutamic acids on plant growth parameters of sunflower

Table 3 presents the influence of pre- and post-inoculation foliar applications of ascorbic acid and glutamic acid on sunflower shoot and root fresh weights 45 days post nematode inoculation. Inoculated control plants exhibited reduced growth with shoot fresh weight of 25.6 ± 1.2 g and root fresh weight of 12.1 ± 0.6 g and this reflects the detrimental effects of *M. incognita* infection.

Pre-inoculation treatment with ascorbic acid significantly enhanced plant growth in a dose-dependent manner. At 1 mg/ml, shoot fresh weight improved by 14.06% to 29.2 ± 1.0 g, and root fresh weight increased by 18.18% to 14.3 ± 0.5 g compared to controls ($p < 0.001$ and $p = 0.003$, respectively). Doubling the concentration to 2 mg/ml further boosted growth with a 21.09% increase in shoot weight (31.0 ± 1.1 g) and 24.79% increase in root weight (15.1 ± 0.6 g). Similarly, glutamic acid application pre-inoculation improved shoot and root growth significantly. Shoot fresh weight increased by 10.55% and 16.02% at 1 mg/ml and 2 mg/ml, respectively, while root fresh weight increased by 15.70% and 23.14% (p -values significant).

The treatments of post-inoculation showed mild but still significant growth promotion influences. Ascorbic acid at 1 mg/ml increased shoot and root fresh weights by 5.47% and 7.44%, respectively. At 2 mg/ml, increases reached 11.33% (shoot) and 11.57% (root). Glutamic acid post-inoculation treatments yielded similar moderate improvements.

Statistical analysis confirmed significant effects of treatment concentration on shoot and root growth ($p < 0.001$ and $p = 0.003$, respectively). Application timing also influenced growth responses, with pre-inoculation applications generally more effective, although all treatments demonstrated better growth performance than the inoculated control.

Table 3 Effect of pre- and post-treatments with ascorbic and glutamic acids on plant growth parameters of sunflower infected with *M. incognita*

Treatment	Application Time	Concentra- tion (mg/ml)	Shoot Fresh Weight (g)	% Increase Shoot FW	Root Fresh Weight (g)	% Increase Root FW
Control (Inoculated only)	-	-	25.6 ± 1.2c	-	12.1 ± 0.6d	-
Ascorbic Acid	Pre-Inoculation	1	29.2 ± 1.0b	14.06	14.3 ± 0.5c	18.18
		2	31.0 ± 1.1a	21.09	15.1 ± 0.6b	24.79
Glutamic Acid		1	28.3 ± 1.3b	10.55	14.0 ± 0.7c	15.70
		2	29.7 ± 1.1ab	16.02	14.9 ± 0.8b	23.14
Ascorbic Acid	Post-Inoculation	1	27.0 ± 1.3c	5.47	13.0 ± 0.5cd	7.44
		2	28.5 ± 1.4b	11.33	13.5 ± 0.6c	11.57
Glutamic Acid		1	26.8 ± 1.2c	4.69	13.2 ± 0.5c	9.09
		2	27.5 ± 1.3bc	7.42	13.8 ± 0.4bc	14.05
<i>p</i> -value			<0.001		<0.003	

Values are means $\pm$ SE of four replicates. Different letters within columns indicate significant differences at $p < 0.05$ by Tukey HSD test

3.4 Effect of pre- and post-treatments with ascorbic and glutamic acids on defense-related enzyme activity in sunflower leaves

Table 4 presents the activities of key defense enzymes peroxidase (POX), phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD), and ascorbate peroxidase (APX) in sunflower leaves following treatment with ascorbic and glutamic acids at two application timings relative to *M. incognita* inoculation. The inoculated control plants exhibited the lowest enzymatic activities, with POX activity at $14.5 \pm 1.2 \mu\text{mol min}^{-1} \text{mg}^{-1}$, PAL at $8.6 \pm 0.8 \mu\text{mol cinnamic acid h}^{-1} \text{mg}^{-1}$, SOD at $25.3 \pm 1.5 \text{ units mg}^{-1} \text{protein}$, and APX at $5.8 \pm 0.5 \mu\text{mol min}^{-1} \text{mg}^{-1}$.

