



EVALUATION THE EFFICACY OF SOME AMINO ACIDS IN NORMAL AND NANO FORMS IN DUCING RESISTANCE IN TOMATO PLANTS AGAINST ROOT-KNOT NEMATODE *MELOIDOGYNE SPP.*

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ABSTRACT

This study was conducted to evaluate the role of certain plant elicitors of normal and Nano amino acids in inducing systemic defenses against root-knot nematodes in tomato plants in greenhouse conditions. All treatments showed high efficacy, and the inhibitory effect of the tested acids on nematode root-knot Reproduction was positively correlated with increasing concentration. Additionally, plant growth was enhanced by all the treatments in general. Arginine and Serine at a concentration 20 and 30 ppm respectively recorded the highest stimulation peroxidase enzyme activity after 9 days of treatment with significant differences in quantity (21.28, 21.56 min / gm fresh) compared to the control treatment that It recorded (19.14 min/g fresh), while the highest stimulation of Phenyl alanine ammonia-lyase enzyme activity was recorded for the same two acids at the same higher concentrations (16.78, 18.31 mg cinnamic per hour) compared to the comparison treatment, which was (11.02 min/fresh weight). The higher concentration of the two acids above was the evidence of complexation. the severity of the infection was (1.41, 1.08) and the severity of the infection were (33.33, 27.08)%. Compared to the control treatment, which was 3.41, as evidenced by the complexity and severity of infection was 85.41%, while all the tested treatments recorded a significant superiority in the wet and dry weight of the shoot and the amount of chlorophyll concentration, compared to comparison treatment. Which recorded the highest percentage of the wet root system, which was to 23.84 g/ wet. All tested amino acids had a significant effect on the development and alleviation of the disease.

Keywords: Tomato, Root-knot nematodes, Phenyl alanine ammonia-lyase and peroxidase enzymes, Amino acids.

* The article is taken from a master's thesis by the first researcher.



تقييم فعالية بعض الاحماض الامينية العادية والنانوية في استحثاث المقاومة الجهازية في نباتات الطماطة ضد نيماتودا تعقد الجذر *Meloidogyne spp*

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الخلاصة

اجريت هذه الدراسة لتقويم دور بعض المحفزات النباتية من الاحماض الامينية العادية والنانوية في استحثاث الدفاعات الجهازية ضد نيماتودا تعقد الجذور في نباتات الطماطة في البيت البلاستيكي، اذ اظهرت جميع المعاملات كفاءة عالية وارتبط التأثير التثبيطي للاحماض المختبرة على تكاثر نيماتودا تعقد الجذور بشكل ايجابي مع زيادة التركيز المستخدم، كما تم تعزيز نمو النبات من خلال جميع المعاملات المستخدمة، بشكل عام سجل الحامضين الارجنين والسيرين بتركيز 20 و30 جزء بالمليون على التوالي اعلى تحفيز لنشاط انزيم البيروكسيداز بعد 9 يوم من المعاملة وبفروق معنوية وبكمية (21.56, 21.28 دقيقة/غم طري) قياسا بمعاملة المقارنة التي سجلت 19.14 دقيقة /غم طري، في حين سجل اعلى تحفيز لنشاط انزيم الفينيل الانين امينوليز لنفس الحامضين بنفس التراكيز اعلاها (18.31, 16.78 ملغم سيناميك /ساعة) قياسا بمعاملة المقارنة التي بلغت 11.02 دقيقة/ وزن طري، سجل التركيز العالي من الحامضين اعلاهما اقل دليل تعقد وشدة اصابة بلغ (1.08, 1.41) وشدة اصابة بلغت (27.08, 33.33) % قياسا بمعاملة المقارنة التي بلغت 3.41 دليل تعقد وشدة اصابة 85.41 % . سجلت المعاملات المختبرة جميعها تفوق معنوي في زيادة الوزن الرطب والجاف للمجموع الخضري وتركيز الكلوروفيل قياسا بمعاملة المقارنة التي سجلت اعلى نسبة للمجموع الجذري الرطب والذي بلغ 23.84 غم /وزن رطب. اعطت جميع الاحماض الامينية المختبرة تأثيرا كبيرا في تطور وتخفيف حدة المرض.

الكلمات المفتاحية : الطماطة، نيماتودا تعقد الجذور، انزيم الفينيل الانين امينو ليز والبيروكسيداز، الاحماض الامينية .

