



## The potential role of some amino acids with different concentrations, application types and times to manage *Meloidogyne javanica* on tomato plants

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### ABSTRACT

In present study four different amino acids i.e. DL-Valine, DL-Methonine, DL-Asparagine and DL-Arginine with five different concentrations (50, 100, 150, 200 and 400 mg/ml) and two different application methods (soil drench and foliar spray) at two different application times (three days before and three days after nematode inoculation) were tested under both laboratory and greenhouse conditions to evaluate their influences on root-knot nematode *Meloidogyne javanica* and growth criteria of treated tomato plants. The results revealed that all tested amino acids at concentration of 400 mg/ml reduced significantly both eggs hatching and juveniles' viability compared to control (nematode treated with tap water) under in vitro conditions. The highest percentage of non-hatched eggs (68%) and dead juveniles (81%) was recorded by DL-Methonine amino acid followed by DL-Asparagine which percentage of non-hatched eggs and dead juveniles recorded 60% and 80%, respectively.

**Key words:** *Meloidogyne*, root knot nematode control, green chemistry, natural control

### INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is considered one of the most popular and nutritious vegetable crop in Egypt. According to FAO stat (Food and Agriculture Organization on United Nations) in 2020 the total planted area with tomato was 428182 faddens which producing about 6751856 tons/Fadden a year of production. This crop is attacking by several serious plant pathogens such as fungi, bacteria, virus and plant parasitic nematodes causing several harmful effects and diseases consequently

severe losses in yield and economic income. Root-knot nematodes *Meloidogyne* species are one of the highly important soil-borne pathogens that cause great economic damages to horticultural and field crops and considered one of the most dangerous plant parasitic nematodes which can infect approximately all the world main crop plants (Oka *et. al.*, 2000). They have the potential to cause up to 80% losses in vegetables in tropical and subtropical counties (Hussain *et.al.*, 2011). Different methods can be utilized for controlling *Meloidogyne*

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species such as cultural practices, resistant cultivars, and chemical nematicides which are easy for application and more effective. Chemical nematicides have harmful effects for air, soil and water (Alam, 1987) and very expensive particularly for poor farmers in Egypt. Thus for these reasons, the safety alternative strategy methods are highly needed to manage root-knot infections. From the alternative methods to manage root-knot nematodes *Meloidogyne* spp. are amino acids. One of their advantages, they are used with very relative doses which allow to use small particular amount for large infestation control. So, our search aimed to use the safety and cheap alternative compounds at different concentrations, application types and times to manage root-knot nematode (*Meloidogyne javanica*) on tomato plants.

## **MATERIALS AND METHODS**

The *in vitro* and *in vivo* assays were conducted at Agric. Botany Dept., Fac. of Agric., Menofia Univ., Egypt, to evaluate the efficacy of four different amino acids i.e. DL-valine, DL-methionine, DL-asparagine and DL-arginine at different concentrations (50,100, 150, 200 and 400 mg/ml) using two different application types i.e. soil drench and foliar spray and two different applications times (three days before and after, nematodes inoculation) to manage root-knot nematode (*M. javanica*) on tomato plants (*Lycopersicum esculentum* cv. Super strain B).

Pure culture of *M. javanica* was established from single egg mass on

tomato plants under greenhouse conditions at 25°C±2. Nematode species identification was carried out according to the perennial patterns technique (Taylor and Sasser, 1978). Root-knot nematode eggs were extracted from heavily galled roots by using 1.5% sodium hypochlorite solution (NaOCl) technique as described by (Hussey and Barker, 1973).

### **I. *In vitro* experiment**

The amino acids were tested for their effectiveness at different concentrations as mentioned before against egg hatching and larvae mortality of *M. javanica* under laboratory conditions.

#### **A. Egg hatching:**

Three hundred eggs in 0.1ml of distilled water placed in 0.9ml of different concentrations of the different amino acids in a 72-well micro-titre plate to evaluate the effectiveness of the different concentrations of amino acids on egg hatching. Each individual treatment replicated 6 times. Eggs placed in distilled water served as a control. The well micro-titre plate was incubated at 25°C±2 under lab. conditions. The percentage of hatched eggs was calculated in 100 eggs after 6 days of incubation under stereomicroscope using counting dish.

#### **B. Larvae mortality:**

Three hundred freshly hatched larvae in 0.1ml distilled water were placed in 0.9 ml of the different amino acids concentrations in a 72 well micro-titre plate to evaluate its effectiveness on percentage of larvae mortality. Each

treatment replicated 6 times. Larvae in distilled water only served as a control. The well micro-titer plate was incubated under lab. conditions at  $25^{\circ}\text{C}\pm 2$  and the percentage of the dead larvae were recorded in 100 larvae after 6 days using counting dish under stereomicroscope. To confirm that the larvae are dead after the incubation periods (6 days), all treated larvae with the different concentrations of the tested amino acids were washed with distilled water and incubated for 24 hours to confirm if they were dead or anesthetic.