Pre-inoculation treatment with ascorbic acid markedly enhanced enzyme activities, with POX activity reaching 33.5 ± 1.8 , PAL at 19.8 ± 1.2 , SOD at 52.4 ± 2.7 , and APX at 15.3 ± 1.0 . This is more than a twofold rise relative to levels of control ($p < 0.001$). The presence of such an induction shows a strong defense system activation of the plant prior to nematode challenge.

The enzyme activities were also significantly elevated by post-inoculation applications of ascorbic acid relative to the control, although to a smaller degree as compared to pre-inoculation treatments (POX 28.0 ± 1.5 , PAL 16.2 ± 1.0 , SOD 44.3 ± 2.3 , APX 12.0 ± 0.8). The treatments of glutamic acid similarly promoted defense enzyme activities. Pre-inoculation applications elevated POX, PAL, SOD, and APX activities to 28.3 ± 2.0 , 17.1 ± 1.1 , 45.7 ± 2.3 , and 12.6 ± 1.1 , respectively. Post-inoculation treatments produced moderate increases (POX 24.5 ± 1.7 , PAL 14.0 ± 0.9 , SOD 38.7 ± 1.8 , APX 10.2 ± 0.7), all significantly above untreated controls ($p < 0.001$).

The p-values of all the enzyme activities were highly significant, implying strong treatment effects. The increase in the activities of defense-related enzymes noted is associated with enhanced resistance mechanisms in sunflower, leading to the suppression of nematodes as well as a reduction in root damage. The highest and most potent induction of

defensive enzymes was recorded following pre-inoculation foliar application of ascorbic acid that indicates its potential use as an effective biological elicitor in nematode management programs.

3.5 Percentage increase in activity of defense-related enzymes in sunflower leaves relative to control

Figure 1 illustrates the percentage increases in the activities of defense-related enzymes peroxidase (POX), phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD), and ascorbate peroxidase (APX) in sunflower leaves following foliar application of ascorbic acid and glutamic acid at different timings relative to *M. incognita* inoculation.

The largest increases in enzyme activities were observed by pre-inoculation treatment with ascorbic acid, as POX activity increased by 131.0, PAL increased by 130.2, SOD increased by 107.5, and APX increased by 163.8 relative to untreated inoculated controls. This high induction indicates a high level of priming of the plant defense system, which allows the plant to be more capable of resisting nematode infection.

The use of ascorbic acid after inoculation also increased enzyme activities to a significant extent of 93.1% (POX), 88.4% (PAL), 75.5% (SOD), and 106.9% (APX), but to a slightly lesser degree. These findings show that ascorbic acid is able to activate antioxidative defenses even when nematode infestation has begun. Glutamic treatments adhered to the same pattern, with pre-inoculation treatment showing increases of 95.2% (POX), 98.8% (PAL), 80.6% (SOD) and 117.2% (APX), and post-inoculation treatment showing increases of 69.0%, 62.8%, 53.0% and 75.9%, respectively.

These results refer ascorbic and glutamic acids promising and green alternatives or supplements to traditional nematocides, which may be used as integrated management to enhance sunflower health and yield. The enhanced enzyme activities are attributed to the increased resistance responses and lesser nematode damage.

Table 4 Activity of defense-related enzymes in sunflower leaves after pre- and post-treatments with ascorbic and glutamic acids (units/mg protein)

Treatment	Application Time	POX ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)	PAL ($\mu\text{mol cinnamic acid h}^{-1} \text{mg}^{-1}$)	SOD (units $\text{mg}^{-1} \text{protein}$)	APX ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)
Control (Inoculated)	-	$14.5 \pm 1.2\text{d}$	$8.6 \pm 0.8\text{d}$	$25.3 \pm 1.5\text{d}$	$5.8 \pm 0.5\text{d}$
Ascorbic Acid	Pre-Inoculation	$33.5 \pm 1.8\text{a}$	$19.8 \pm 1.2\text{a}$	$52.4 \pm 2.7\text{a}$	$15.3 \pm 1.0\text{a}$
	Post-Inoculation	$28.0 \pm 1.5\text{b}$	$16.2 \pm 1.0\text{b}$	$44.3 \pm 2.3\text{b}$	$12.0 \pm 0.8\text{b}$
Glutamic Acid	Pre-Inoculation	$28.3 \pm 2.0\text{b}$	$17.1 \pm 1.1\text{b}$	$45.7 \pm 2.3\text{b}$	$12.6 \pm 1.1\text{b}$
	Post-Inoculation	$24.5 \pm 1.7\text{c}$	$14.0 \pm 0.9\text{c}$	$38.7 \pm 1.8\text{c}$	$10.2 \pm 0.7\text{c}$
p-value		<0.001	<0.001	<0.001	<0.001