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is considered the second most important vegetable crop worldwide, belonging to the *Solaneaceae* family. Tomato cultivation has expanded in Iraq the year 2020, with a production estimate of 754,000 tons, an increase of 21% compared to the previous season, which was estimated at 615000 tons (Central Statistical Organization, 2020).

Nematodes are characterized by their global spread, wide host range, and high reproductive efficiency, making them difficult to control. Their damage includes root knots, reducing the root's efficiency in nutrients absorption, accompanied by stunted growth, yellowing, and wilting of the shoot parts, resulting in a decrease in yield and quality (Moens et al., 2009; Sikora et al., 2005).

The severity of this pathogen increases when it overlaps with other pathogens such as fungi and bacteria, causing significant losses compared to single infections and contributing to the loss of crop resistance to other pathogens (Elbadri et al., 2009).

Various methods have been used to control this pathogen, including chemical methods, which have shown high effectiveness in its control. However, their high cost, environmental impact, and long persistence have limited their use due to their adverse effects on non-target organisms (Ghini & Kimati, 2000). Among the alternative methods used to manage this pest is the principle of induction of systemic acquired resistance, which has attracted the attention of many researchers (Walters, 2009). Studies have indicated that the application of amino acids affects RKN and reduces the development of its females in the host (Blümel et al., 2018).



In recent years, nanoparticles, or what is known as nanotechnology, have revolutionized as one of the distinct means and methods in managing nematodes and their efficiency in control, as these materials, which are less than 100 nanometers in size, are considered the most protective in reducing pollution, but what is taken from them is their high cost (El-Sheriff, *et al.*, 2019; Chaudhary, *et al.*, 2021).

The Nano particles in some prepared compounds act as antioxidants, and some of them have plant-stimulating activity and possess free radicals compared to extracts from compounds (Al-Jubouri *et al.*, 2022). In agriculture, studies have confirmed the impact of Nanoparticles on improving plant productivity and resistance to disease-causing agents in various approaches, such as direct spraying on plants, soil application, and treating stored fruits in therapeutic and preventive conditions (Abdul-Karim & Hussein, 2022).

Sadiq & Mohammed (2022) mentioned in a study of the use of the boron element in its nano and conventional forms at a concentration of 10 mg L⁻¹ and 15 mg L⁻¹ in bean plants, as it led to a significant increase in growth parameters and an increase in the number of branches, seed yield, and number of flowers.

Also, in a study conducted to find out the effect spraying different combinations of organic emulsifier (Appetizer) and NPK nano fertilizer with urea fertilizer on the growth and yield of yellow corn varieties, there are significant differences between the different fertilization treatments, if the nano fertilization treatment achieved the highest average in most the growth indicators in addition to The highest mean in the number of days cultivation, the highest plant height, the number of leaves, and the chlorophyll content (AL-Mafrajee & EL-Rubae, 2022).

Due to the limited research on safe alternatives for managing RKN disease in vegetable crops in the Arab world in general and Iraq in particular, this study aimed to "Evaluate the efficacy of certain amino acids in normal and Nano forms in inducing systemic resistance in tomato plants against the nematode-induced root-knot disease caused by *Meloidogyne* spp. by stimulating the production of peroxidase and phenylalanine ammonia-lyase enzymes". These enzymes play a key role in plant resistance to pathogenic agents, including RKNs under greenhouse conditions.

MATERIALS AND METHODS

1. Preparation of amino acids (Arginine, Serine, and Leucine): A standard stock solution of each amino acid was prepared by dissolving 0.015 mg of the amino acid in 500 ml flask containing distilled water. The solution was continuously stirred until complete dissolution to obtain a concentration of 30 ppm as a standard solution. The desired concentrations were prepared from this solution using the dilution law of Dalton, " $M_1V_1 = M_2V_2$ "

2. Nano Preparation of amino acids (Arginine, Serine, and Leucine): Concentrations of the laboratory amino acids were prepared Nanoscale at ppm levels (10,20,30ppm) using an ultrasonic device the homogenizer company (OMNISONICRUPTOR400), in the Mycotoxicology Laboratory, Plant Protection Department, College of Agricultural Engineering Sciences, University of Baghdad. The transformed Nanoparticles were confirmed by device (AFM) AL-Kora Company (Angstrom AA 2000), at "the College of Science, University of Al-Nahrain, Department of Chemistry", as shown in Figure 1, with a



diameter of 10.633 Nanometers. The effective concentration of normal and nano-acids was chosen based on the laboratory experience of eggs and juveniles.

