



EFFECT OF SOME CHEMICAL AND BIOLOGICAL FACTORS ON ROOT KNOTS NEMATODE *MELOIDOGYNE* spp. IN VITRO

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ABSTRACT

This study, conducted at the University of Baghdad, College of Agricultural Sciences and Engineering, Department of Plant Protection, aimed to evaluate both chemical and biological factors in inhibiting the hatching of *Meloidogyne* spp. nematode eggs using ascorbic acid and potassium phosphate at three different concentrations (50, 30, 10 mM). The study aimed to select the effective concentration that significantly impacts the vitality of nematode eggs and juveniles. Additionally, some earthworm-derived products were tested in both solid and liquid states, comparing the results with a commercial compost powder.

The results indicated that the vermicompost produced in this study (liquid and dry) exhibited the highest inhibition percentage for *Meloidogyne* spp. egg hatching at concentrations of 0.7 and 0.07 gm, respectively. The inhibition percentage reached 100% after 72 hours under laboratory conditions.

Keywords: Nematode Root Knot, Earth Worm, Salt, Organic Fertilizers, Acid.

تأثير بعض العوامل الكيميائية والاحيائية في نيماتودا تعقد الجذور. *Meloidogyne* spp.

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الخلاصة

نفدت هذه التجربة في جامعة بغداد. كلية علوم الهندسة الزراعية. قسم وقاية النبات، اذ تناولت تقييم العوامل الكيميائية والاحيائية في تثبيط نفس بيوض ويافيعات نيماتودا تعقد الجذور *Meloidogyne* spp. باستخدام حامض الاسكوربيك وفوسفات البوتاسيوم الثاني على ثلاث تراكيز مختلفة (50 ، 30 ، 10mm) لاختيار التركيز الفعال والذي يؤثر بشكل كبير على حيوية بيوض ويافيعات نيماتودا تعقد الجذور. تم أيضاً استخدام بعض المنتجات المشتقة من دودة الأرض واختبارها في الحالتين، (سائل وجاف) ومقارنة النتائج مع المسحوق التجاري للكومبوست. أظهرت النتائج أن السماد الدودي المصنوع في هذه الدراسة (السائل والجاف) قد أعطى أعلى نسبة تثبيط لنفس بيوض نيماتودا تعقد الجذور ويافيعاتها عند التركيز (0.7، 0.07 غم) على التوالي، حيث بلغت نسبة التثبيط 100% بعد 72 ساعة في الظروف المختبرية.

الكلمات المفتاحية: نيماتودا تعقد الجذور، دودة الأرض، ملح، اسمده عضوية، حامض.

* This article is taken from the first researcher's master's thesis.



INTRODUCTION

Cucumber (*Cucumis sativus*) is one of the most important vegetable belonging to the family Cucurbitaceae. It is widely cultivated due to its fast growth, early maturity, high yield, and continuous demand for consumption. Contain approximately 4-6% dry matter, 2-3% carbohydrates, 1-1.5% proteins, 0.6% fiber, 0.1% fats, and 6% mineral salts (Al-Bahash, 2005). Cucumber cultivation in Iraq is extensive, occurring in both protected greenhouses and open fields. The cultivated area for cucumbers reached 69,502 dunums, with an average yield of 2,152.3 kg/dunum, according to the Central Statistical Organization in 2019. (Central Statistical Organization, 2019).

Cucumbers are infected with various insect pests and diseases, with powdery and downy mildews and root rot being among the most significant diseases the root-knot nematode, *Meloidogyne* spp., is known to be the most dangerous among plant-parasitic nematodes due to its global distribution and wide host range it causes a significant reduction in quantity and quality of cucumber production, greatly affecting the marketability of these crops (Gharabadiyan *et al.*, 2013).

