Veterinary Medicine and Public Health Journal (VMPH)

Volume.5, Issue.2, pp: 134-141 Article 14
May (2024)

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Received: 15/12/2023 **Revised:** 15/1/2024 **Accepted:** 17/2/2024

DOI: https://doi.org/10.31559/VMPH2024.5.2.14

www.refaad.com

Veterinary Medicine and Public Health Journal (VMPH)

Journal Homepage: https://www.refaad.com/Journal/Index/7

E-ISSN 2707-7195 | P-ISSN 2707-7187

Evaluation of the Efficiency of some Nanoparticles and Chemical Nematicides in Controlling Root-Knot Nematodes Meloidogyne Incognita (on Eggplant)

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How to cite this article: Abdalreda, E., et al. (2024). Evaluation of the Efficiency of some Nanoparticles and Chemical Nematicides in Controlling Root-Knot Nematodes Meloidogyne Incognita (on Eggplant). Veterinary Medicine and Public Health Journal, 5(2), 134-141.

Abstract:

Objectives: This study was carried out to investigate the effectiveness of some nanoparticles in inhibiting the growth of the root knot nematode.

Methods: This study was carried out to investigate the effectiveness of aluminum oxide Alo-NPs and silver Ag-NPs, and chemical nematicides (Velum prime and Furadan granules), in inhibiting the growth of the root knot nematode (Meloidogyne incognita) on two different types of eggplant cultivars, Barcelona and Thuraya.

Results: The result obtained indicated that chemical nematicides and nanoparticles were highly effective in lowering the nematode invasion level expressed as number of knots per root and a gall index based on a 0-5 scale. Velum prime at concentrations of 300 and 400 ppm was the most effective and gave 0.67 and 0.17 knots/root average on both eggplant cultivars, respectively. In addition, the ALO-NPs treatment reduced infection to 1.17 knots/root compared to initial infection of 37.33 knots/root. The use of Ag-NPs at the concentration of 4000 ppm reduced infection to a low average of 2.83 knots/root for both eggplant varieties. All treatments average reduced the gall index to 0.95 (cv. Barcelona) and 1.20 (cv. Thuraya) compared to 3.33 and 4.00 for the infected non-treated controls, respectively.

Conclusions: Nanoparticles have a role in controlling root-knot galls as alternatives to chemical pesticides that are dangerous to the environment and humans, and reducing the number of galls on the plant indicates the inhibition of eggs and second stage juveniles (J2).

Keywords: *Meloidogyne incognita; Alo-NPs; Ag-NPs; chemical nematicides.*

1 Introduction

The aubergine one of the most significant vegetable crops, *Solanum melongena*, is found in both temperate and tropical climates. The largest producers of eggplant worldwide are China and India. Over 50 million tons of eggplant are grown each year on more than 1,800,000 hectares worldwide (Gürbüz *et al*., 2018). Due to its high fiber content, water-soluble carbohydrates that control blood sugar, and phenols that block diabetes-related enzymes, eggplant possesses antioxidant properties. Eggplant is a storehouse of calcium, iron and other important elements Besides vitamins, minerals, and amino acids. Plants and human health (Rodriguez-Jimenez *et al*., 2018) Most farmed eggplant cultivars are susceptible to a wide range of diseases, especially those brought on by the root-knot nematode *Meloidogyne* spp. Root-knot nematodes, which also cause annual losses to most crops of about $100 billion globally, harm more than 3000 plant species. The most deadly species include *M.incognita, M.javanica, M.arenaria,* and *M.hapla*. This is especially true of Iraq. The vegetative system's wilting and yellowing of the leaves, as well as the development of knots and swelling on the root system, are symptoms. *Meloidogyne incognita* may cause a complete crop failure if it is not controlled. (Ghareeb *et al*., 2020) and (Hada *et al*., 2022). Due to the small size of these galls, the large number of species of more than 110 species, and the similarity of a large number of their species to morphological characteristics, overlapping signs and morphometric measurements, which increased the difficulty of diagnosing them for more than one method, including diagnosis using genetic fingerprinting, chemical method and morphological method. The oldest and most widely used methods depend on anatomical differences, including body length, female shape, spear shape, perineal pattern planning in the back of the mature female body and others (Barros *et al*., 2018) and (Cunha *et al*., 2018). In order to reduce the damage of these galls to below the level of economic damage, various means were used to combat them, including chemical methods, which proved successful despite the fact that they cause pollution to the environment in addition to killing beneficial organisms in the soil. One of the best chemicals that were used and gave effective results is methyl bromide, but it was withdrawn. Because to its risk to people and extended longevity, it is removed from the market in most countries (Noling & Becker, 1994) and (Dhananjayan *et al*., 2020). Recently, scientists and farmers have begun using nanomaterials to lessen negative environmental effects, reduce pollution in water and the environment, and produce better agricultural food that reflects positively on the economic return. No previous technology has received much attention, such as nanotechnology as the magic key to progress and economic growth (Sanjay and Pandey 2017) and (Al-Hadede 2020). Silver nanoparticles (AgNPs) have been used as catalysts and anti-cancer materials and have emerged as an effective nanotechnology product in recent years due to their chemical stability, catalytic and antibacterial activity and are harmless to humans and the environment (El-Deen & El-Deeb 2018). Although aluminum oxide nanoparticles have been investigated for a long time, the extent of their toxicity is still unknown. Some researchers have hypothesized that DNA damage and oxidative stress are to blame for aluminum oxide nanoparticles' cytotoxic effects. absence of control (Dağlioğlu *et al*., 2020) and (Ingle & Gupta, 2021).