3. **Preparation of root-knot nematode inoculum:** The samples were collected from the roots of eggplant exhibiting yellowing, stunting, and the appearance of knots on the root system. The plants were collected from vegetable farms in “the Yusufiya area, south of Baghdad”. The nematode inoculum nematodes eggs and second-stage juveniles (J2) according to the method described by **Hussey & Barker (1973)**. The roots were washed with water to remove any soil particles, then cut into small pieces measuring 2-3 cm in length. Fifty grams of the roots were placed in a 1-liter container and 200 ml of 1% sodium hypo chloride (NaOCl) solution was added. The solution was stirred for 3-4 minutes, and then the contents were passed through sieves with sizes of 212mm and 25mm for isolation process. The contents of the second sieve were washed with water to remove any remaining NaOCl. To obtain second-stage juveniles, the eggs were incubated in glass plates containing distilled water at room temperature for five days. Afterward, the juveniles were collected and counted using a Peters' counting slide.

Field experiment:

A. Evaluation of adding treatments on the activity of **POD and *PAL enzymes, calculation of the knot index, infection intensity, and growth parameters in the plastic greenhouse.** In this experiment, polyethylene bags with capacity of 2-3 kg were filled with soil taken from the plastic greenhouse after being well plowed and prepared. Tomato seedlings with 3-4 true leaves were planted in the bags and left for 4 days under plastic house conditions. Then, the seedlings were treated with Root-knot Nematode inoculum, which was prepared previously, and added to the soil through three longitudinal holes, 2 cm away from the plant stem and 3 cm deep (**Yass, 2015**).

The inoculum was added at a rate of 2500 ± 50 eggs/juveniles/plant. After three days, the treatments of normal and Nano-amino respectively were applied to the seedlings at the tested concentrations, at a rate of 15 ml per seedling, in addition to the control treatment, which contained inoculum and distilled water at a rate of 5 ml without additives. The experiment was conducted according to a completely randomized block design (CRBD) with four replicates per treatment and one plant per pot.

After two months of treatment, the knot index was calculated according to the **Dube & Smart (1987)**, which consists of five levels (0 = no root knot, 1 = knot occupies 1-25% of the affected root volume, 2 = knot occupies 26-50% of the root area, 3 = knot occupies 51-75% of the root volume, 4 = knot occupies 76-100% of the affected root volume). The percentage of infection severity was calculated using the **McKinney (1923)** equation:

$$\text{Infection severity (\%)} = (\text{Sum of (number of infected plants * infection level)}) / (\text{Total number of plants * highest level}) * 100\%$$

The fresh and dry weights of the shoot and root were determined after washing the roots to remove soil particles and excess water and allowing them to air dry on filter paper. The shoot and root weights were measured separately, and for dry weight determination: the samples were placed in an oven at 70°C for 72 hours or until a constant weight was reached



(Shahin & Ramadan, 2001). Chlorophyll content was measured after 45 days using a *SPAD device "Soil Plant Analysis Development".

**POD stands for Peroxidase, which is an enzyme found in plants, animals, and microorganisms. It plays a crucial role in various biological processes, including defense against pathogens, lignin biosynthesis, and oxidative stress responses. Peroxidase catalyzes the oxidation of various substrates using hydrogen peroxide as a co-substrate, producing reactive oxygen species (ROS) in the process. It is involved in the detoxification of ROS and the formation of cross-linking compounds, contributing to cell wall reinforcement and plant defense mechanisms.

*** PAL stands for Phenylalanine Ammonia-Lyase, which is another enzyme found in plants, specifically in the phenylpropanoid pathway. PAL catalyzes the deamination of phenylalanine, an amino acid, to produce trans-cinnamic acid and ammonia. This reaction is the initial step in the biosynthesis of various secondary metabolites, including flavonoids, lignin, and other phenolic compounds. PAL is involved in plant growth, development, and defense responses to biotic and abiotic stresses. It plays a key role in the production of phytoalexins, compounds that help plants combat pathogens and environmental challenges.

*The SPAD device, also known as a SPAD meter, is a portable instrument used for measuring the relative chlorophyll content in plant leaves. SPAD stands for "Soil Plant Analysis Development." The device utilizes a non-destructive method to estimate chlorophyll levels, which is an indicator of plant health and photosynthetic activity. It works by measuring the absorption of light at specific wavelengths by chlorophyll in the leaf. The SPAD meter provides a numerical reading that represents the chlorophyll content, with higher values indicating higher chlorophyll levels and typically healthier plants. It is commonly used in agriculture, horticulture, and plant research to assess plant nutrient status, monitor crop health, and guide fertilization practices.