The genus *Meloidogyne* spp. is the most important and widespread among nematodes. Most vegetables crops, including cucumbers, suffer significant losses due to root-knot nematode infestation, which can lead to complete crop failure under severe infection (Abu-Ghribieh *et al.*, 2010). There are over 90 species belonging to the genus *Meloidogyne* spp. worldwide, with four widely distributed species representing 95% of root-knot nematodes in agricultural lands: *M. javanica*, *M. hapla*, *M. arenaria*, and *M. incognita*. These four species are responsible for 90% of the losses caused by root-knot nematode infestation (Castagnone-Sereno, 2002). In Iraq, 120 plant families have been recorded as hosts of this pathogen, making it the most prevalent nematode pathogen group (Antoine, 2014). Controlling the spread of *Meloidogyne* spp. requires the implementation of integrated management approaches, as the use of chemical pesticides is no longer a suitable option due to their high cost and environmental and health risks. Crop rotation has not shown positive results in managing root-knot nematodes, primarily due to the extensive (Toumi *et al.*, 2014).

Several chemical pesticides were used to control root-knot nematodes, including Vidate and Furadan, which proved effective against this pathogen. However, their acute and chronic toxicity contributed to severe environmental contamination, posing a threat to human health, as well as long persistence and toxicity to non-target organisms (Chakraborty & Sukul, 2013). Therefore, the use of chemical and biological alternatives has been considered to reduce the damage caused by nematodes (Al-Bayati, 2005). Study to evaluating the efficacy of biological pesticides, Biocont-T and *Paecilomyces lilacinus*, as well as the chemical pesticide Rygbe, against root-knot nematodes (*Meloidogyne* spp), the results indicated positive effects of all control factors in reducing the number of eggs and juveniles compared to the control treatment (Al-Agidi & Al-Mashhadani, 2013). Recent efforts have focused on investigating the induction of plant's self-defense mechanisms against various plant pathogens, due to its environmental and economic significance (Bakker *et al.*, 2007). Plant resistance against a specific disease can be enhanced by pre-treating the plant with various factors, including physical, chemical, and biological agents (Bakker *et al.*, 2007).

Used of biological agents to stimulate plant resistance has a positive effect on reducing the root-knot index caused by *M. javanica* and the severity of infection in plants. Among these factors are bacteria such as *Glomus* spp and *Pseudomonas* sp (Aljuboori *et al.*, 2022).



Translation: In another study mentioned by **Hussein et al. (2022)**, chemical control using certain nutrients yielded effective results in inhibiting widespread fungi causing plant diseases.

DiPotassium hydrogen phosphite (DKP) is considered an important compound in agriculture not only because it is a good source of potassium and phosphorus but also because it induces resistance against many plant pathogens (**Reuveni et al., 1994**). The study by (**AL-Mayahi & Alaa 2021**) reported that potassium phosphate (K_2HPO_4) achieved complete inhibition, reaching 100%, of fungal growth at a concentration of 200 mM.

Chemical compounds have been widely used to induce systemic resistance in plants against various pathogens, including plant-parasitic nematodes. Ascorbic acid has been used to stimulate resistance in plants (**Abdel-el-Karem et al., 2013**). It is known for its ability to control various fungal diseases (**Abdel-Kader et al., 2012; Shahda, 2000**) and combat invasive nematodes such as root rot and root-knot nematodes (**Anter et al., 2014**).

Mohammed & Abdul Malek, (2020) demonstrated in a study that folic acid and magnesium oxide had a significant effect against root-knot nematodes (*Meloidogyne* spp). Treatment with different concentrations of FA and Mgo (1000, 2000, 3000 ppm) in the laboratory led to a significant decrease in egg hatching and juveniles' viability after 3 and 7 days compared to the control treatment.

In another study evaluating the efficacy of some organic stimulants, such as humic acid, against root-knot nematodes, the results showed that it had a significant lethal effect on juveniles, which was correlated with increasing concentrations compared to the control treatment. It also led to an increase in growth parameters compared to the control treatment, including an increase in fresh root weight (**Yass et al., 2020**).

