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Nematicidal performance of β-aminobutyric and ascorbic acids against the root-knot nematode, *Meloidogyne incognita* on tomato plants under greenhouse conditions

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ABSTRACT

The technique of induced systemic resistance (ISR) is one of the most novel and durable approaches for managing plant parasitic nematodes. The current trial was conducted to study the efficacy of ascorbic acid and β-aminobutyric acid (BABA) against the root-knot nematode, *Meloidogyne incognita* under *in vitro* and *in vivo* conditions on tomato plants. The obtained results elucidated that ascorbic acid (LC_{50} = 3426.76 and 965.66 mg l^{-1}) was more effective or toxic than BABA (LC_{50} = 16028 and 8405.68 mg l^{-1}) towards the juveniles' mortality of the root-knot nematode (*M. incognita*) after 24 and 48 hrs of exposure, respectively. However, the estimated LT_{50} values of ascorbic acid were in the range of 193.02 to 17.95 hrs, while BABA recorded LT_{50} values in the range of 159.04 to 33.61 hrs. On the other hand, application of ascorbic acid suppressed tomato root galls, egg masses, and soil populations at 88.46, 91.79, and 43.67%, while BABA recorded 87.18, 73.40 and 49.52%, respectively. Some of the applied treatments relatively improved tomato growth indices. The effect of BABA and ascorbic acid was also evaluated as inducers on tomato plants against *M. incognita*. The results elucidated that BABA and ascorbic acid (at two rates) increased the total soluble phenol (TSN), total soluble protein (TSP), polyphenol oxidase (PPO) and peroxidase (POD), significantly.

Key words: ISR, *Meloidogyne incognita*, β-aminobutyric acid (BABA), ascorbic acid, Tomato plants,

INTRODUCTION

Tomato is a unique crop that has the ability to be produced in all the governorates of Egypt and is available all year round. According to the FAO, Egypt recorded production in tomato yield that reached up to 6.3 million metric tons in 2021. Meanwhile, the cultivated area of the tomato crop in Egypt was approximately 360.261 feddan (FAOSTAT, 2021). Egypt is the fifth-largest tomato producer worldwide. Certain pests, such as insects, mites, viruses, bacteria, fungi, and plant nematodes, are attacking tomato plants in open fields or protected houses. The most famous plant parasitic nematodes are the root-knot nematodes, (*Meloidogyne* spp.)

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that invade the roots of most vegetables, especially tomatoes, causing damage estimated at approximately 40% (Blok *et al*., 2008).

The most predominant species of root-knot nematodes in soils is *Meloidogyne incognita*, which causes changes in the production of chlorophyll, amino acids and organic acids, reactive oxygen species, water metabolism, defense enzymes, or even the physical priors (Eves-van den Akker, 2021). Several agricultural practices are available to manage plant parasitic nematodes. These practices are intended to improve plant growth, quality, and yield by keeping the nematode population below the economical threshold level (Khalil *et al*., 2022).

One of the new trends is using β-aminobutyric acid (BABA), which is considered a non-protein amino acid, to induce systemic resistance of the plants defense system against a wide range of biotic or abiotic stress factors (Pastor *et al*., 2014). The mechanisms of BABA-induced resistance (BABA-IR) are diverse and appear to be pathogen- and plant-specific (Piekna-Grochala and Kepczynska, 2013). The inhibitory effect of BABA has also been reported on root-knot nematode infection (root galls, number of egg masses, and number of eggs per egg mass) on cucumber (Sahebani *et al*., 2011).

Meanwhile, ascorbic acid is one of the organic acids that recorded nematicidal activity, as well as improving plant growth and/or inducing resistance to susceptible plants (Refaei, 2009; Bakr and Hewedy ,2018). Therefore, this study aimed to investigate the impact of ascorbic and β-aminobutyric acids on the development of the root-knot nematode, (*Meloidogyne incognita*), and tomato growth and the induction of plant systemic resistance under greenhouse conditions.