Values are means $\pm$ SE of four replicates. Different letters within columns indicate significant differences at $p < 0.05$ by Tukey HSD test

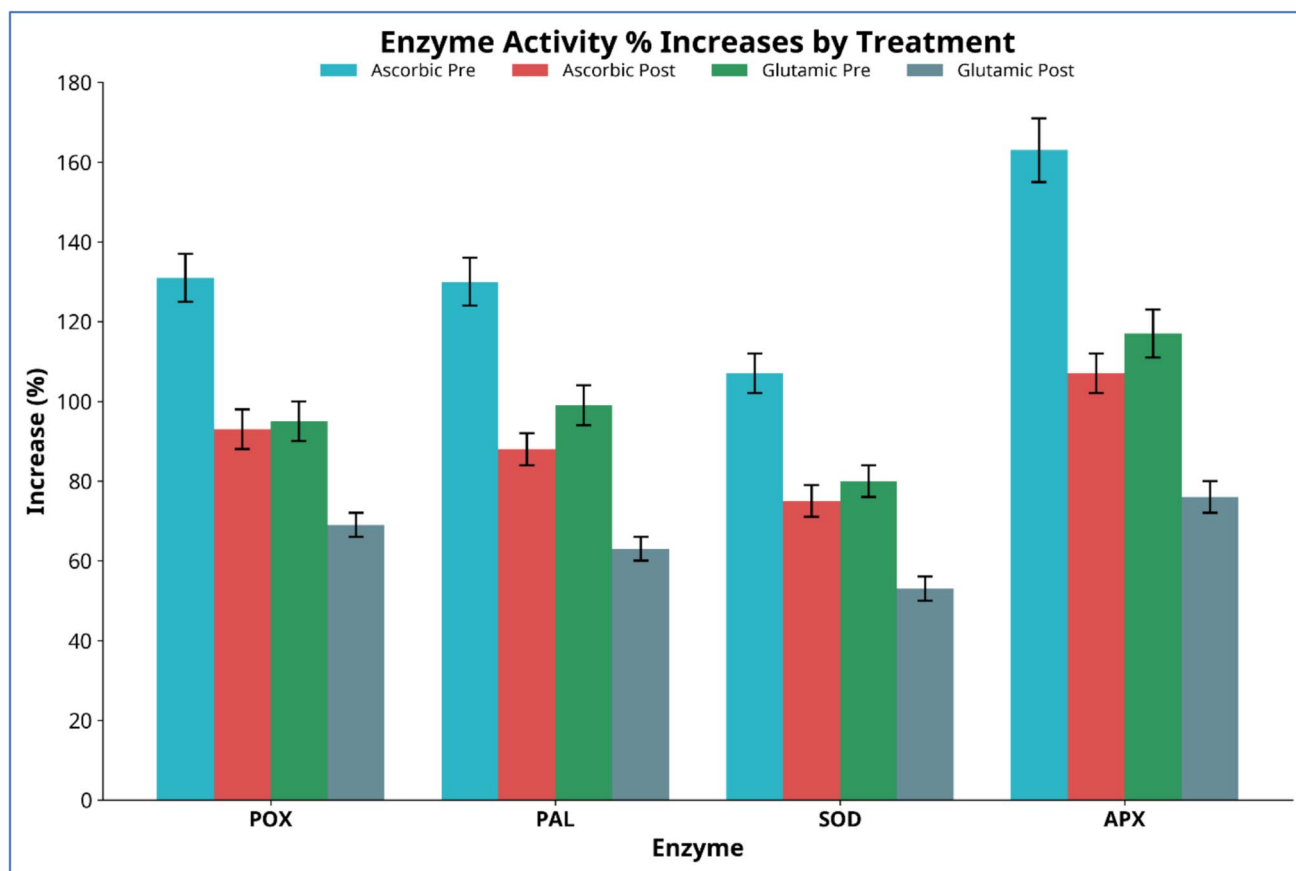


Fig. 1 Percentage increase in activity of defense-related enzymes in sunflower leaves relative to control

4 Discussion

The current study demonstrates the effect of ascorbic acid and glutamic acid in inhibiting *M. incognita* egg hatching, juvenile mortality, reduction in the severity of nematode infection, and promoting host plant resistance in sunflower. The concentration-dependent inhibition of egg hatch and the high mortality of juvenile limnicoles is a direct effect of nematicidal action, likely associated with the disruption of key physiological processes in the nematode (Neeraj et al. 2017; Singh et al. 2021).