B. Effect of adding treatments on the activity of (PAL) and (POD).

1. Estimation of phenylalanine ammonia-lyase (PAL) enzyme activity was measured by its conversion rate to cinnamic acid at 290 Nanometers (Dickerson *et al.*, 1984).

One gram of plant material was ground with 5 ml of sodium phosphate solution (0.1 M, pH 7) containing 0.1 grams of Polyvinylpyrrolidone (PVP). The extract was filtered through two layers of tulle, and the filtrate was centrifuged at 20,000 rpm for 20 minutes. The supernatant (surface layer), which served as the enzyme source, was mixed with 0.4 ml of sodium borate solution (0.1 M, pH 8.8) and 0.5 ml of 12 M phenylalanine for 30 minutes at 30°C.

Then, 0.4 ml of the enzyme solution was mixed with 1 ml of the borate solution to blank the spectrophotometer. The enzyme activity was measured based on the amount of cinnamic acid formed (g-1. nM.min-1 of fresh tissue) using an extinction coefficient of 9.630 M.cm-1.

2. Estimation of peroxidase (Pod) enzyme activity was performed by grinding 1 gram of stimulated plant material with 2 ml of sodium phosphate buffer (0.01 M, pH 6.5) at 4°C.

The mixture was filtered through four layers of tulle, and the filtrate was centrifuged at 6000 rpm for 20 minutes at 4°C using a centrifuge. The supernatant was used as the enzyme source. Peroxidase activity was determined according to the method of Hammerschmidt *et al.* (1982)



using 100 μ l of the enzyme extract with 1.5 ml of 0.05 M pyroknolol in a spectrophotometer tube.

To initiate the reaction, 100 μ l of 1% hydrogen peroxide was added, and the absorbance was recorded at 420 nm using a spectrophotometer every 30 seconds for 300 seconds. The change in absorbance was calculated using the following equation: Change in absorbance = $\Delta A / \Delta T$ / gram fresh weight (ΔA = change in absorbance, ΔT = change in time in minutes).

Statistical Analysis

The average of the treatments was compared by using the east significant difference (LSD) test at a significance level of 0.05, and the Genestat program was used for statistical analysis.

RESULTS AND DISCUSSION

1. qualitative analysis for the normal amino acids arginine, serine, and leucine, (those had been conducted at the College of Science/University of Al-Nahrain/Department of Chemistry);

It's been indicated that the diameter of the Nano- particles reached a size of 10.633 nanometers. It was confirmed that the particles reached the Nano scale through atomic force microscopy (AFM) analysis, which provides atomic-resolution images to measure surface topography.

The analysis results are in line with the European Union's definition of engineered Nanomaterials, which states that engineered Nanomaterials are intentionally produced materials with one or more dimensions measuring 100 nanometers or less. There is a more specific definition of Nanomaterial, which is "any natural, manufactured, or related material that contains particles in an unspecified, aggregated, or agglomerated state, where 50% or more of its particles have one or more external dimensions in the range of 1-100 Nanometers" (Peters *et al.*, 2014).

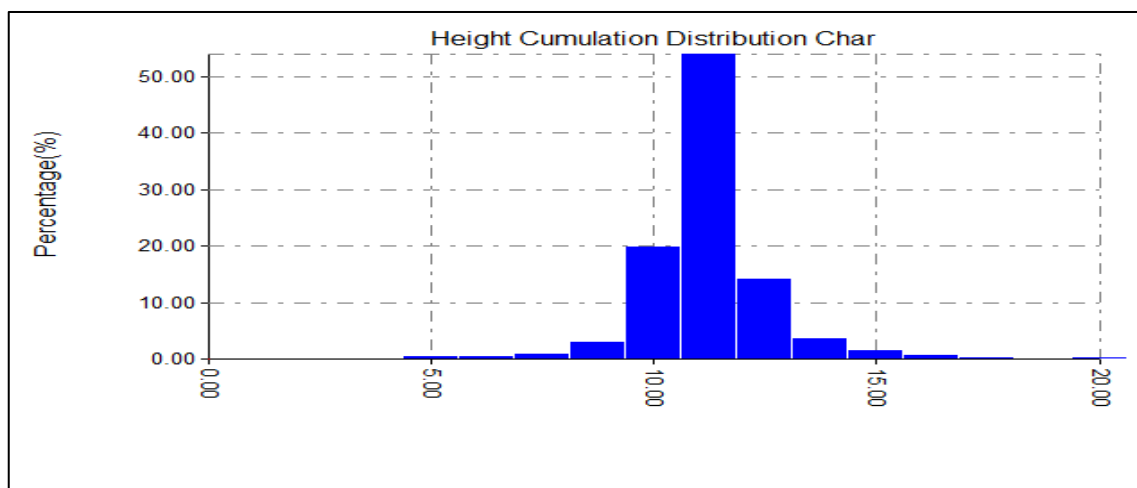


Figure (1): The particles reach the nano.