## II. *In vivo* experiment:

Tomato cultivar Super strain B which is susceptible to the root-knot nematode was used in greenhouse experiment. Three weeks old tomato seedlings were grown in plastic pots (15cm in diameter) filled with 2 kg unsterilized mixture of sandy-clay soil (2:1, v/v). Each pot contained one seedling. All four different amino acids were applied at a one single dose (400 mg/ml) as it was the effective dose according to *in vitro* test. Amino acids were applied at two different application types separately, (a) soil drench by receiving each plant 10 ml around root zone, (b) foliar spraying where each plant was sprayed with 10 ml of each individual amino acid on a whole foliar system of plant. Amino acids were applied in two different application times, (a) three days before nematode inoculation, (b) three days after nematode inoculation. *M. javanica* eggs were extracted as mentioned before by the technique of (Hussey and Barker, 1973). Three

thousands nematodes eggs were pipetting into 4 holes made around tomato roots. Un-treated plants served as a control. Each treatment was replicated 3 times. Plants were arranged in a completely randomized block design under greenhouse conditions at  $25^{\circ}\text{C}\pm 2$ . Plants were watered daily and fertilized weekly by receiving 5 ml of 2g/L NPK (20:20:20). Two months after nematode inoculation, nematode parameters i.e. No. of galls, egg masses/root system, No. of larvae/250g soil (Goodey, 1957), nematode final population (Pf), reproduction factor (Rf) and root galling were determined. Rate of nematode reproduction factor was recorded according to the equation:  $Rf = pf/pi$  where: pi = initial inoculation (Norton, 1978).

Root galling index was calculated according to (Taylor and Sasser, 1978) where:

- 0= no galls or egg masses
- 1= 1 to 2 galls or egg masses
- 2= 3-10 galls or egg masses
- 3= 11-30 galls or egg masses
- 4= 31-100 galls or egg masses
- 5= more than 100 galls or egg masses.

Egg masses were stained prior to counting by dipping the infected roots in Phloxin-B solution (0.015%) for 20 minutes as described by Daykin and Hussey (1985). Plant growth characters i.e. shoot and root fresh weights (g) as well as plant height (cm) were recorded. Chemical constitutions i.e. antioxidant enzyme activities of peroxidase (PO), polyphenol oxidase (PPO), catalase were also estimated in fresh leaves according to methods of

Broesh (1954), Fehrman and Dimond (1967), Bach and Oparin (1968), respectively.

### Statistical analysis

All obtained data were analyzed using analysis of variance (ANOVA) among treatments. Means were compared by least significant differences (LSD) at  $p \leq 0.05$  as described by Song and Keane (2006).

### RESULTS

Effect of five different concentrations i.e. 50, 100, 150, 200 and 400 mg/ml from each individual tested amino acid (DL-Valine, DL-Methonine, DL-Asparagine, DL-Arginine) on egg hatching of *M. javanica* nematode was evaluated under laboratory conditions. The obtained data illustrated in (Fig. 1) revealed that most of the tested amino acids significantly inhibited egg hatching compared to control (eggs treated with tap water). Moreover, results confirmed that increasing the concentration of tested amino acids resulted in increasing the inhibition of nematode eggs hatching. Thus the highest inhibition of eggs hatching was observed with concentration of 400 mg/ml of tested amino acids. Indeed, the lowest percentage (32 and 40%) of hatched eggs at concentration of 400mg/ml was recorded with DL-Methonine and DL-Asparagine, respectively (Fig. 1).

Similar results were recorded with aspect to influence of the tested amino acids at five different concentrations i.e. 50, 100, 150, 200 and 400 mg/ml on mortality of nematode juveniles (Fig. 2). The highest mortality percentage (81 and 80%) was detected at concentration of 400mg/ml with DL-Methonine and DL-Asparagine, respectively (Fig. 2). Contrary, the lowest percentage of

dead juveniles (51%) at same concentration (400mg/ml) was detected with DL-Arginine, followed by DL-Valine (57%).

The influence of four different amino acids applied with two different application methods (soil drench and foliar spray) at two different application times (three days before and three days after nematode inoculation) on physiological criteria i.e. plant height (cm), shoot and root fresh weights (g) were determined under greenhouse conditions. The obtained results showed that except of DL-Asparagine amino acid, the three other amino acids tested (DL-Valine, DL-Methonine and DL-Arginine) increased the plant height of treated plants compared to control (inoculated plants with nematode only) when amino acids were applied as soil drench 3 days before nematode inoculation (Fig.3). On the other hand, only significant deference in plant height was recorded with DL-Valine amino acid in aspect to influence on plant height parameter with soil drench application method when amino acids used 3 days after nematode inoculation (Fig.3). With foliar spray of amino acids 3 days before nematode inoculation, the results showed that only two amino acids (DL-Valine and DL-Methonine) increased plant height of treated tomato plants compared to control (inoculated with nematode only) (Fig.3). Contrary, no significant differences were recorded between tomato plants sprayed individually with the four tested amino acids 3 days after nematode inoculation and control plants (Fig.3).