In another study the results showed wheat's response to potassium fertilization and foliar application of iron and manganese. Treatment with K120 and foliar application of Mn25 with Fe100 twice during the elongation and heading stages resulted in the highest grain yield of 6240 kg.ha^{-1} . This was associated with the highest nitrogen concentration of 3.39%, phosphorus concentration of 0.36%, and potassium concentration of 2.68%, which correlated with the best N, P, K indices of 0.1, 0.34, and 0.41 respectively. The total cumulative index for N, P, and K indicators amounted to 1. (**Abdul Majeed, et al., 2005**)

On the other hand, the use of natural and microbial agents has become significantly important as alternatives to nematicides, which have negative effects on human health and the environment (**Hubbard et al., 2014**). The presence of earthworms in the soil is a good indicator of soil quality, as it indicates the availability of nutrients resulting from their feeding, making plants grown in such soil more resistant to pathogens (**Scott-Fordsmand & Weeks, 2000; Sala et al., 2000**).

In a study conducted to assess the effects of different formulations of organic emulsion (Appetizer) and nanoscale NPK fertilizer combined with urea fertilizer on the growth and yield of yellow corn varieties, significant differences were observed among the different fertilizer treatments. The nanoscale fertilizer treatment achieved the highest average in most growth parameters, including the number of days from planting to harvest, plant height, leaf number, and chlorophyll content (**Al-Mafrajee & El-Rubaee, 2022**).

Despite the importance of the diverse soil organisms in biological control, there is a lack of sufficient studies and research on their significance in combating these pests (**Fournil et al., 2018**). Soil organisms contribute to creating a favorable environment for plant development and an unfavorable environment for pathogenic organisms (bacteria, fungi, nematodes), while maintaining a balanced community that does not negatively affect the crop (**Baker, 1991**;



Barrios, 2007). The main function of these organisms is to reduce the amount of primary and secondary inoculum produced by pathogenic agents, inhibit or prevent plant penetration, promote plant growth, increase plant disease resistance, and prevent infection (Fokkema, 1995).

Microbial-based bio-products are considered potential candidates in integrated pest management programs as they are beneficial for plant families and environmentally friendly (Rehman *et al.*, 2009).

The study aimed to assess three concentrations of factors used in the experiment (biological and chemical factors) on the viability of eggs and juveniles of the second generation of root-knot nematodes in a laboratory setting.

MATERIALS AND METHODS

The method described by Hussey & Barker (1973) was followed to extract root-knot nematode (RKN) inoculum. Infected cucumber plant roots were collected from a field in the Shirqat district, Salah al-Din Governorate, after observing symptoms of infection such as yellowing and wilting of leaves. The root samples were washed with water to remove adhering soil, and then cut into small pieces (2-3 cm) using sterile scissors. The root pieces were placed in a 1000 ml flask with a 1% sodium hypochlorite (NaOCl) solution. The flask was thoroughly shaken for 3 minutes to ensure homogenization of the solution. The solution was passed through a 25-micron sieve, and the sieve contents were washed several times with water to remove traces of sodium hypochlorite. The eggs and second-stage juveniles (J2) were collected. The solution was placed dish in an incubator at a temperature of $25 \pm 2^{\circ}\text{C}$ for 72 hours. Based on the inoculum rate, whether from eggs or J2, the inoculum rate was calculated by taking 1 ml of the suspension using a sterile pipette and counting it on a counting slide (Peters, 1 ml ell Worm) under a compound light microscope at a magnification of 40x.

Experimental Treatments:

Manufactured Vermicompost (Dry):

Earthworms of the Lumbricidae family were collected from a farm established in January 2022. The farm was set up using a device designed by the researcher and supervisor (a device for breeding and propagating earthworms and converting their waste into organic fertilizers). After breeding and growing the worms, they were collected from the farm and transported to the laboratory for use in research experiments. The worms were cleaned and rinsed with sterile distilled water to remove impurities and accumulated mud on their bodies. They were then placed in a Petri dish containing 10% ethyl alcohol and left for 10 minutes. Afterward, they were transferred to another Petri dish containing ethyl alcohol. The worms were placed on tissues for drying and then transferred to an oven at a temperature of 40°C until completely dried. They were then ground into powder using an electric grinder. Three dry weights (0.3, 0.5, 0.7) grams were taken for use in the experiment to select the effective concentration.