2. Evaluation of adding treatments on the activity of POD and PAL enzymes; The results in Tables (1,2) showed that all treatments significantly exceeded the control treatment in



inducing systemic resistance of the plant. This was achieved through the activation of certain enzyme pathways and the accumulation of phenolic compounds. The results demonstrated the efficacy of both normal and Nano treatments in elevating the levels of PAL and POD enzymes after 3 and 9 days of treatment following vaccination that showed that;

The highest activity PAL enzyme was recorded in the arginine and serine normal treatments at concentrations of 20 and 30ppm respectively (10.21, 16.78 and 15.59, 18.31) respectively, compared to the control treatment, which recorded (8.00 and 11.02). Similarly, the highest activity of the POD enzyme was observed in the same two acids at the same concentrations (15.59, 21.28 and 16.10, 21.56) respectively, compared to the control treatment, which recorded (6.74, 19.14), after 3 and 9 days of treatment. This confirms that the induction increases with higher concentrations of the treatments, which affects the development and vitality of root-knot nematodes.

Basha *et al.* (2006) mentioned about the increase in PAL enzyme concentration being associated with the increase in phenolic compounds such as tannic, knotic, caffeic, chlorogenic, and cinnamic acids. **Ahmed (2016)** also confirmed an increase in PAL enzyme activity when treating pepper plants with phenolic compounds, including tannic acid.

Table (1) the effect of treating tomato plants with normal and Nano-sized amino acids, specifically arginine, serine, and leucine, on the estimation of the enzyme (PAL) in the plastic green house.

Treatments	Concentration ppm	PAL enzyme activity mg cinnamic acid/ h/g fresh weight		
		3d	9d	15d
Arg Nano T1	10	10.44	14.23	5.96
Ser Nano T2	10	10.34	12.89	6.05
Lue Nano T3	10	10.43	12.73	6.18
Arg normal T4	20	10.21	16.78	8.45
Ser normal T5	30	15.59	18.31	8.75
Lue normal T6	10	9.92	11.28	5.51
Control infect T7		8.00	11.02	4.18
Control helth T8		6.38	9.15	3.86
Treatments LSD 0.05		2.0631*	2.9418*	2.6322*

Each number in the table represents the average of four replicates, with each replicate consisting of two plants inoculated with 50 ± 2500 eggs and second-stage juveniles (J2) of the root-knot nematode.



Table (2): the effect of treating tomato plants with normal and Nano-sized arginine, serine, and leucine amino acids on the estimation of enzyme (POD) in the Plastic house.

Treatments	Concentration ppm	peroxidase enzyme activity Absorbance change / min / g fresh weight		
		3d	9d	15d
Arg Nano T1	10	15.62	21.26	6.74
Ser Nano T2	10	13.67	21.15	8.68
Lue Nano T3	10	14.75	21.11	6.49
Arg normal T4	20	15.59	21.28	10.06
Ser normal T5	30	16.10	21.56	8.45
Lue normal T6	10	13.41	20.94	10.51
Control infect T7		6.74	19.14	8.79
Control helth T8		4.76	6.67	5.21
Treatments LSD 0.05		1.0075*	1.0039*	2.12*

Each number in the table represents the average of four replicates, with each replicate consisting of two plants inoculated with root-knot nematodes (50 ± 2500) eggs and second-stage juveniles (J2).

3. The effect of adding treatments on the indicators of root-knot nematode infestation and severity was studied in tomato plants in the plastic greenhouse:

The results in Table (3) indicated a significant reduction in the root-knot index and infection severity in tomato plants treated with normal and Nano-amino acids compared to the control treatment. The root-knot index values were significantly reduced to 1.08% and 1.41% for the normal serine and normal arginine treatments at concentrations of 30 and 20ppm, respectively, compared to 3.41% in the control treatment, which only contained inoculum and distilled water.