MATERIALS AND METHODS

1-The root-knot nematode inoculum

The root-knot nematode inoculum used in the present study was obtained from the infested roots of Okra (Abelmoschus esculentus), which were collected from the El-Nubarya region of El-Behaira Governorate, Egypt. To establish and maintain the culture of root-knot nematodes, a single egg mass of gall containing a single female was removed from the root. To increase nematodes population, a single egg mass was inoculated into a pot containing a 4-week-old tomato plant (*Solanum lycopersicum* cv. Golden Stone) in sterilized loamy sand soil and kept for about 4 months. The perineal patterns method (Taylor and Nelscher, 1974) was used for the identification of the species for root-knot nematode inoculum, and the inoculum species was recognized as *Meloidogyne incognita*. To isolate the eggs from root-galls, the sodium hypochlorite (NaOCl) method was used (Hussey and Barker, 1973). The egg suspension was passed through a 200-mesh sieve nested upon a 400-mesh sieve. The eggs on the 400-mesh sieve were washed to free the residue

of NaOCl via a slow stream of tap water before inoculation. To obtain the second-stage juveniles (J_2), the eggs were hatched under laboratory conditions (30 ± 2 ºC) using the Baermann plate technique (Ayoub, 1980), and the juveniles were collected after 48 hrs. The larvae of root-knot nematodes in the suspension were collected on 325-mesh sieves. The retained nematodes were collected on the sieve by back washing into a 250-ml beaker using tap water. The collected J_2S were used for the laboratory experiments.

2-The laboratory assays

The efficacy of ascorbic and β-aminobutyric acids was assessed on the mortality of the second stage juveniles' (J_2) of *M. incognita* under laboratory conditions (25±2 ºC). Each treatment was replicated four times, and each replicate (vial, ca. 15 ml) contained about 250 J_2. The intervals for juveniles' mortality were recorded after 24 and 48 hrs of exposure to calculate the lethal concentrations (LC), while lethal time (LT) was calculated after 24, 36, 48, and 72 hrs of exposure. Ascorbic acid was tested at 250, 500, 1000, 2000, 3000, and 6000 mg l^{-1} to calculate the lethal concentration (LC) and at 250, 500, 1000, 2000, 3000 and 6000 mg l^{-1} to calculate the lethal time (LT). Furthermore, β-aminobutyric acid was assessed at serial dilutions of 1000, 2000, 4000, 6000, 8000, and 12000 mg l^{-1} to calculate both lethal concentration and lethal time. The numbers of both dead and live juveniles were counted, and the mortality % was calculated. The untreated replicates contained distilled water, served as a control.

3-The pots experiment

The nematicidal activity of ascorbic and β-aminobutyric acids was investigated against *M. incognita* on tomato plants in a pot experiment. The standard nematicide used during the experiment was fosthiazate (Fosimex® 10% EC). Ascorbic acid (97% purity) was obtained from El-GOMHOURIA company in Alexandria, Egypt. While β-aminobutyric acid (98% purity) was obtained from Zhengzhou DeLong Chemical Company Limited, China. The black plastic bags were filled with 2 kg of infested loamy sand soil. One seedling of 40 days old tomato (*Solanum lycopersicum* L., cv. 086) was transplanted in each pot (plastic bags). Ascorbic acid was applied as a soil drench at rate of 7.5 and 15 g l^{-1} which represent the value of LC_{90} and its fold after 48 hrs of exposure. Also, β-aminobutyric acid applied as a soil drench at rate of 8.5 and 17 g l^{-1} which represent the value of LC_{50} and its fold after 48 hrs of exposure. The non-fumigant nematicide (Fosimex® 10% EC) was applied at the field rate (12L/feddan), that recommended by the Agricultural Pesticide Committee, Ministry of Agriculture and Land Reclamation, Egypt. All the treatments were applied after two days of transplanting. However, the used soil in this experiment was naturally infested, and the average number of the root-knot nematode (*M. incognita*) was 2900