2 Materials and Methods

2.1 Collection samples of nematode

Samples of eggplant roots infected with several Meloidgyne species. Figure 1 shows that samples were collected from a field in the Karbala/Al-Hussainiya district and put on plastic bags for transportation. While there, samples were sliced into little pieces after being cleaned with tap water to eliminate soil (1-2 cm) and blended in hypochlorite sodium (NaOCl) 1% to preserve eggs in accordance with the manufacturer's instructions (Hussey and Barker, 1973). The solution's components each immediately passed through a 75 m, 50 m, and 25 m sieve. In the experiment, eggs were gathered from 25 mm sieves. According per (40) technique species of Meloidogyne used in this study were identified based on the morphological features of adult females have a straight perineal cuticle. According to (Eisenback *et al*., 1985).'s approach, the perineal pattern of mature laying eggmass females was removed from each infected root system, and the diagnosis was made based on the perineal pattern's straight cuticle. This was then studied under a microscope at 40X and subsequently 100X magnification.

Figure 1: Root samples of eggplant were infected by root knot nematode in Karbala / Al-Hussainya district field.

2.2 Soil Sterilization

A piece of thick plastic was used to cover the soil after it had been properly mixed with 1% formalin and combined in a 1:2 ratio with the peat moss. To eliminate any remaining formalin, the mixture was then carefully sealed for 72 hours. The soil was then packed in 2 kg plastic pots until the time of culture (Hizam, 2002).

2.3 Preparation of seeds

For this investigation, Barcelona and Thuraya eggplant cultivar seeds were employed. 1% sodium hypochlorite solution was used to sterilize the seeds for one minute, after which they were washed with sterile distilled water and placed in Petri dishes on filter paper to dry. The seeds were then planted in plastic trays filled with a 1:2 mixture of soil and peatmoss that had been sterilized in an autoclave under the proper temperature and humidity conditions for use in field experiments.

2.4 The Field Experience

The seedlings were transplanted into plastic pots filled with the previously prepared soil mixture and peatmoss at a rate of 3 seedlings per pot based on a cultivar with three replications. The pots were then left for a week before being inoculated with RKN galls at a rate of 2000 20 second-stage juveniles (J2) and eggs for each pot. By using a sterile pipette, nematode inoculum was poured to the soil and inserted during the creation of 4 holes 2 cm from the plant's roots and 3 cm deep. The holes were then covered with light dirt and watered as needed. Nanoparticles, Silver nanoparticles after a week (Ag-NPs) and aluminum oxide (ALO-NPs), were added three times, three days apart, at the same 4000 ppm concentration for them. According to the instructions, the chemical pesticide (Velum Prime) from the German company BAYER was applied twice, 20 days apart, at doses of 400 and 300 ppm/liter. According to recommendations, Furadan granules (Carbofuran), a chemical pesticide manufactured by the US corporation FMC, were applied twice, 21 days apart, at a dosage of 1000 ppm. 100 cc of chemical pesticide and nanoparticles were applied per plant. Negative control involved simply irrigation, while positive control involved nematode inoculum alone. Following the 65-day trial period, the number of knots and gall index were determined using the (Taylor and Sasser, 1978) scale, which has five degrees, as shown in the table below.

Table 1: Root gall index according to (39)

Description degree	Description
0	There are no knots on the roots
1	Number of nodes 1 - 2 knots on the root
2	Number of nodes 3-10 nodes on the root
3	Number of nodes 11-30 nodes on the root
4	Number of nodes 31-100 nodes on the root
5	Number of nodes more than 100 nodes on the root

2.5 The Statistic Analysis

With the help of the statistical program SPSS 25.0, the findings were statistically evaluated to perform an analysis of variance and to assess the significance of any differences between the results at a 5% level of significance.