The decrease in the root-knot index was accompanied by a corresponding decrease in the infection severity. The infection severity values for the same two treatments at concentrations of 30 and 20ppm were 27.08% and 33.33%, respectively, compared to 85.41% in the control treatment. There were no significant differences between other treatments, including Nano-arginine, serine, and leucine at a concentration of 10 parts per million, compared to the control treatment that recorded the highest index and severity of infection.

Osman (1993) found a high mortality rate in *Meloidogyne javanica* with increasing concentrations of arginine and glutamic acid from 1000 to 2000 parts per million. **Mohammed & Yass (2020)** also pointed out the efficacy of folic acid (FA) and magnesium oxide against *Meloidogyne spp.* root-knot nematodes, showing that all treatments were effective in reducing the root-knot index and infection severity at concentrations of 1000, 2000, and 3000 parts per million compared to the control treatment.

The reduction in the root-knot index and infection severity may be attributed to the toxic and direct effects of these factors on the eggs and juveniles of root-knot nematodes. This decrease was accompanied by a reduction in the number of juveniles penetrating the roots, leading to a decrease in the number of root knots and subsequently reducing the severity of infection.

It is also possible that these factors stimulated plant's self-defense mechanisms (inducing systemic resistance) and the production of compounds that are toxic or antagonistic to root-knot nematodes, including phytoalexins, resulting in a decrease in the number of



juveniles penetrating the roots and, consequently, reducing the root-knot index and infection severity. These results partially agree with the findings of researchers who have concluded that amino acids are lethal to the survival of J2 juveniles of root-knot nematodes, *Meloidogyne spp.*, at varying concentrations.

Table (3): Effect of treating tomato plants with normal and Nano-amino acids (arginine, serine, leucine) on Root-knot index and infection severity in pots “field experiments.

Treatments	Concentration ppm	Knots Index	Severity of infection %
Arg Nano T1	10	2.25	56.25
Ser Nano T2	10	2.33	58.33
Lue Nano T3	10	2.25	56.25
Arg normal T4	20	1.41	33.33
Ser normal T5	30	1.08	27.08
Lue normal T6	10	2.33	58.33
Control infect T7		3.41	85.41
Control helth T8		0.00	0
Treatments LSD 0.05		0.3151*	

Each number in the table represents the average of three replicates, with each replicate consisting of four tomato plants inoculated with root-knot nematodes (50 ± 2500) eggs and second-stage juveniles (J2). The infection was performed when the plants had 4-5 true leaves.

4. The effect of adding treatments on calculating some growth parameters under greenhouse conditions was assessed.

The treatment of tomato plants inoculated with *Meloidogyne spp.* eggs and second-stage juveniles (J2) nematodes, using normal and Nano-sized amino acids through addition method at a concentration of 15 ml/plant, showed a significant increase in growth parameters (chlorophyll content, fresh and dry weight of shoot and root) compared to the control group, except for the fresh weight of the total root, which was higher in only nematode-infected plants (Table 4).

The chlorophyll content reached 48.25 in the plants treated with inoculation, compared to other treatments, which recorded higher percentages for the amino acids arginine and serine at concentrations of 20 and 30ppm (86.48, 85.05). The fresh weight of the shoot and root biomass was 42.55 and 23.84 grams, respectively, in the inoculated treatment plants, significantly different from the control. The dry weight of the shoot and root biomass was 6.58 and 2.97 grams, respectively, in the infected plants compared to the treatment plants.

The increase in fresh weight of the total root in plants infected with root-knot nematodes may be due to the large number of root knots resulting from the penetration of second-stage juveniles of root-knot nematodes. Treating the infected plants with amino acids led to a significant increase in all studied growth parameters, except for the fresh weight of the total root, which was higher in the untreated infected plants compared to the infected and treated plants.

It is worth noting that the amino acid arginine is nitrogen-rich and helps in the activity of Rhizobacteria, which have the ability to fix atmospheric nitrogen. It also helps in the solubilization of phosphate compounds and the production of iron and indole-3-acetic acid (IAA), as well as reducing the reaction degree and releasing nutrients, including potassium.



Additionally, it secretes many growth regulators and organic acids, which have the ability to attract elements and increase their concentration in the soil, which positively affects plant growth. These bacteria also enhance plant growth by facilitating biotic and abiotic stress tolerance (Deka *et al.*, 2015; Dixit *et al.*, 2017; Guerrieri *et al.*, 2020; Basu *et al.*, 2021). The results of this study agree with a study conducted to evaluate the efficiency of some organic catalysts, humic acid, where the results showed that it has a significant lethal effect on adolescent second-stage juveniles (J2). It was associated with an increase in the concentrations used compared to the control treatment, which recorded the highest number of root nodes. which recorded an increase in wet root weight (Yass *et al.*, 2020).