Aqueous Extract of Manufactured Vermicompost

The following weights (0.3, 0.5, 0.7) grams were taken and placed in a glass flask, and the volume was completed to 100 ml with distilled water. The flask was placed on an electric magnetic stirrer for 24 hours to homogenize the solution. It was then filtered through two



layers of filter cloth. The extract was transferred to plastic tubes and centrifuged at a speed of 3000 rpm for 10 minutes using a centrifuge to obtain a supernatant. The supernatant was stored in a bottle until used in the experiments (Al-Sakani, 2019; Sahi, 2019).

Commercial Compost:

It was obtained from a nursery in Baghdad (Dafaf), and three weights (0.03, 0.05, 0.07) grams were taken from it.

Preparation of Ascorbic Acid:

The following formula was used to convert millimolar concentration of ascorbic acid to grams:

$$M = w / (M \times WT) = 1000 / (V \text{ ml})$$

$50 = w / 176.12 = 1000 / 200 = 1.761.2 \text{ g}$ 1.761.2 g of ascorbic acid was taken and dissolved in 200 ml of distilled water. The solution was stored in dark bottles to prevent oxidation by light. Then, other concentrations were prepared according to the dilution formula.

$$N1V1 = N2V2, 50 V1 = 100 \times 20 = 40 \text{ ml}$$

The concentration was taken and the volume was completed to 100 ml (60 ml of distilled water + 40 ml of stock solution).

$$N1V1 = N2V2, 50 V1 = 100 \times 10 = 20 \text{ ml}$$

The concentration was taken and the volume was completed to 100 ml (80 ml of distilled water + 20 ml of stock solution).

These solutions were placed in dark bottles, labeled, and stored for use in the research experiments.

Preparation of K_2HPO_4 Salt

It was prepared using the same method as ascorbic acid, using the molarity and dilution formula. Its molecular weight is 174.2, and the concentrations used were 50, 20, and 10 ml molar. The solutions were stored in special containers for use in the experiments.

Labrotary experiments

Five treatments were prepared, with three replicates for each treatment, AC + Nematode inoculum consisting of 100 eggs and 100 juveniles + 10 ml of ascorbic acid concentration (10, 20, 50 Mm).

DKP + Nematode inoculum consisting of 100 eggs and 100 juveniles + 10 ml of salt concentration (10, 20, 50 Mm)..

Liquid Manufactured Vermicompost + Nematode inoculum consisting of 100 eggs and 100 juveniles + 10 ml of compost concentration (0.3, 0.5, 0.7 gm)

Dry Manufactured Vermicompost + Nematode inoculum consisting of 100 eggs and 100 juveniles + weight of compost (0.03, 0.05, 0.07 gm).

Dry Commercial Compost + Nematode inoculum consisting of 100 eggs and 100 juveniles + weight of compost (0.03, 0.05, 0.07 gm).

The experiment was conducted with three replicates for each treatment and incubated at a temperature of 25°C. Readings were taken at the following time intervals: 24 hours, 48 hours, and 72 hours.

RESULTS

Study virto on the Effect of Chemical and Biological Factors on Inhibiting Egg Hatching and Mortality of Second-Stage Juveniles (J2) of *Meloidogyne* spp. Nematodes Causing Root Knot Disease in Cucumber, in the Laboratory showed.



Showed that the effect of the factors varied according to the type and concentration of the tested substance and the time after treatment. The results were detailed in Tables 1 and 2.

Study on the Effect of Chemical and Biological Factors on Inhibiting Nematode Egg Hatching (*Meloidogyne* spp.) Causing Root Knot Disease, in the Laboratory.