J_2 / 2 kg soil. Each treatment was replicated five times and arranged in a complete randomized design under greenhouse conditions (photoperiod; 14:10 L:D, 30±3 ºC and 55% RH). The inoculated and uninoculated plants were served as controls. The irrigation and fertilization were made when needed. After 50 days of transplanting, the plants were uprooted, and the roots were washed to be free from soil. The shoot or root weights and lengths were recorded. Furthermore, the number of J_2/250 g soil and egg-masses were recorded. Also, the utilized root gall index scale was from 0 to 10 according to Bridge and Page (1980). The second stage juveniles (J_2) were counted after being extracted from the soil by sieving and the Baermann plate technique (Ayoub, 1980). To count the egg masses, the roots were stained with an aqueous solution of Phloxine B (0.15 g/l water) for 15 minutes, then washed with running water to remove excess stain (Holbrook et al., 1983). The pot trial included the following treatments:

-Uninoculated check (healthy plants).

- Inoculated with *M. incognita* alone (untreated check).

-*M. incognita* + Fosthiazate

-*M. incognita* + Ascorbic acid (7.5 g l^{-1})

-*M. incognita* + Ascorbic acid (15 g l^{-1})

-*M. incognita* + β-aminobutyric acid (8.5 g l^{-1})

-*M. incognita* + β-aminobutyric acid (17 g l^{-1})

4-Determination of total soluble phenol

The total soluble phenol contents of tomato leaves were extracted according to Slinkard and Singleton (1997). Samples of 1 g of fresh tomato leaves were mixed with 16 mL of methanol 80% and kept overnight. The suspension was filtered, and the filtrate was diluted to 20 mL. This solution served as a stock solution for subsequent analysis. 0.2 mL of the stock solution was mixed with 1.4 mL of distilled water and 0.1 mL of 50% (1N) Folin-Ciocalteu phenol reagent. After 30 seconds (at least), 0.3 mL of 20% (w/v) sodium carbonate was added. The reaction mixture was allowed to stand for two hours, and the absorbance at 765 nm was estimated by using a spectrophotometer (Tuner, model 390). The total soluble phenol content was standardized against tannic acid, and absorbance values were converted to µg of phenols per gram fresh weight of tomato leaves. Each value was the average of three replicates. The results were expressed as tannic equivalents according to the following formula:

mg tannic acid /gm fresh weight =

(A/K *(20/(0.2))/ 1) Where:

A= absorbance at 765 nm.

K= the extension coefficient=0.016898 μg^{-1}.

5-Determination the total protein

Total protein was estimated according to the method described by Bradford (1976), with slight modifications proposed by Dixon (1985). About 0.25g of fresh tomato leaves were added to 5 ml of acetone. The plant samples were transferred to 5 ml of 1N NaOH and heated at 85 °C for 1.5 hours. The produced solution after filtration contained the hydrolyzed

protein, which was determined using Bio-Rad assay dye [100µl protein + 1 ml bio-rad. mixture (1 ml bio-rad: 5 ml distilled water)]. The developed color was measured at 595 nm by using a spectrophotometer (Tuner, model 390). The reading was related to a standard curve prepared from the known concentration of BSA protein.

mg protein/gm fresh weight = ((O/K)*100)/0.25)

Where: K = 0. 29 mg/ml. O =

The absorption at 595 nm.

6-Estimation of Polyphenol oxidase (PPO) activity

Polyphenol oxidase activity was determined, according to Broesch (1954) as follows: 0.5g of fresh tomato leaves were homogenized in 3 ml of borate buffer, PH 9. The homogenate was centrifuged at 4000 r.p.m. for 15 min. The supernatant was used as a crude enzyme. One ml of the supernatant was mixed with 2 ml of borate (PH=9), 1 ml of para-aminobenzoic acid, ethanol 1%, and 2 ml of catechol. The reaction mixture was incubated for 1 h at 45 °C. After briefly overtaxing, the absorbance at 575 nm was estimated by using a spectrophotometer (Tuner, model 390). The activity as a percentage of control was calculated. Each value was the average of three replicates.