3 Results and Discussion

3.1 Diagnosing the Type of Nematode

Figure 2 illustrates the diagnosis' findings, which indicated that *Meloidogyne incognita* is the type of nematode isolated from one of the Karbala/Al-Hussainiya district's orchards, which was used in the field experiment. More than one sample was diagnosed from more than one different site in the field, and they all belong to the species *M. incognita*. The females of this pattern can be identified by the curve of the anterior back, the presence of a squarish dorsal arch at the top of the dorsal side of the perineal pattern, undefined side fields, and zigzag, jagged, and jagged cuticle lines. Splits and folds distinguish it from other species, and the anus is forward and the tail end is smooth (17); (18). This confirms a number of investigations that discovered the presence of this species on Iraqi soil (Al-Kubaisi, 2013; Murad *et al*., 2020; Mohammed, 2021). Diagnosis of root knot galls *Meloidogyne* spp. on tomato in some areas of Karbala governorate and evaluation of some biological and non-biological control factors. Master's thesis - College of Agriculture - University of Karbala. (Kaur and Attri, 2013; Al-Kubaisi, 2013; Murad *et al*., 2020).

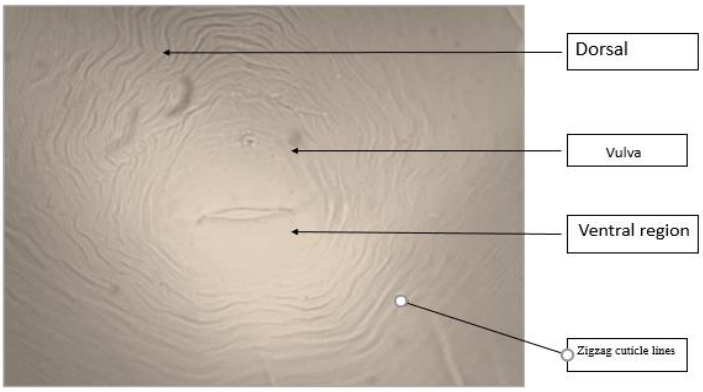

Figure 2: Perineal pattern of female of *Meloidogyne incognita*

3.2 Field Experience

The results of field control on the eggplant crop demonstrated that the use of chemical pesticides and nanoparticles had a beneficial impact because they effectively reduced the infestation of the root-knot galls *Meloidogyne incognita* and decreased the number of nodules on the roots of the eggplant cultivars used in the experiment (Figure 1). This, in turn, led to a reduction in the severity of nematode infection. All of the treatments outperformed the nematode-only control treatment in terms of lowering the number of galls on roots and the severity of infection. According to the scale, the average number of galls was 37.33, and the level of infection was 3.66. The Barcelona type was likewise shown to be the most effective, with the fewest knots at a rate of 4.37 and an infection level of 0.95. The chemical pesticide Velum Prime treatment with a concentration of 400ppm and 300ppm was superior to the other treatments in reducing root nodes to 0.00 and 0.33 compared to the contaminated comparison treatment, which amounted to 29.00 knots/root, in comparison to the other variety (Thuraya), which reached 6.96 and a degree of infection of 1.20, The treatment of ALO-NPs, the chemical pesticide Furadan, and the treatment of Ag-NPs were done in that order, and the results were 1.00, 1.33, and 2.00, respectively, with no appreciable variations between them. With regard to the Thuraya variety, the 400ppm and 300ppm Velum Prime treatments outperformed the other treatments in lowering the root knots index to 0.33 and 1.00, respectively, as opposed to the contaminated control treatment, which resulted in 45.67 knots/root. The treatment of ALO-NPs, Furadan, then Ag-NPs, with respective amounts of 1.33, 1.67, 2.00, and 3.67, came next, these results did not substantially differ from the comparison uncontaminated treatment, which did not record any galls infestation, and they did not significantly differ from one another. These findings are in line with earlier research demonstrating that green synthesis—the use of nanoparticles such as silver nanoparticles, zinc, aluminum oxide, etc.—is safe, non-toxic to the environment, chemically stable, and increases antimicrobial activity and pathogen-fighting efficiency, including against root-knot nematodes *Meloidogyne* spp. affecting the juveniles and eggs of the second stage J2 plant infection. (Taylor and Sasser, 1978; Cromwell *et al*., 2014; Soliman *et al*.,