The results in (Table, 1) demonstrated the effect of different concentrations of ascorbic acid, potassium salt, liquid and dry vermicompost on the percentage of nematode egg hatching inhibition. It was found that the rates of egg hatching inhibition varied according to the type and concentration of the added substance and the time after treatment, with significant differences between the treatments at a significance level of ($P \leq 0.05$). The treatment of liquid vermicompost gave the highest egg inhibition rates at a concentration of 0.7g. The inhibition rates reached 87.33%, 92.67%, and 100% after 24, 48, and 72 hours of treatment, respectively, showing significant differences compared to the other treatments. This was followed by the treatment of dry vermicompost, which achieved inhibition rates of 84.33%, 90.33%, and 100% at a concentration of 0.07g, after 24, 48, and 72 hours of treatment, respectively, with significant differences compared to the other treatments. However, there were no significant differences in the egg hatching inhibition rates between the two treatments after 48 and 72 hours. The commercial compost treatment, at the lowest concentration used (0.3g), gave the least inhibition percentage for nematode egg hatching, with rates of 15%, 25.67%, and 30.33% after 24, 48, and 72 hours of treatment, respectively, showing significant differences compared to the other treatments.

The effectiveness of the tested treatments in reducing egg hatching increased with longer incubation periods, with significant differences between the incubation periods. The longer the incubation period, the more effective the substance used in reducing nematode egg hatching rates. This result can be explained by the increased exposure of the eggs to the active factors in the test medium.

The results indicate that the addition of the tested factors to the nematode egg medium causing root knot disease resulted in varying levels of inhibition of egg hatching, depending on the type and concentration of the added substance and the incubation period, with significant differences between the treatments at a significance level of ($P \leq 0.05$).

Moreover, treatment with ascorbic acid and K_2HPO_4 at a concentration of 10 mM gave the least inhibition rates for nematode egg hatching in the laboratory, without significant differences between them. The inhibition rates were 7.33% and 10.67%, 12.67% and 23.67%, and 19.33% and 28.33% after 24, 48, and 72 hours of treatment, respectively, in sequence.

The inhibition rates of nematode egg hatching increased with increasing concentrations of the factors used in the incubation medium. Furthermore, the inhibition rates of egg hatching increased with longer exposure periods, with significant differences between the exposure periods for all treatments.



Table (1): Effect of Chemical and Biological Factors on the Inhibition Rates of Nematode Egg Hatching (*Meloidogyne* sp.).

| Treatment | concentration | Time after Treatment | | | Average |
|---------------------|---------------|---|-------|--------|---------|
| | | 24 | 48 | 72 | |
| | | Percentage of Inhibition of Egg Hatching(%) | | | |
| Ascorbic Acid | 50Mm | 27.00 | 41.00 | 70.67 | 46.22 |
| | 20Mm | 15.33 | 31.00 | 54.67 | 33.67 |
| | 10Mm | 7.33 | 12.67 | 19.33 | 13.11 |
| k_2hpo_4 | 50Mm | 26.33 | 31.67 | 44.33 | 34.11 |
| | 20Mm | 16.33 | 26.00 | 32.67 | 25.00 |
| | 10Mm | 10.67 | 23.67 | 28.33 | 20.89 |
| Liquid Vermicompost | 0.7% | 87.33 | 92.67 | 100.00 | 93.33 |
| | 0.5% | 83.00 | 88.33 | 94.67 | 88.67 |
| | 0.3% | 71.67 | 77.67 | 84.33 | 77.89 |
| Dry Vermicompost | 0.07gm | 84.33 | 90.33 | 100.00 | 91.55 |
| | 0.05gm | 83.00 | 84.67 | 90.33 | 86.00 |
| | 0.03gm | 41.67 | 42.00 | 76.67 | 53.45 |
| Compost | 0.07gm | 35.00 | 36.00 | 43.67 | 38.22 |
| | 0.05gm | 18.00 | 34.67 | 35.33 | 29.33 |
| | 0.03gm | 15.00 | 25.67 | 30.33 | 23.67 |
| Control | - | 0.00 | 0.00 | 0.00 | 0.00 |
| L.S.D (0.05) | - | 10.63 | | | 6.14** |
| Average | - | 38.87 | 64.12 | 56.58 | |
| L.S.D (0.05) | - | 2.66** | | | |

Each number in the table represents the average of three replicates, and each replicate consists of three dishes. The experiment was conducted in the laboratory at a temperature of (25 ± 2) degrees Celsius. Each replicate contained 100 eggs. The experiment was designed using a completely randomized design (CRD).