The observed suppression of nematode reproduction and root galling by ascorbic and glutamic acids suggests interference with both nematode development and host immune modulation. These findings align with those of Singh et al. (2021), who reported similar suppressive effects in rice treated with ascorbate, and extend them to the sunflower-*M. incognita* system. Notably, the greater efficacy of ascorbic acid compared to glutamic acid at equivalent concentrations (2 mg/ml reduced galling by 59% vs. 50%) may reflect its more direct role in both ROS detoxification and signalling pathways that activate plant defences (Akram et al. 2017).

The superior efficacy of pre-inoculation application compared to post-inoculation treatments indicates that priming the plant prior to nematode attack enhances subsequent defense responses. This finding is consistent with the established paradigm of induce resistance, where timely elicitor application activates the plant's immune system, leading to upregulation of defense pathways (Mauch-Mani et al. 2017; Pieterse et al. 2014). Our results suggest that the enhanced enzyme activities observed in pre-treated plants reflect this primed state, enabling a more rapid robust response to nematode invasion. Increased activities of defense-related enzymes, such as peroxidase (POX), phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD), and ascorbate peroxidase (APX) are biochemical predictors of an active defense state (Singh et al. 2021). These enzymes regulate the balance of reactive oxygen species (ROS) and the production of antimicrobial complexes, which are important in limiting the development and settlement of nematodes (Pandey et al. 2018). The significant rise in enzyme activities at pre-inoculation ascorbic acid treatment indicates strong elicitor activity, which is in line with earlier findings that vitamins could

also serve as stress signals to promote pathogen resistance (Al-Sayed and Montasser 1986).

Mechanistically, nematodes locate infection sites by sensing host root exudates (Mendy et al. 2017), while plants perceive nematode invasion through pattern recognition receptors, triggering defensive responses (Zhou et al. 2023). It is plausible that the exogenous application of ascorbic and glutamic acids enhances these perception and signaling processes, leading to the heightened defense enzyme activities observed in our study. However, the direct causal link between enzyme induction and nematode suppression, while strongly suggested by our correlative data, requires further validation through targeted molecular studies, such as gene expression analysis or the use of specific enzyme inhibitors. Co-evolutionary arms race between root-knot nematodes and their host plants involves structural, as well as biochemical adaptations based on the innate immune system of the plant and the arsenal of effector proteins of the nematode (Jones and Dangl 2006; Mitchum et al. 2013). Recent research indicates that nematode effectors attack host defense to support the formation of feeding sites, and plants evolve enzymes and secondary metabolites to counter these effectors (Phani et al. 2021). Our results indicate that the foliar application of ascorbic and glutamic acids enhances the defensive biochemical armory system of the host plant, which leans towards resistance.

5 Conclusion

This study demonstrates that foliar applications of ascorbic acid and glutamic acid reduces the *M. incognita* egg hatching and the survival of juvenile *in-vitro*, and suppresses root gallings and nematode populations in sunflower plants under greenhouse conditions. The increase in defense-related enzyme activities (POX, PAL, SOD, APX) following treatments is associated with induce systemic resistance, contributing to the observed nematode suppression. Pre-inoculation treatment with ascorbic acid at 2 mg/ml was the most effective, highlighting the importance of application timing. These finding suggest that ascorbic and glutamic acids are promising candidates for inclusion in integrated nematode management programs for sunflower. However, field validation is necessary to confirm efficacy under diverse environmental conditions. Further studies are required to optimize the application protocols (e.g., frequency, formulation) and elucidating the molecular signaling pathways involved to fully harness their potential for sustainable protection.

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Data availability The tables in this research article show all the data that was collected.

Declarations

Competing interests The authors declare no competing interests.

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