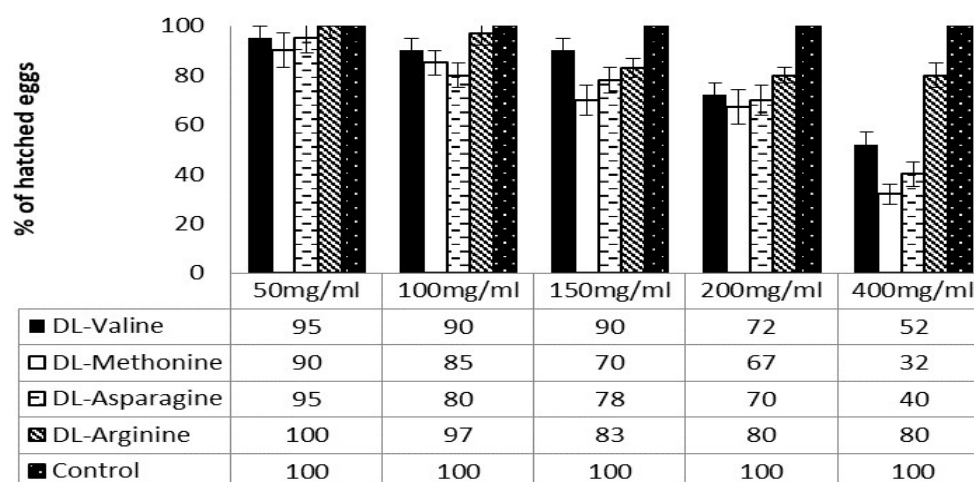


Fig. (1). Effect of different amino acids at different concentrations on percentage of eggs hatching of *Meloidogyne javanica* under *in vitro* conditions

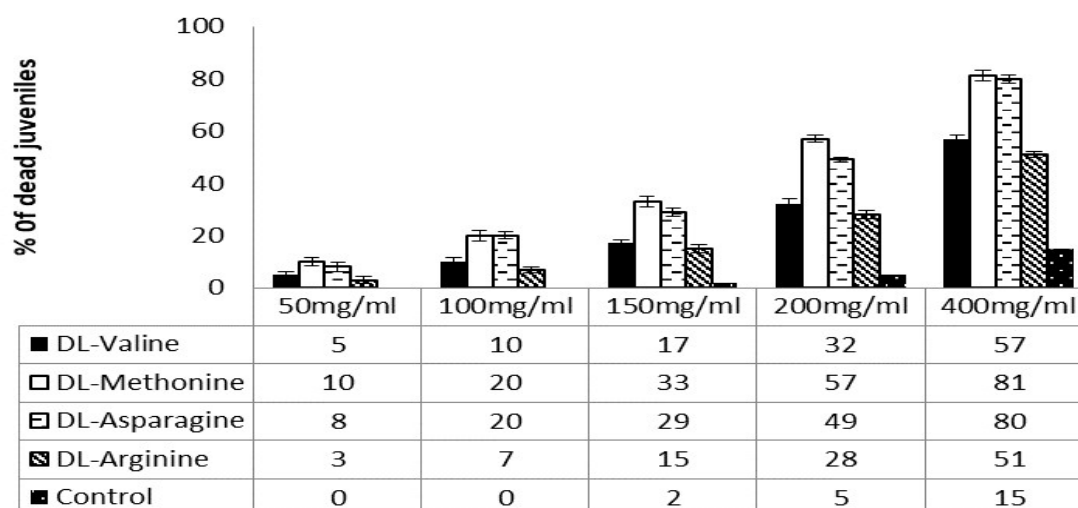


Fig. (2). Mortality percentage of *Meloidogyne javanica* juveniles treated with different concentrations of five different amino acids under *in vitro* conditions.

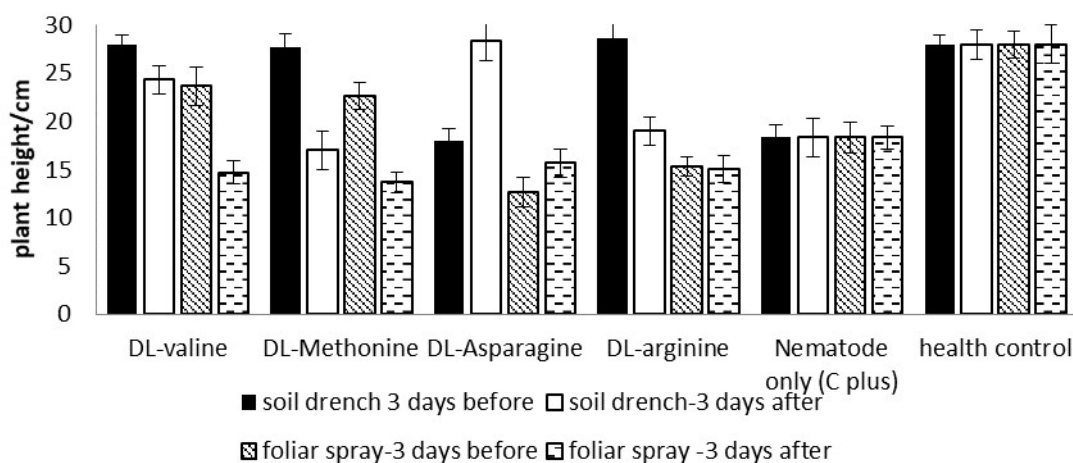


Fig. (3). Plant height recorded with treated and untreated tomato plants with valine, methonine, asparagine and arginine amino acids using soil drench and foliar spray application methods at 3 days before and after nematode inoculation times.

The data recorded with shoot weight revealed that, the all tested amino acids except of methonine affected positively the shoot weight of treated plants either with soil drench or foliar application at 3 days before and 3 days after nematode inoculation compared to the control plants (Fig. 4).

The highest shoot weight (31.2 and 28.2 g) was recorded with asparagine amino acid as soil drench 3 days before nematode inoculation and with foliar spray 3 days after nematode inoculation treatments, respectively (Fig. 4).

Similar results recorded with shoot weight, the obtained data showed that all tested amino acids expect methonine increased significantly root fresh weight of treated plants in all treatments compared to control.