% Activity = ((A1/A2)*100)

Where:

A1: Absorbance of the treatment sample at 575 nm,

A2: Absorbance of the control sample at 575 nm.

7-Estimation of Peroxidase (POD) activity

One g of fresh leaves was ground into powder using a mortar and pestle with 10 ml of sodium phosphate buffer (0.1 M, pH 7.5) containing 0.2 M sodium meta sulfite and sodium chloride. Homogenates were centrifuged at 10,000 r.p.m. for 15 min at 4 °C. The supernatant was used to determine enzyme activities (Tuzun et al., 1989). Peroxides activity was determined according to Murage and Masuda (1997) with a modified reaction mixture containing 200 µl of enzyme extract, 0.1M sodium phosphate buffer (PH 6.1, 1.5 ml of 20% H2O2, and 1.5 ml of catechol 0.04 M. The initial rate of increase in absorbance was measured within 1 min at 470 nm and determined by using spectrophotometer (Tuner, model 390). POD activity as a percentage of control was calculated. Each value was the average of three replicates.

Activity (%) = ((A1/A2)*100)

Where:

A1: Absorbance of the treatment sample at 470 nm,

A2: Absorbance of the control sample at 470 nm.

Statistical analysis

The data from the pot trial were subjected to the analysis of variance test (ANOVA) as a complete randomized design (CRD). The least significant differences (LSD) at the 5% level of probability were determined using a computer program, Costat Version 6.303 (2005). The values of $LC_{50\&90}$ and

$LT_{50\&90}$ of each compound were calculated based on the larval mortality of *M. incognita* by using a probit analysis program according to Finney (1971). To correct the larval mortality in untreated check, Abbott formula (1925) was used.

RESULTS

The nematicidal activity efficacy oof β-amino butyric acid (BABA) and ascorbic acid was assessed on the juveniles' mortality of *M. incognita* juveniles for 24 and 48 hrs under laboratory conditions (Table 1). After 24 hrs of exposure, results showed that both BABA and ascorbic acid recorded values of LC_{50} estimated at 16028 and 3426.76 mg l^{-1}, respectively. The recorded values of LC_{90} values were 144871.20 and 5177.72 mg l^{-1} with BABA and ascorbic acid, respectively. However, after 48 hrs of exposure, the recorded values of LC_{50} values were 8405.68 and 965.66 mg l^{-1}, while LC_{90} values were 114812.40 and 7368.23 mg l^{-1} with BABA and ascorbic acid, consecutively. Generally, the obtained results indicated that ascorbic acid was more effective or toxic than BABA towards the juveniles' mortality of the root-knot nematode (*M. incognita*) after 24 and 48 hrs of exposure under laboratory conditions.

Table (1): LC_{50}, LC_{90}, fiducial limits and slop of β-amino butyric and ascorbic acids tested against *Meloidogyne incognita* juveniles for 24 and 48 hrs under laboratory conditions.

Treatment	LC_{50} mg/l	Fiducial Limits (Lower-Upper)	LC_{90} mg/l	Fiducial Limits (Lower-Upper)	Slope ±SE
24 hrs					
BABA	16028	11671-26429.96	144871.2	68993.92 - 516856	1.13 ±0.16
Ascorbic acid	3426.76	2644.98-4836.40	51077.72	25952.73-146458.50	1.09 ± 0.13
48 hrs					
BABA	8405.68	6456.34-12224.92	114812.40	54772.62 - 411506.	1.13 ±0.16
Ascorbic acid	965.66	816.306 - 1141.269	7368.23	5521.23- 10622.04	1.452 ± 0.102

The nematicidal efficacy of β-aminobutyric acid (BABA) and ascorbic acid was assessed on the juveniles' mortality of *M. incognita* for 24, 36, 48, and 72 hrs to calculate the lethal time under laboratory conditions (Table 2). The impact of BABA was assessed at concentrations of 2000, 4000, 6000, 8000, and 12000 mg l^{-1} and recorded lethal time (LT_{50}) values in the range of 159.04 to 33.61 hrs, while the estimated values of LT_{90} were at range of 709.65 to 94.44 hrs. In respect to ascorbic acid, the tested concentrations were 250, 500, 1000, 2000, 3000, and 6000 mg l^{-1}. The estimated LT_{50} values of ascorbic acid were in the range of 193.02 to 17.95 hrs, while the estimated values of LT_{90} were in the range of 1510.94 to 62.57 hrs. However, the obtained

results exhibited that ascorbic acid was more toxic than BABA against the second-stage juveniles (J_2) of the root-knot nematode (*M. incognita*).