The results in (Table, 2) showed that the factors added to the J2 incubation medium had varied effects on the mortality of juveniles, depending on the type, concentration of the substance, and incubation period. The liquid and dry vermicompost treatments at concentrations of 0.7 and 0.07 showed the highest effect on the mortality of second-stage juveniles of *Meloidogyne* spp., with no significant difference between the two treatments. The percentages of juvenile mortality were 91% and 89% (after 24 hours), 97% and 95% (after 48 hours), and 100% and 100% (after 72 hours) for the liquid and dry vermicompost treatments, respectively. The liquid and dry vermicompost treatments at concentrations of 0.5 and 0.05 also had positive effects on the mortality of second-stage juveniles of *Meloidogyne* sp., with no significant difference between the two treatments after 48 and 72 hours of incubation. The percentages of juvenile mortality were 90.33% and 88.33% (after 48 hours) and 94% and



92.67% (after 72 hours) for the liquid and dry vermicompost treatments, respectively. The compost treatment at a concentration of 0.03 showed the least mortality of second-stage juveniles of *Meloidogyne* sp., with significant differences compared to the other treatments at a significance level of ($P \leq 0.05$). The percentages of juvenile mortality were 5.67%, 10.33%, and 13.67% (after 24, 48, and 72 hours, respectively). This was followed by the compound K_2HPO_4 at a concentration of 10 mM, and then ascorbic acid at a concentration of 10 mM. The results also indicate that the factors added to the J2 incubation medium had varied efficacy in terms of the percentages of juvenile mortality, depending on the type, concentration of the substance, and incubation period (Table, 2).

Furthermore, the lethal effect of the compounds on second-stage juveniles of *Meloidogyne* sp. increased with increasing concentration and incubation time, with significant differences. After 72 hours of exposure to the tested factors, the highest efficacy in increasing the percentages of mortality in second-stage juveniles was observed, with significant differences between incubation periods ($P \leq 0.05$).

Table (2): Effect of chemical and biological factors on the mortality percentages of second-stage juveniles (J2) of *Meloidogyne* spp.,

| Treatment | concentration | Incubation Time (hours) | | | Average |
|---------------------|---------------|---|-------|--------|---------|
| | | 24 | 48 | 72 | |
| | | (%)(J2) percentages of juvenile mortality | | | |
| Ascorbic Acid | 50Mm | 34 | 47.00 | 78.33 | 53.11 |
| | 20Mm | 31.67 | 48.33 | 68.67 | 49.56 |
| | 10Mm | 21.33 | 32.33 | 52.33 | 35.33 |
| K_2HPO_4 | 50Mm | 24.00 | 22.00 | 38.33 | 28.11 |
| | 20Mm | 12.00 | 25.00 | 27.33 | 21.44 |
| | 10Mm | 5.33 | 18.33 | 27.33 | 17.00 |
| Liquid Vermicompost | 0.7% | 91.00 | 97.00 | 100.00 | 96.00 |
| | 0.5% | 86.33 | 90.33 | 94.00 | 90.22 |
| | 0.3% | 75.67 | 83.67 | 89.00 | 82.78 |
| Dry Vermicompost | 0.07gm | 89.00 | 95.00 | 100.00 | 94.67 |
| | 0.05gm | 82.00 | 88.33 | 92.67 | 87.67 |
| | 0.03gm | 71.33 | 73.33 | 84.67 | 76.44 |
| Compost | 0.07gm | 15.33 | 24.33 | 26.67 | 22.11 |
| | 0.05gm | 10.33 | 15.67 | 20.33 | 15.44 |
| | 0.03gm | 5.67 | 10.33 | 13.67 | 9.89 |
| Control | - | 0.00 | 0.33 | 3.67 | 1.33 |
| L.S.D (0.05) | - | 7.21** | | | 4.16** |
| Average | - | 40.93 | 48.20 | 57.31 | |
| L.S.D (0.05) | - | 1.80** | | | |