The highest absolute values of root fresh weight (8.2 and 5.4 g) were recorded with asparagine and arginine amino acids with the treatment of soil drench three days before nematode application, respectively (Fig. 5).

The influences of the four tested amino acids on nematode infection were assessed under greenhouse conditions. Significant differences between amino acids (valine, methonine, arginine and asparagine) as well as between application methods (soil drench and foliar

spray) at two different application times (three days before and three days after nematode inoculation) were recorded.

The relative lowest number of galls/root system i.e. 9, 16 and 17 was recorded with arginine, valine and methonine amino acids at soil drench three days before nematode inoculation treatments, respectively (Fig. 6).

Contrary, the highest number of galls/root system (85 and 79) was recorded with methonine and asparagine amino acids in foliar spray three days after nematode inoculation treatments, respectively (Fig. 6).

Similar to the results recorded with number of galls, the lowest number of egg masses (1.3 and 4.3) was recorded with arginine amino acid in soil drench treatments three days before nematode inoculation and three days after nematode inoculation times, respectively (Fig. 7).

The obtained results illustrated that all tested amino acids reduced significantly compared to the control either when they applied as soil drench or foliar spray (fig.8). The absolute lowest number of second stage juveniles per 250g soil was recorded with DL-arginine (fig.8).

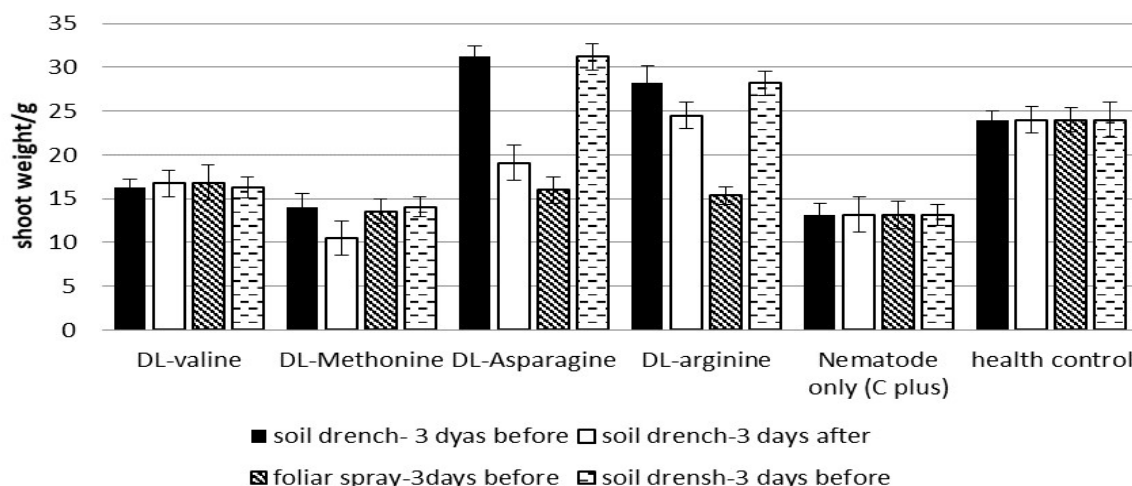


Fig. (4). Shoot fresh weight recorded with treated and untreated tomato plants with valine, methonine, asparagine and arginine amino acids using soil drench and foliar spray application methods at 3 days before and after nematode inoculation times.

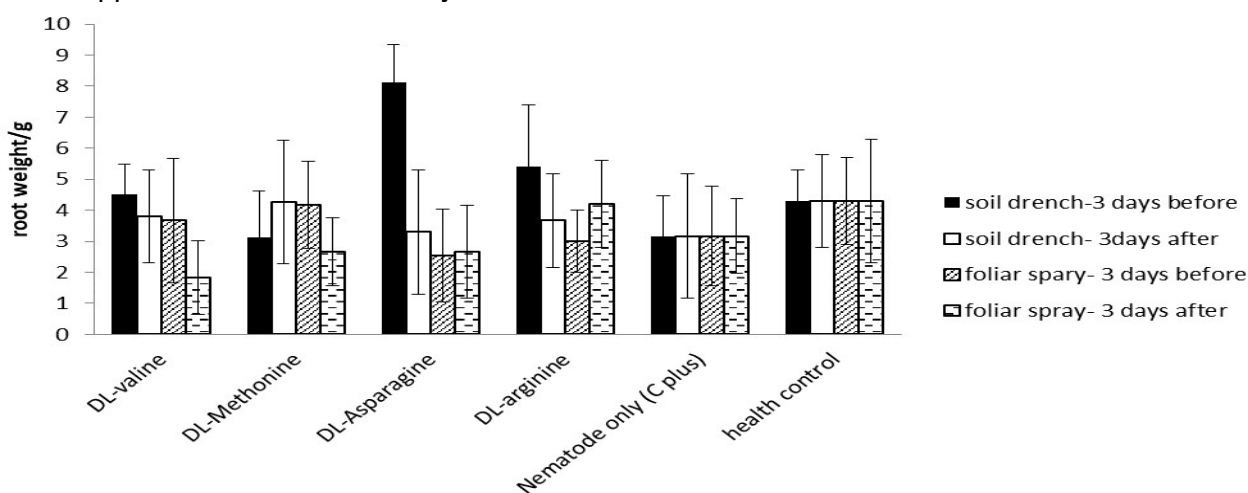


Fig. (5). Root weight recorded with treated and un-treated tomato plants with valine, methonine, asparagine and arginine amino acids using soil drench and foliar spray application methods at 3 days before and after nematode inoculation times.