Table (2): LT_{50}, LT_{90}, fiducial limits and slop of β-amino butyric and ascorbic acids tested against *Meloidogyne incognita* juveniles post 24, 36, 48 and 72 hrs under laboratory conditions.

Concentrations	LT_{50} (hrs)	Fiducial Limits (Lower-Upper)	LT_{90} (hrs)	Fiducial Limits (Lower-Upper)	Slope ±SE
BABA					
2000 mg l^{-1}	159.04	103.09 – 207.47	709.65	289.79 – 8877.81	1.97± 0.48
4000 mg l^{-1}	112.42	82.86 - 227.78	497.11	240.07 – 2962.31	1.98±0.43
6000 mg l^{-1}	59.33	50.40 – 78.14	270.65	159.9 – 862.22	1.94±0.37
8000 mg l^{-1}	45.47	39.70 -54.10	192.24	126.43 – 456.81	2.05±0.37
12000 mg l^{-1}	33.61	29.39-37.38	94.44	76.92-132.81	2.86±0.40
Ascorbic acid					
250 mg l^{-1}	193.02	108.25-1735.36	1510.94	399.99-279639.20	1.43±0.44
500 mg l^{-1}	100.97	70.39-329.32	945.30	303.41-57671.45	1.32±0.38
1000 mg l^{-1}	48.32	41.48-59.11	229.52	140.53-680.08	1.89±0.37
2000 mg l^{-1}	34.37	28.08-39.81	152.09	104.34-337.08	1.98±0.38
3000 mg l^{-1}	21.34	14.31-26.24	85.33	66.64-140.64	2.13±0.40
6000 mg l^{-1}	17.95	11.25-22.64	62.57	51.80-89.55	2.36±0.45

The presented data in Table 3 demonstrated the effectiveness of β-amino butyric acid (BABA) and ascorbic acid against the root-knot nematode (*Meloidogyne incognita*) at two rates on the tomato plants. The non-fumigant registered nematicide fosthiazate (Fosimex® 10% EC) was used as a standard. The recorded results indicated that ascorbic acid was the most effective treatment, with a general mean reduction (GMR) in tomato gall index estimated at 88.46%, followed by BABA with a GMR of 87.18%. However, the highest rate of ascorbic acid, or BABA, recorded the highest reductions of tomato gall index by 92.31 and 89.74%, respectively, compared with the standard nematicide, fosthiazate, that recorded 61.54%.

On the other hand, the soil population densities (J_2/250g soil) of *M. incognita* were also recorded. BABA and ascorbic acid recorded GMR of 49.52 and 43.67%, consecutively, while fosthiazate recorded a 71.77%

reduction. No significant differences were noted between the high and low rates of BABA or ascorbic acid in gall index or soil populations.

In respect to egg masses, the fosthiazate treatment recorded a decrease of 65.91%. Whereas BABA and ascorbic acid recorded GMR estimated at 73.40 and 91.79%, respectively. However, ascorbic acid at high rate exhibited high efficacy against egg masses and recorded a 100% reduction (Table 3).