2017; Bernard, *et al*., 2019). silver Ag-NPs cause nematode membrane damage, oxidative stress, and adenosine triphosphate production (ATP), Silver is regarded as an essential component for plant growth and development, and it can have an impact on bacteria and nematodes that are linked with the roots, such as *Gaeumannomyces graminis* and *Rhizoctonia solani* (Das and Pattanayak, 2020; Abbassy *et al*., Abdel-Rasoul, M. A., Nassar, A. M., & Soliman, B. S. 2017). ALO-NPs were found to have a beneficial effect on lowering the number of knots over silver nanoparticles, nematodes, minerals, but more recently they have been introduced as insect and fungus repellents, despite the fact that there have only been a few studies on the influence of this material on root-knots (Nazir *et al*., 2019), Many studies on the toxicity of ALO-NPs and the concentration at which they are present on a surfacehave addressed both their chemical and physical properties. As stated by the writers, despite delivering beneficial results, ALO-NPs may be severely poisonous and affect consumers and workers. They can have harmful effects on a number of organisms, including microalgae, invertebrates, and various types of bacteria, such as *Bacillus subtillis*, *Escherichia coli*, and *Pseudomonas fluorescens* (Ismail *et al*., 2021). By inhibiting the respiratory chain in the mitochondria and stopping the production of (ATP), the substance that provides energy in living cells, the chemical pesticide Velum Prime reduces the number of knots, which has the effect of stopping the vital processes in nematodes and causing their quick death. It is a non-selective pesticide that has an impact on the digestive system and palpation route (B bayer Crop Science. (2019). Several studies have also demonstrated how well the insecticide Velum Prime works to eradicate some nematode species and lessen the harm they cause to a variety of plants, including eggplant, tomato, soybean, and others. even when used in low doses below what is advised, whether in the field or greenhouse, and reduces nematode density. (Faske and Hurd, 2015; Dahlin *et al*., 2019; Hawk, 2019). In line with earlier research (Di Sanzo, 1973; Akter *et al*., 2021), the results of the Furadan treatment likewise shown a reduction in the number of knots compared to the inoculum treatment. Because of its toxicity, it has been outlawed in developed nations (Faruk *et al*., 2012; Arain *et al*., 2015).

This study provides proof that nanoparticles are an effective alternative to chemical pesticides that are harmful to both humans and the environment. Reducing the amount of galls on the plant also suggests that eggs and second stage juveniles (J2) are being inhibited.

1 2

Figure 3: Eggplant roots inoculated with root knot galls 1-Pesticide treatment 2-not treated with pesticide.

Table (2): Effect of treating two eggplant cultivars infested with root knot nematodes (RKN) by nanoparticles and chemical nematicides expressed by number of knots/roots.

	Number of knots/roots		Cultivar average
Treatments	**Barcelona**	**Thuraya**	
Ag-NPs(4000PPM)	2.00	3.67	2.83
ALO- NPs (4000 ppm)	1.00	1.33	1.17
Velum Prime (300 ppm)	0.33	1.00	0.67
Velum Prime (400 ppm)	0.00	0.33	0.17
Furadan (1000 ppm)	1.33	1.67	1.50
Furadan (500 ppm)	1.33	2.00	1.67
Infected control (untreated)	29.00	45.67	37.33
Healthy control	0.00	0.00	0.00
Treatments average	4.37		6 .96
L.S.D. at P (0.05)	For cultivars= 4.354, For treatments= 2.177For interaction=6.157; * Values represent average of three replications.		

Table 3: Effect of using Nanoparticles and chemical nematicides on the root knot gall index (based on a 0-5 scale) of eggplants infested with the root knot nematode(RKN).

	Cultivar		Average
Treatments	**Barcelona**	**Thuraya**	
Ag-NPs (4000 ppm)	1.33	1.66	1.50
ALO- NPs (4000 ppm)	0.66	0.66	0.66
Velum Prime (300 ppm)	0.33	0.66	0.50
Velum Prime (400 ppm)	0.00	0.33	0.16
Furadan (1000 ppm)	1.00	1.00	1.00
Furadan (500 ppm)	1.00	0.33	1.16
Infected contro	3.33	4.00	3.66
Healthy control	0.00	0.00	0.00
Average	0.95	1.20	
L.S.D. at P (0.05)	For cultivars= 0.5143, For treatments = 0.2512		
	For interaction=0.7274; * Values are means for three replications.		

4 Conclusions:

Nanoparticles have a role in controlling root-knot galls as alternatives to chemical pesticides that are dangerous to the environment and humans, and reducing the number of galls on the plant indicates the inhibition of eggs and second stage juveniles (J2).

Recommendations:

Nanoparticles are the most Application in plant protaction from many pathogens such as nematode cuased rot knot nematode.

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