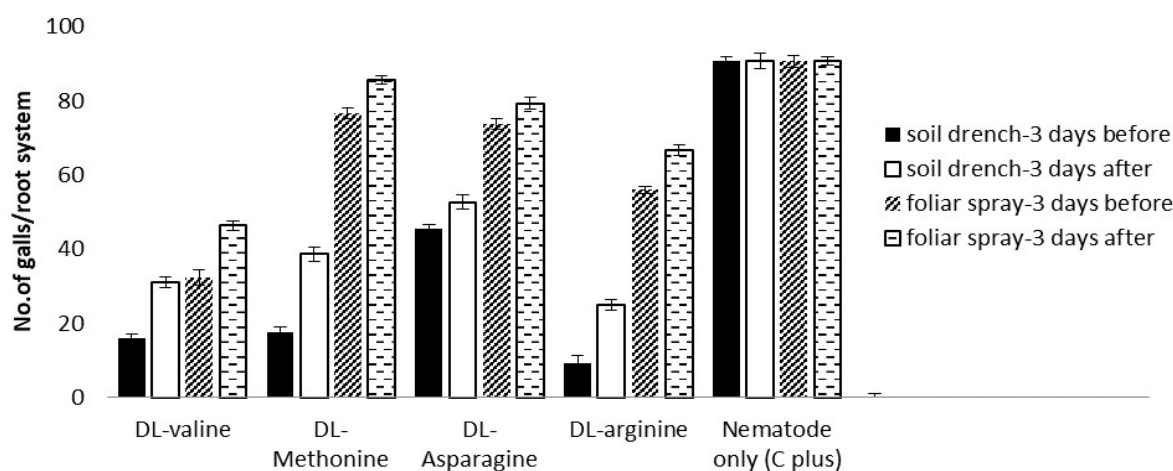


Fig. (6). Number of galls/root system recorded with treated and un-treated tomato plants with valine, methonine, asparagine and arginine amino acids using soil drench and foliar spray application methods at 3 days before and after nematode inoculation times.

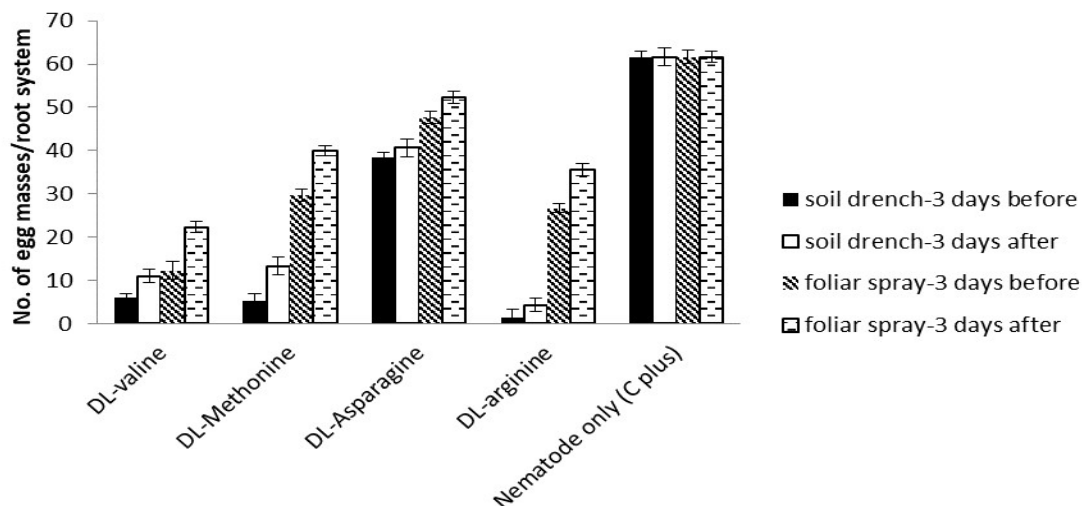


Fig. (7). Number of egg masses/root system recorded with treated and un-treated tomato plants with valine, methonine, asparagine and arginine amino acids using soil drench and foliar spray application methods at 3 days before and after nematode inoculation times.