Table (3): The nematicidal effect of β-amino butyric acid (BABA) and ascorbic acid on gall index, soil population (J_2/250g) and egg mass of *Meloidogyne incognita* on tomato plants

Treatments	Gall index/ root system			J_2/250g soil			Egg masses/root system		
	means	R %	GMR %	means	R %	GMR %	means	R %	GMR %
BABA-L	1.4 bc	82.05	87.18	185.6 b	44.26	49.52	17.60 c	81.93	73.40
BABA-H	0.6 c	92.31		150.6 bc	54.77		34.21 b	64.88	
Ascorbic acid-L	1.0 bc	87.18	88.46	182.8 b	45.11	43.67	16.00 c	83.57	91.79
Ascorbic acid-H	0.8 c	89.74		192.40 b	42.22		0.00 d	100.00	
Fosthiazate	3.0 b	61.54	61.54	94.0 c	71.77	71.77	33.20 b	65.91	65.91
Untreated check	7.8 a	-----	-----	333.0 a	-----	-----	97.40 a	-----	-----

Means in each column followed with the same letter are not significantly different at 5 % level. L= low rate; H = high rate; R % = Reduction Percent; GMR (%) = general mean reduction percent of high and low rates with BABA or ascorbic acid.

According to the data in Table 4**,** the healthy plant (untreated uninoculated plants) indices such as shoot height, shoot weight, and root weight were increased by 10.03, 10.75, and 3.39%, respectively, while the root length was decreased by 2.56%. Furthermore, application of ascorbic acid or BABA recorded reductions in shoot height of 0.86 and 5.63%, respectively, while fosthiazate recorded a reduction of 17.48%. The shoot weight recorded GMI estimated by 5.47% with BABA, while ascorbic acid and fosthiazate recorded reductions at 1.07 and 4.77%, respectively. Despite the differences between the high and low rates of ascorbic acid on shoot weight, there weren't any significant differences. Application of BABA and ascorbic acid recorded GMI in the root length estimated by 34.63 and 18.93%, respectively. Also, fosthiazate showed an augmentation in root length estimated at 42.50%. For root weight, BABA and ascorbic acid applications recorded reductions estimated at 17.80 and 39.13%, consecutively. In contrast, fosthiazate exhibited an increase of 50.56% in root weight.

On the other hand, the efficacy of BABA and ascorbic acid on the activities of total soluble phenol (TSN), total soluble proteins (TSP), polyphenol oxidase (PPO%), and peroxidase (POD%) were estimated in the leaves of tomato plants (Table 5). The healthy plants showed augmentation in TSN (68.73%) and TSP (86.04%), and vice versa, PPO and POD were suppressed in healthy plants by 4.03 and 11.09%, respectively. Furthermore, the synthetic nematicide

fosthiazate had recorded increases in TSN (57.77%), TSP (89.28%), PPO (1.16%), and POD (31.45%).

In respect to BABA and ascorbic acid treatments, the GMI in TSN was recorded at 176.54 and 119.37%, respectively. Whereas TSP recorded GMI with BABA and ascorbic acid estimated at 162.27 and 135.40%, respectively. However, application of BABA and ascorbic acid to tomato plants recorded GMI in PPO, estimated at 81.03 and 53.75, while POD recorded GMI at 87.57 and 69.50%, respectively.

Generally, it could be noticed that the performance of using BABA was more effective than that of ascorbic acid. However, the applied high rate exhibited more activity than the low rate, despite the most significant differentiation between them for both BABA and ascorbic acid on tomato plants infested by root-knot nematode (*Meloidogyne incognita*).

Table (4): The effect of β-amino butyric acid (BABA) and ascorbic acid on shoot height, shoot weight, root length and root weight of tomato plants under greenhouse conditions.

Treatments	Shoot height			Shoot weight			Root length			Root weight		
	Means (cm)	I %	GMI %	Means (g)	I %	GMI %	Means (cm)	I %	GMI %	Means (g)	I %	GMI %
BABA-L	126.20 a	9.17	-0.86	45.5 a	-4.73	5.47	46.4 a	45.00	34.63	6.18 b	-15.21	-17.80
BABA-H	105.80 bc	-8.48		53.06a	10.20		51.8 a	61.88		6.94 b	-2.59	
Ascorbic acid-L	117.00 ab	1.21	-5.63	50.10 a	4.89	-1.07	45.40 a	41.88	18.93	5.67 b	-25.57	-39.13
Ascorbic acid-H	108.20 bc	-6.84		44.97 a	-5.96		34.8 b	8.75		6.27 b	-13.56	
Fosthiazate	98.40 c	-14.88	-14.88	45.48 a	-4.77	-4.77	45.60 a	42.50	42.50	10.72 a	50.56	50.56
Healthy plants	127.20 a	10.03	10.03	52.77 a	10.75	10.75	31.2 b	-2.56	-2.56	7.37 b	3.39	3.39
Untreated check	115.60 ab	-----	-----	47.65 a	-----	-----	32.0 b	-----	-----	7.12 b	-----	-----