Table (4) the effect of treating tomato plants with normal and Nano-sized amino acids (arginine, serine, leucine) on assessing growth parameters in the plastic greenhouse.

Treatments	Concentration ppm	Chlorophyll contents	Shoot Biomass fresh/g	Shoot Biomass Dry/g	Root Biomass fresh/g	Root Biomass Dry/g
Arg nano T1	10	74.50	54.74	12.14	18.48	3.40
Ser nano T2	10	74.14	52.47	10.71	16.48	4.13
Lue nano T3	10	76.94	49.89	10.67	17.68	4.24
Arg normal T4	20	86.48	52.27	10.52	18.98	4.32
Ser normal T5	30	85.05	52.28	10.00	15.27	3.41
Lue normal T6	10	58.52	50.53	11.26	19.34	4.47
Contr infect T7		48.25	42.55	6.58	23.84	2.97
Contr helth T8		55.84	48.96	9.58	14.75	1.97
Treat LSD 0.05		11.844*	9.0884*	1.4003*	4.3602*	1.2505*

Each number in the table represents the average of three replicates, with each replicate consisting of four tomato plants inoculated with root-knot nematodes (*Meloidogyne* spp.) containing 50 ± 2500 eggs and second-stage juveniles (J2).

CONCLUSIONS

- It is possible that the use of amino acids in normal or nano form can affect female fertility and adult development of root-knot nematode RKN that infects vegetable crops.
- Nanotechnology and the use of minerals, metals, plant extracts and many others are promising and environmentally friendly methods, but it is too early to use them extensively without knowing their residues, which depend on the concentration and the age of the plant.
- This study presents environmentally friendly nematode inhibitors as well as plant growth stimulants that are useful in reducing environmental pollution by reducing the use of pesticides and improving the use of sustainable farming methods.



REFERENCES

1. Abdul-Karim, E. K., & Hussein, H. Z. (2022). The biosynthesis of nanoparticles by fungi and the role of nanoparticles in resisting of pathogenic fungi to plants: a review. *Basrah Journal of Agricultural Sciences*, 35(1), 243-256.
2. Ahmed, G. A. (2016). Evaluation the efficacy of some phenolic compounds in controlling bacterial spot disease and biochemical changes associated in pepper plants under greenhouse conditions. *Journal of Plant Protection and Pathology*, 7(10), 655-662.
3. Al-Jubouri, a. k., al-saadi, n. h., & kadhim, m. a. (2022). Green synthesis of copper nanoparticles from myrtus communis leaves extract: characterization, antioxidant and catalytic activity. *Iraqi journal of agricultural sciences*, 53(2), 471-486.
4. Al-Mafrajee, W. M., & El-Rubae, F. A. (2022). Effect of spraying organic emulsion (appetizer) and nano npk with urea on some growth characteristics of three synthetic cultivars of maize. *Iraqi Journal of Market Research and Consumer Protection*, 14(1), 108-117.
5. Basha, S. A., Sarma, B. K., Singh, D. P., Annapurna, K., & Singh, U. P. (2006). Differential methods of inoculation of plant growth-promoting rhizobacteria induce synthesis of phenylalanine ammonia-lyase and phenolic compounds differentially in chickpea. *Folia Microbiologica*, 51, 463-468.
6. Basu, A., Prasad, P., Das, S. N., Kalam, S., Sayyed, R. Z., Reddy, M. S., & El Enshasy, H. (2021). Plant growth promoting rhizobacteria (PGPR) as green bio inoculants: recent developments, constraints, and prospects. *Sustainability*, 13(3), 1140.
7. Blümel, R. C., Fischer, D. F., & Grundler, F. M. (2018). Effects of exogenous amino acid applications on the plant-parasitic nematode *Heterodera schachtii*. *Nematology*, 20(8), 713-727.
8. Central Statistical Organization. (2020). *Statistical Group*. Iraq. Production of secondary crops and vegetables.
9. Chaudhary, S., Sarkar, N., & Kaushik, M. (2021). Recent advances in nanotechnology for accomplishing sustainable agriculture. *Water Conservation in the Era of Global Climate Change*, 1, 147-166.
10. Deka, H., Deka, S., & Baruah, C. K. (2015). Plant growth promoting rhizobacteria for value addition: mechanism of action. *Plant-growth-promoting rhizobacteria and medicinal plants*, 42, 305-321.
11. Dickerson, D. P., Pascholati, S. F., Hagerman, A. E., Butler, L. G., & Nicholson, R. L. (1984). Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. *Physiological plant pathology*, 25(2), 111-123.
12. Dixit, S., Kuttan, K. A., & Shrivastava, R. (2017). Isolation and characterization of phosphorus solubilizing bacteria from manganese mining area of Balaghat and Chhindwara. *Current Science*, 113(3), 500-504.
13. Dube, B. & Smart, G.C. (1987). Biological control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. *Journal of Nematology*, 19(2), 222-227.
14. Elbadri, G. A. A., Lee, D. W., Park, J. C., & Choo, H. Y. (2009). Nematicidal efficacy of herbal powders on *Meloidogyne incognita* (Tylenchida: Meloidogynidae) on potted watermelon. *Journal of Asia-Pacific Entomology*, 12(1), 37-39.