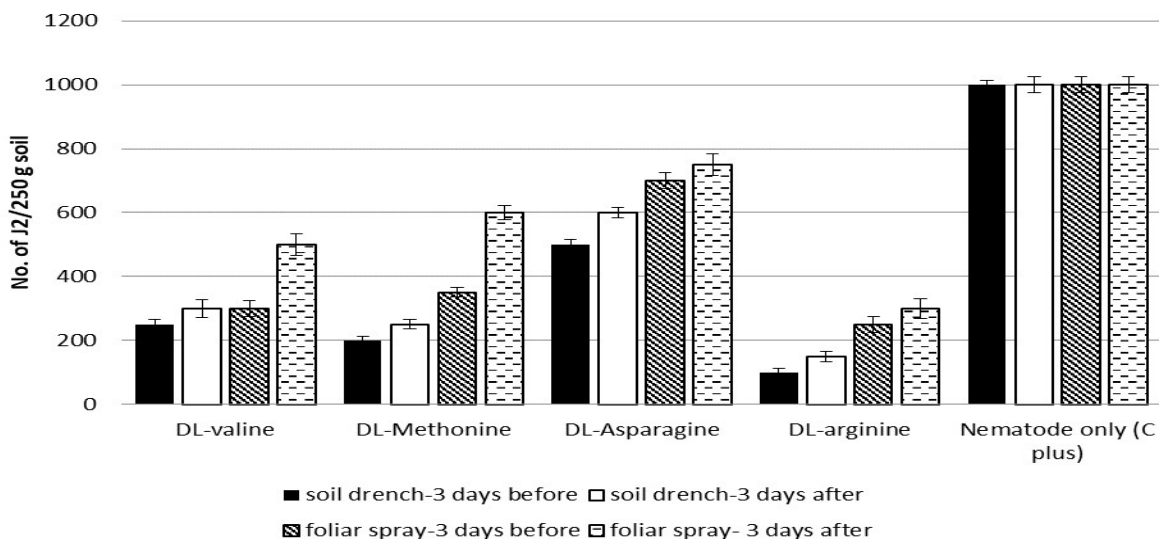


Fig. (8). Number of J<sub>2</sub>/250g soil recorded with treated and un-treated tomato plants with valine, methonine, asparagine and arginine amino acids using soil drench and foliar spray application methods at three days before and after nematode inoculation times.

The results showed that the highest peroxidase activity (0.95) was recorded with DL-Arginine when applied as a soil drench 3 days before nematode inoculation (table 1), while the lowest enzyme value was recorded with DL-Methonine as a foliar spray at 3 days post nematode inoculation treatment. Similar results were recorded also with aspect to polyphenoloxidase activity. Thus the highest enzyme activity (0.92) was

recorded with DL-Arginine and DL-Valine when applied as a soil drench at 3 days before nematode inoculation time (table1). The analysis of catalase enzyme was consistent with the other evaluated enzymes (peroxidase and ployphenoloxidase) as the arginine amino acid used as soil drench at 3 days before nematode inoculation resulted in highest catalase activity (0.38).



Table (1). Effect of the tested amino acids applied as soil drench and foliar spray at three days before and after nematode inoculation on the activity of antioxidant enzymes, peroxidase, polyphenoloxidase and catalase in tomato plants.

Treatments	Type of application	Time of application	Peroxidase (O.D. g <sup>-1</sup> fr.wt. after 2 min.)	Polyphenoloxidase (O.D. g <sup>-1</sup> fr.wt. after 45 min.)	Catalase (mg/g <sup>-1</sup> fr.wt.)
DL-Valine	Soil drench	3 days before	0.90	0.92	0.25
		3 days after	0.86	0.86	0.22
	Foliar spray	3 days before	0.80	0.83	0.20
		3 days after	26.87	0.81	0.20
DL-Methonine	Soil drench	3 days before	0.93	0.93	0.29
		3 days after	0.81	0.84	0.19
	Foliar spray	3 days before	0.59	0.60	0.18
		3 days after	0.28	0.27	0.20
DL-Asparagine	Soil drench	3 days before	0.70	0.69	0.25
		3 days after	0.54	0.71	0.23
	Foliar spray	3 days before	0.56	0.56	0.22
		3 days after	0.37	0.31	0.20
DL-Arginine	Soil drench	3 days before	0.95	0.92	0.38
		3 days after	0.89	0.90	0.30
	Foliar spray	3 days before	0.71	0.69	0.22
		3 days after	0.69	0.69	0.23
Nematode alone (C+)			0.18	0.21	0.16
Health control			0.33	0.36	0.21

Results in Fig. (9) revealed that all tested amino acids significantly reduced the nematode final population compared to plants treated with nematode alone.

The highest reduction obtained with DL-arginine amino acid when applied as a soil drench 3 days before nematode inoculation, followed by DL-methonine as a soil drench 3 days before nematode inoculation recorded. The lowest effect recorded with DL-asparagine.

Results in Fig. (10) confirmed that DL-arginine was the effective one in reducing the nematode reproduction when compared with the other amino acids. DL-valine came in the second rank

after DL-arginine, whereas DL-asparagine the least effective one.

Results revealed also that applying the amino acids generally as a soil drench 3 days before nematode inoculation achieved highest reduction in nematode reproduction, followed by soil drench 3 days after nematode inoculation. Application the amino acids as a foliar spray 3 days after nematode inoculation was the lowest one in reducing the nematode reproduction as shown in Fig. (11).