Means in each column followed with the same letter are not significantly different at 5 % level.
L= low rate; H = high rate; GMI (%) = general mean increases percent of high and low rates with BABA or ascorbic acid; I (%) = increase percent.

Table (5): The efficacy of β-amino butyric acid (BABA) and ascorbic acid on total soluble phenol and proteins, as well as activity (%) of polyphenol oxidase and peroxidase in tomato leaves under greenhouse conditions.

Treatments	Total soluble phenol (mg tannic acid /g Fresh weight)			Total soluble protein (mg protein/g Fresh weight)			Polyphenol oxidase		Peroxidase	
	Mean	I%	GMI (%)	Mean	I%	GMI (%)	Activity (%)	GMI (%)	Activity (%)	GMI (%)
BABA-L	3374.23 b	164.34	176.54	516.32 a	158.39	162.27	76.64 a	81.03	88.14 a	87.57
BABA-H	3875.26 a	188.75		541.61 a	166.15		85.42 a		86.99 ab	
Ascorbic acid-L	2496.93 c	121.61	119.37	433.56 b	133.00	135.40	37.84 b	53.75	65.70 b	69.50
Ascorbic acid-H	2404.91c	117.13		449.20b	137.80		69.66 a		73.30 ab	
Fosthiazate	1186.09 f	57.77	57.77	291.03 d	89.28	89.28	1.16 c	1.16	31.45 c	31.45
Healthy plants	1411.04 e	68.73	68.73	280.46 d	86.04	86.04	-4.03 c	-4.03	-11.09 d	-11.09
Untreated check	2053.17 d	-----	-----	325.98 c	-----	-----	0.00 c	-----	0.00 d	-----

Means in each column followed with the same letter are not significantly different at 5 % level.
L= low rate; H = high rate; GMI (%) = general mean increases percent of high and low rates with BABA or ascorbic acid; I (%) = increase percent.

DISCUSSION

In our study, it could be noticed that tested plant elicitors may act directly or indirectly through the stimulus of plant chemical or physical defense systems or by reducing the juveniles ability to find or penetrate the plant roots. Certain studies have reported that β-aminobutyric acid (BABA) succeeded in suppressing the cucumber, pineapple, and tomato root galls formed by *Meloidogyne javanica* (Sahebani *et al*., 2011; Sahebani and Hadavi, 2009; Chinnasri *et al*., 2006). Also, application of BABA on cucumber (Sahebani *et al*., 2011) or tomato (Sahebani and Hadavi, 2009) induced the production of H_2O_2 against *M. javanica*. In the same context, application of BABA on rice plants induced H_2O_2, lignin, and callose accumulation against *M. graminicola* (Ji *et al*., 2015). Moreover, BABA can suppress the penetration of *M. graminicola*, which may be attributed to induce the production of lignin in the cell wall. Lignin can decrease the ability of secreted nematode enzymes' during penetration and migration in plant roots (Gheysen and Jones, 2006). In addition, genes involved in lignin biosynthesis were more strongly expressed in BABA-treated plants (Ji *et al*., 2015).

According to our study, the application of BABA, or ascorbic acid, showed increases in phenolics, proteins, polyphenol oxidase, and peroxidase in the leaves of tomato plants. Phenolic compounds are essential for pigmentation, growth, reproduction, resistance to pathogens, and many other functions. At the same time, phenolic compounds may be directly responsible for resistance, but the compounds that bestow resistance may have linked metabolic pathways with phenols (Gill *et al*., 1983). However, the accumulated proteins in plant parts help plants to endurance the adverse conditions during or between growing seasons and may provide nutrients to support the growth of new plants as seedlings or shoots (Shewry, 2003).