15. El-Sheriff, A. G., Gad, S. B., Megahed, A. A., & Sergany, M. I. (2019). Induction of tomato plants resistance to *Meloidogyne incognita* infection by mineral and Nano-fertilizer. *Journal of Entomology and Nematology*, 11(2), 21-26.
16. Ghini, R., & Kimati, H. (2000). *Resistência de fungos a fungicidas*. Jaguariúna: Embrapa Meio Ambiente.78.
17. Guerrieri, M. C., Fanfoni, E., Fiorini, A., Trevisan, M., & Puglisi, E. (2020). Isolation and screening of extracellular PGPR from the rhizosphere of tomato plants after long-term reduced tillage and cover crops. *Plants*, 9(5), 668.
18. Hammerschmidt, R., Nuckles, E. M., & Kuć, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology*, 20(1), 73-82.
19. Hussey, R.S.& Barker, K.R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter*. 57,1025-1028.
20. Mckinney, R. H. (1923). Influence on soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativus*. *Journal of Agricultural Research*, 26, 195-218.
21. Moens, M., Perry, R. N., & Starr, J. L. (2009). *Meloidogyne* species-a diverse group of novel and important plant parasites. In *Root-knot nematodes*. Wallingford UK: CABI.1-17.
22. Mohammed, h. j., & Yass, s. t. a. m. (2020). activity of fulvic acid and magnesium oxide against root knots nematode *Meloidogyne* spp. development and disease incidence on tomato. *Biochemical & Cellular Archives*, 20(1) ,397-401.
23. Osman, G. Y. (1993). Effect of amino acids and ascorbic acid on *Meloidogyne javanica* Chitw.(Tylenchidae, Nematoda). *Anzeiger Für Schädlingskunde, Pflanzenschutz, Umweltschutz*, 66, 140-142.
24. Peters, R., Brandhoff, P., Weigel, S., Marvin, H., Bouwmeester, H., Aschberger, K., & Mech, A. (2014). Inventory of Nanotechnology applications in the agricultural, feed and food sector. European Food Safety Authority. *Supporting Publications* EN-621, 11(7), 1-125.
25. Sadiq, S. M., & Mohammed, A. A. (2022). Response of faba bean to planting distance between plants and spraing with nano and traditional boron. *Iraqi Journal of Market Research and Consumer Protection*, 14(1), 84-93.
26. Shahin, R. R., & Ramadan, K, N. (2001). *Practical exercises in plant nutrition*. Riyadh.King Saud University.232.
27. Sikora, R. A., & Fernandez, E. (2005). Nematode parasites of vegetables. In *Plant parasitic nematodes in subtropical and tropical agriculture*. Wallingford UK: CABI. 319-392.
28. Walters, D. R. (2009). Are plants in the field already induced? Implications for practical disease control. *Crop Protection*, 28(6), 459-465.
29. Yass, S. T., Aish, A. A., Al-Sandooq, D. L., & Mostafa, M. M. (2020). Activity of humic acid against root knot nematodes on tomato. *Plant Archives*, 20(1), 1-3.
30. Yass A., S. T. (2015). *The interaction between Trichoderma harzianum and a glyphosate pesticide preparation and its effect on the disease complex between the fungus Fusarium oxysporum f.sp.lycopersici and the root-knot worm Meloidogyne spp on the tomato*. PhD thesis, College of Agriculture. University of Baghdad, Iraq.