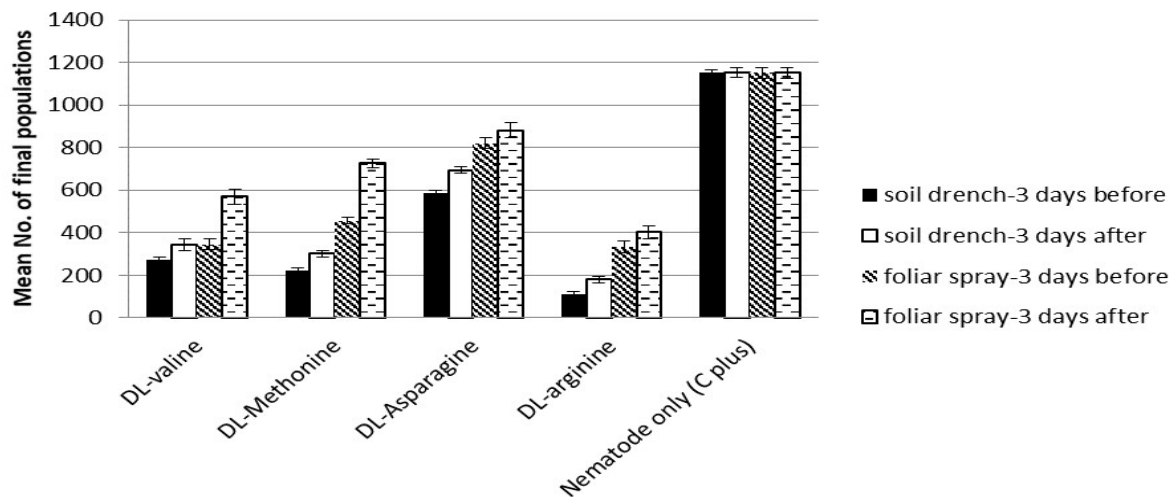


Fig. (9). Nematode final population recorded with treated and un-treated tomato plants with valine, methonine, asparagine and arginine amino acids using soil drench and foliar spray application methods at 3 days before and after nematode inoculation times.

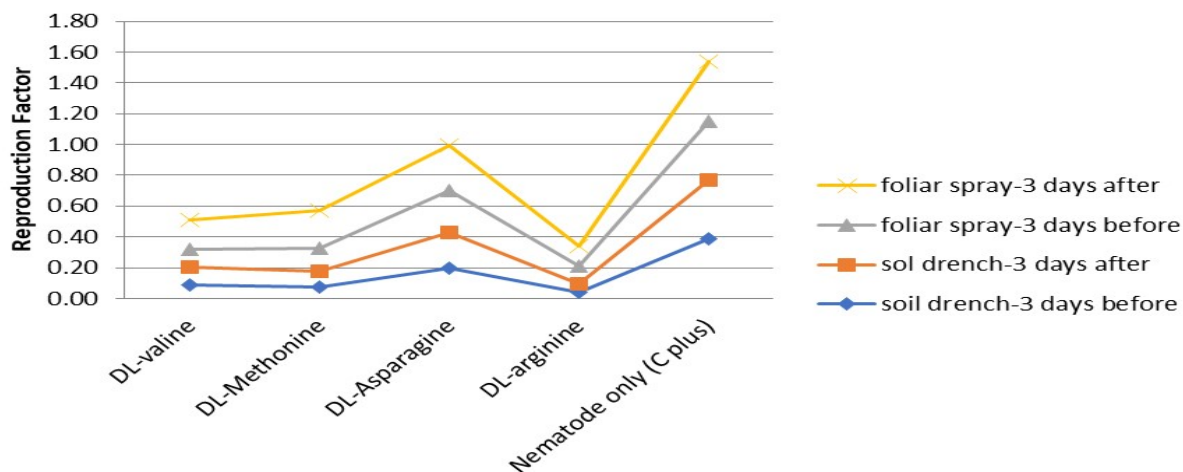


Fig. (10). Reduction factor (Rf) recorded with treated and un-treated tomato plants with valine, methonine, asparagine and arginine amino acids using soil drench and foliar spray application methods at 3 days before and after nematode inoculation.

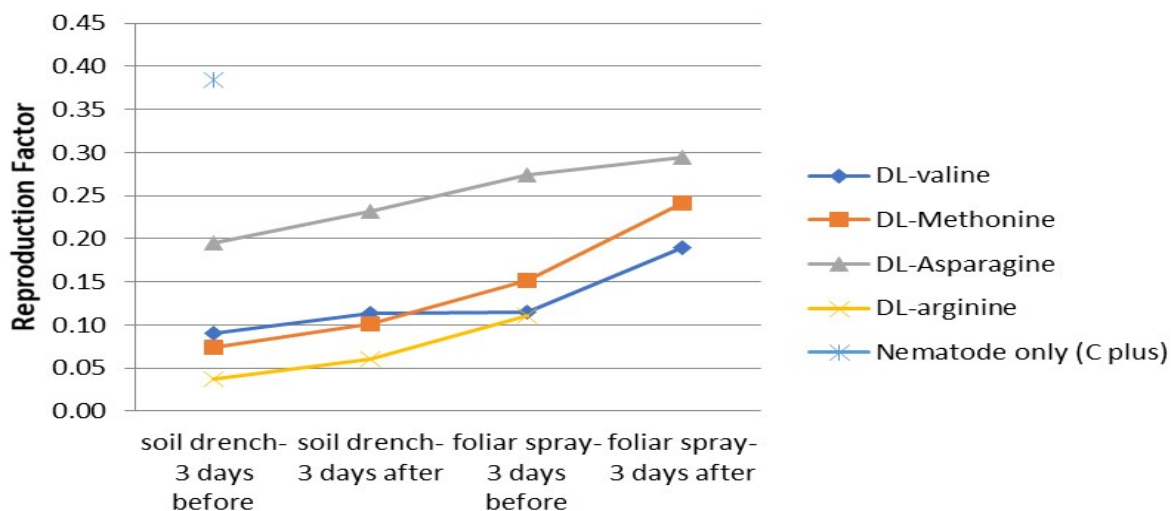


Fig. (11). Effect of different application types and times of four different amino acids on reproduction factor of *M. javanica* on tomato plants.