Polyphenol oxidase (PPO) is a part of the plant defense mechanism against pests and pathogens. PPO is not only activated in response to abiotic and biotic stimuli, but it also shows a differential cell-specific response to different signaling molecules, such as salicylic acid, jasmonate, and ethylene (Thipyapong and Steffens, 1997). However, peroxidase (POD) plays an important role in certain metabolic processes in plants, such as the catabolism of auxins, the formation of bridges between components of the cell wall, and the oxidation of the cinnamyl alcohols before their polymerization during lignin and suberin formation (Quiroga *et al*., 2000).

In the same context, when BABA was applied as a soil drench treatment, the number of egg masses and root-gall index of *M. incognita* in tomato plants were suppressed (Asif *et al*., 2021). Also, the tomato plant growth indices, such as plant length, fresh and dry matter, were significantly induced. Meanwhile, the concentration of total phenol was increased from 72.00 to 96.2 µg mg-1

compared with the control (60.0 µg mg-1).

The present results are also in accordance with those of Refaei (2009), who investigated the effectiveness of ascorbic acid, citric acid, folic acid, nicotinic acid, pyridoxine, and thiamine in comparison with oxamyl against *Meloidogyne incognita* infecting okra plants under greenhouse conditions. The obtained results indicated that ascorbic acid, folic acid, nicotinic acid, and thiamine reduced the root galls (ranged from 82.7 to 75.7%) and egg masses (ranged from 96.6 to 90.5%) significantly. While using ascorbic acid at 1g l-1 as a foliar spray reduced the presence of *M. incognita*-infected soybean plants (Saeed, 2005).

Our results were also confirmed by Radwan et al. (2017), who found that oxalic and ascorbic acids were effective against tomato root galls and soil population and recorded reductions estimated at 58.07 & 56.92% and 75.82 & 74.92%, respectively. Furthermore, the tomato growth characteristics (shoot length, shoot weight, root length, and root weight) were increased over control. The application of acetylsalicylic acid, ascorbic acid, and salicylic acid as soil drench three days pre-inoculation was more effective in reducing the nematode population than at or post-inoculation time (Anter *et al*., 2014).

Similar results were introduced by Osman *et al*. (2016) and Maareg *et al*. (2014), who found that application of ascorbic acid suppressed the soil population and development of *M. javanica* in the potato crop and *M. incognita* in the sugar beet crop, respectively, and improved the plant yields. Several mechanisms were suggested for the possible actions of organic acids, which may be attributed to stimulating plant growth and increasing the resistance against plant nematode infestation (Radwan *et al*., 2017).

The synthetic nematicide, fosthiazate was used to reduce the root-knot nematode (*M. incognita*) indices such as galls, soil population, and egg masses significantly by 63.81, 90.31, and 67.50% on tomato plants (Saad *et al*., 2017). Similarly, fosthiazate was found to be a very efficient nematicide on the tomato crop, with a 97.52% reduction in galls and 96.45% reduction in soil population, while cadusafos was relatively least effective, causing 77.51 and 86.63% reductions in galling and J_2 population, respectively (Radwan *et al*., 2012). However, comparative study by Khalil (2013) elucidated that fosthiazate moderately minified root galls (51.50%) and egg masses (59.27%) compared with the bio-product *Bacillus thuringiensis*, which recorded reductions of 71.60 and 77.78% with galls and egg masses, respectively.

Therefore, the findings of our study concluded that both β-aminobutyric acid (BABA) and ascorbic acid were very effective against the root-knot nematode (*Meloidogyne incognita*) and relatively enhanced the tomato growth, in addition to induce the systemic resistance. Also, these results suggested

that the application of BABA or ascorbic acid is a promising tactic could be developed to use in integrated nematodes management programs in the future.

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