Each number in the table represents the average of three replicates, with each replicate consisting of three plates. The experiments were conducted in the laboratory at a temperature of (25±2) degrees Celsius. Each replicate contained one hundred juveniles. The experiment was designed using a Completely Randomized Design (CRD).

DISCUSSION

From the results in Tables 1 and 2, it was found that the tested factors showed variation in their inhibitory effect on egg hatching and mortality of second-stage juveniles of *Meloidogyne* spp. The treatments can be arranged in descending order of their effectiveness at the active concentration as follows: liquid vermicompost ≤ dry vermicompost > ascorbic acid > K_2HPO_4 > compost.

Furthermore, the effects of the treatments on inhibiting egg hatching and mortality of the tested nematodes increased with increasing concentrations of the factors used, with significant differences observed among the means of hatching rates for all the studied treatments. This result can be explained by the increased presence of active factors in the test medium due to higher concentrations. The effects of the tested factors in reducing egg hatching rates and increasing juvenile mortality rates of the nematodes also increased with longer incubation periods, with significant differences observed among the incubation periods. The longer the incubation period, the more effective the substance used in reducing egg hatching rates and increasing the mortality rates of the nematode juveniles. This result can be explained by the increased exposure of the eggs to the active factors in the test medium. Additionally, changes in the acidity level of the medium where the eggs or juveniles are present negatively affect the hatching rate and viability of the second-stage juveniles of *Meloidogyne* sp. A suitable pH level for egg hatching and the growth of second-stage juveniles of root knot nematodes is considered to be 6.5 pH= (Loewenberg *et al.*, 1960). These results are consistent with (Osman, 1993), who found that ascorbic acid has an inhibitory effect on egg hatching and reduces the viability of second-stage juveniles of *Meloidogyne javanica*, with the effect increasing with higher concentrations (Tsai, 2008). demonstrated that the addition of aqueous extracts from lemon, orange, and grapefruit peels rich in ascorbic acid caused mortality in second-stage juveniles of *Meloidogyne incognita* at rates exceeding 80%. Moreover, the infusion of sweet orange peels showed effectiveness in reducing egg hatching and causing mortality in second-stage juveniles of *Meloidogyne incognita* (Abolusoro *et al.*, 2010).

Habash, & Al-Banna (2011) reported that phosphorous and potassium fertilizers inhibit egg hatching and cause mortality in second-stage juveniles for tow species (M. javanica and M. incognita), with the effect increasing with higher concentrations and longer incubation periods.

Hemmati, & Saeedizadeh (2020) demonstrated that vermicompost, potassium fertilizer, and plant leaf residues inhibited egg hatching and caused mortality in second-stage juveniles of *Meloidogyne javanica* in a dose-dependent manner, with the effect increasing with higher concentrations and longer incubation periods. Another study evaluating the efficiency of some organic stimulants, such as humic acid, showed that it had a significant lethal effect on juveniles, which was associated with increasing concentrations compared to the control treatment, which had the highest number of root knots. Additionally, it led to an increase in growth parameters compared to the control treatment, which recorded an increase in fresh root weight (Yass *et al.*, 2020)



CONCLUSION

The effectiveness of vermicompost in both liquid and solid forms at concentrations of 0.7 and 0.07 revealed the highest inhibition rate against eggs and juveniles of the second generation. A 100% inhibition rate against eggs and juveniles of the second generation was achieved after 72 hours with the highest concentration of vermicompost treatment. The results also showed the effectiveness of ascorbic acid Dkp at a concentration of 50 mm against eggs and juveniles of the second-generation root-knot nematodes.

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