## DISCUSSION

Some amino acids have been found to inhibit egg hatching and juveniles survival as well as nematode galling caused of *Meloidogyne* species under both *in vitro* and *in vivo* conditions (Osman, 2013). He reported also, different amino acids and vitamins application have been resulted in reducing the hatching of *Meloidogyne incognita* eggs, number of root galls as well as mortality and fecundity of nematode females. Role of amino acids in resistance mechanisms of host plants against root-knot nematodes have been reported by many studies (Osman, 2013; Amdadul *et al.*, 2014). Furthermore, Osman (2013) reported also that amino acids may have a repellent effect toward *M. javanica* larvae. He suggested also that L-arginine and ascorbic acid have nematicidal activity and might produce promising some anti-metabolites which can play role in biological control of *M. javanica*.

Amdadul *et al.*, (2014) found that the effect of 6 amino acids viz. DL-methionine, DL-valine, DL-serine, DL-phenylalanine, DL-proline, and DL-histidine were found to give significantly lower response in relation to hatching of egg masses and survival of J<sub>2</sub> compared to the control treatment. Significant variations in hatching and survival response were also found among the treatments from lower to higher doses.

Reddy (1975) reported the inhibition of egg masses of *M. incognita* was referred to contact toxicity of DL-amino acids. With the increase of doses, the variation in hatching was found to be reduced among the treatment indicating their

lethal effect on hatching. Inhibition of egg hatching increased with increasing doses of all amino acids (Rekha *et al.*, 1993). Reddy *et al.*, (1975) reported that DL-amino acids had some contact toxicity *in vitro* to the second stage juveniles of *M. incognita*. Decrease in survival J<sub>2</sub> was thought to be due to lethal effect as many of the DL-amino acids like DL-methionine and DL-phenyl-alanine as well as L-proline and L-histidine were found to be lethal to J<sub>2</sub> as stated by Reddy *et al.*, (1974).

Osman (1993) found 100% mortality of *M. javanica* with increasing concentrations of L-arginine and L-glutamic acid from 1000 to 2000 ppm. Amdadul *et al.*, (2014) investigate six different amino acids and found that the all tested amino acids were lethal to the survival of J<sub>2</sub> juveniles of *Meloidogyne* spp. to varying degrees at their different concentrations. They added also, DL-phenyl-alanine appeared to give maximum response in plant growth characters like length of shoot and root, fresh weight of shoot and root correspondingly with the lowest galling incidence. Evans and Trudgill (1971) forward that amino acids have chemotherapeutic effects on some diseased plants and they accumulated in the giant cells of nematode-infested plants and they might be used as nematicides. They revealed also some amino acids inhibited reproduction of *Trichodorus christiei* Allen and *Helicotylenchus nannus* Steiner and decreased galling of tomato roots by *M. incognita*.

Eight amino acids tested against four species of nematodes, DL-methionine and DL-alanine decreased number of

*Heterodera avenae* (Woll) cysts on oats and the numbers of *Ditylenchus dipsaci* (Kuehn) on oats, while DL-butyric acid decreased the number of *Aphelenchoids ritzemabosi* (Schwartz) on lucerne (Evans and Trudgill, 1971). Kim and Whang (2012) investigated the lethal effects of J<sub>2</sub> juveniles for 19 types of commercial amino acids. Five kinds of amino acids (L-asparagine, L-aspartic, L-methionine, L-tyrosine, L-cystein) showed mortality rate of more than 50% at a concentration of 50 mM, while L-asparagine showed the highest mortality rate at 94%.

Kesba (2003) indicated the accumulating of protein in roots as a result of grape roots chemical analysis that accumulation of proteins in roots may reach toxic limits due to amino acids spray application and may have a direct toxic action as a result of exuberance of such chemicals in giant cells. Amdadul *et al.*, (2014) revealed that with the increase of the doses, the variation in hatching was found to be reduced among the treatments indicating their lethal effect on egg hatching.

Masood and Husain (1975) observed the best plant growth with phenyl-alanine applied as soil drench to eggplant inoculated with *M. incognita*. They also observed higher plant growth with proline applied as soil drench. Amdadul *et al.*, (2014) found that with respect to development of *M. javanica* in six amino acids treated tomato plants, other than egg masses all the amino acids gave identical response with a reduced number of adult females, J<sub>2</sub> and J<sub>4</sub> juvenile stages compared to control.

Prasad and Webster (1967) revealed that several amino acids were used as anti-metabolites for nematodes control. They found that also application of DL-amino butyric to soil effectively reduced the number of galls induced by *Nacobbus serendipiticus* on tomato roots without the host plant even through a much higher concentration of the amino acid (150mg/10ml) was applied to the soil 2 weeks after inoculation with the nematode. Tsai and Van Gundy (1988) found that a soil drench with DL-methionine, which is not nematicide, reduced the size of galls caused by *M. incognita*. They revealed also that DL-methionine was believed to interact with root exudates, subsequently immobilizing the nematode.

Prasad and Webster (1967) revealed that the exact mechanism of nematodes control by amino acids has not clarified, but D-amino acids are believed to concentrate near root tips, the initial nematode feeding site thereby blocking essential metabolic pathways in either the plant or nematode and interfering with nutrition. Several amino acids such as DL-methionine, DL-amino butyric acid, DL- and L- proline have been used in soil drenches as anti-metabolites for nematode and have reduced nematode damage as reported by (Epstein, 1972; Prasad and Webster, 1967; Tasi and Van Gundy, 1988).

Applications of amino acids as foliar sprays inhibited reproduction, galling by *M. incognita* and *M. javanica* and retarded nematode development maturity, egg mass formation, egg productions and improved plant growth as reported by (Al-Sayed, 1992 and Kesba 1999 and 2003).

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