Evaluation the efficiency of some tomato hybrids, biological and chemical factors against tomato root knot caused by *Meloidogyne* spp.

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Abstract:

This study was conducted to evaluate an integration control strategy in controlling of tomato root knot nematodesby using resistant hybrids, the biological pesticide Verox, Nano-zinc oxide [ZnO NPs] and the chemical pesticide Velum under laboratory and field conditions. The results of the biocidal Verox at concentration of 2000 parts per million and ZnO-NPs at concentration of 4000 parts per million showed a significant decrease in the hatching of nematode eggs at a rate of 94.67 and 87.34%, respectively. compared to the untreated control treatment However The use of 400 ppm, 2000 ppm, and 4000 ppm of the nematicidal Velum prime reached 100%. On the other hands cultivars of tomato plants Samar , Meyameya and Maysaloun, significantly reduced the formation of root nodes and the level of infestation with caecilians compared to the control treatment. Maysaloun cultivar was the best among the other cultivars with a rate of root nodule formation of 0.14 and infection rate of 0.09.

Keywords: Tomato hybrids, biological and chemical agents, root knot, hematology, *Meloidogyne* spp.

Introduction

The tomato, *Solanum lycopersicum* L., which belongs to the Solanaceae family, is the second most consumed vegetable crop after the potato, whether for local use or for export. It is native to the Andes Mountain in South America and from there it spreaded to Europe. Currently, China is the main producer of tomatoes, followed by India and the United States of America [1]. Tomatoes are grown all over the world with an annual production of 182 million tons within an area estimated at 4.8 million hectares [2]. The fruits of tomato have a high nutritional value because they contain many vitamins such as A and C and mineral elements such as iron and phosphorous as well as the antioxidants [3].

The tomato crop is infected in various regions of the world and throughout the seasons of the year by a group of fungi, bacteria and viruses, as well as other pests, including root-knot nematodes [4]. The first infection with root-knot nematode was recorded on the tomato plant at the end of the nineteenth century in Italy, and the disease was attributed to *Heterodera radicicola*, which is the former name of the nematode *Meloidogyne javanica* [5]. Root-knot nematodes [RKN] affect plant health and

growth by inducing the formation of giant cells in the root zone, which result in a decrease of plant nutrition and water absorption leading to a decrease in the rate of photosynthesis accompanied by the appearance of symptoms on the vegetative group such as wilting, stunting and yellowing [6]. These damages can be reduced by decreasing the number of RKN in the soil into below the level of economic damage. This can be achieved by following one or more methods of control.

The pesticides are the most common exploited method in controlling of this nematode. However, their negative impact on humans and environment, toxicity to non-target organisms, long survival and high costs have leaded to a complete ban or restriction of the use of them in several countries worldwide [7]. Therefore, it was necessary to exploration alternative methods that are effective, safe for the environment, and inexpensive at the same time. Biological control has received a wide attention by using of living organisms or their products to remove or reduce the influence of many harmful bacteria, fungi and nematodes. For example, *Rhizobacteria* is one of the most important biological factors such as *Pseudomanas* and *Streptomyces* that have reduced the number of nematodes [8]. In recent years, nanoparticles have contributed as an option for nematode management. These nanomaterials are more environmentally friendly and less polluting [9]. Therefore, this study aimed to control the RKN using some varieties of tomato approved for cultivation besides some biological and chemical agents.

Material and methods

Preparation of tomato hybrids used in the field experiments

Three hybrids certified plant tomato, a Maysaloun, Samar, Meyameya, in addition to the class Super marmond, were soaked with distilled water for 4 hours and then put on tissue paper in Petri dishes to dry then planted in plastic pots filled with a mixture of soil and compost in the ratio of 1:2 that were sterilized with an Autoclave and watered daily until the conducting of the field experimental and using the seedlings raised .

Preparation of root knot nematodes *Meloidogyne* spp. inoculum

A previous procedure [10] was followed to extract RKN inoculum. This was by collecting infected tomato plants from one of the orchards in Al-Hussainiya sub-district /Karbala Province, Iraq after observing the symptoms of infection on the vegetative group with yellowing and wilting of leaves. The samples were transferred to the laboratory, then the affected roots were washed with water to remove the suspended dust, and they were cut with sterile scissors into small pieces 2-3 cm and placed in a glass beaker with a capacity of 1000 ml of sodium hypochlorite solution [NAOCL 1% concentration] for 3 minutes and after shaking for a period of three minutes. It was placed in the electric mixer for 30 seconds, then the solution was passed through a series of sieves of varying diameters100, 150, 250, 400 mm. Second stage juveniles J2 After collecting the inoculum in a beaker,1 ml of the suspension was taken by a sterile pipette

to the counting slide and the numbers of eggs and second stage juveniles were calculated using the installed optical microscope 40 X

Evaluation of the effectiveness of different concentrations of the biocidal Verox and Zinc oxide nanoparticles ZnO-NPS against the root knot nematodes *Meloidogyne* spp.

Plastic dishes [Petri dishes] were prepared, and 5 ml was added for each of the materials used in the experiment, at three concentrations [1000, 1500, 2000,] ppm of zinc oxide nanoparticles [ZnO-NPs] after being dissolved in acetic acid HCL and the volume was supplemented to 7 ml by adding 100 eggs / dish. The Velum Prime was used at a concentration of 400 ppm according to the manufacturer's recommendation, the dishes were placed in the incubator at a temperature of 28 ± 2 C, then the hatched eggs were counted under the microscope lens with a powerful magnification over three times 24, 48, 72 hours according to the following equation:

Number of rotten eggs = Number of rotten eggs/Total number of eggs ×100

The corrected percentage of inactivated eggs was calculated [11]

Comparison in egg damper= Corrected percentage of hatching inhibition- Treatment in egg damper/comparison in the egg damper -100×100

Assessment of the influence of different concentrations of the biocidal Verox and ZnO-NPS on the root-knot nematodes *Meloidogyne* spp.

A 3 ml of the solution containing 100 juveniles [J2] of Meloidogyne spp. were placed in a petri and the volume was increased to 10 ml for each of the materials used in the experiment and with three concentrations of each This experiment was carried out according to a Complete Randomized Design [CRD] and with three replications for each concentration with a comparison work using sterile distilled water They were paced in the incubator at a temperature of 28 + 2 C, then the readings were taken after 24, 48, 72 hours by counting the non-moving larvae [adults] in the dish [the larvae are considered dead if they are pricked in addition to having a straight hair and brown [12] and the percentage of deaths was calculated .

By adopting the following equation:

The number of immobile juveniles = Percentage of adolescent deaths/Percentage of adolescent deaths×100 [13]

Control of root-knot nematodes *Meloidogyne* spp. using hybrids of tomato under greenhouse conditions

The seedlings of tomato plants aged 5-6 real leaves were transferred to 1.5 kg plastic pots filled with a mixture of soil and peat moss at a ratio of 1:2 sterilized with formalin at a concentration of 1% [the soil was sifted with sieves to remove large lumps, and stones were added to Peat moss organic fertilizer in a ratio of 1:2 soil/fertilizer, then it

was placed over a piece of polyethylene nylon and well moistened with formalin and covered tightly for 48 hours, then the soil was exposed for a week to get rid of formalin residues according to the random design, at a random rate of 4 Design Complete with three replicates / cultivar and left for a week to stabilize the status of the plants, then inoculated with root-knot nematodes RKN by 1500 + 50 eggs and juveniles / pot, the nematode inoculum was added using a sterilized, graduated pipette tube to extract the follicles [14] and covered with light soil and watered the plants when needed and fertilized with NPK fertilizer [20:20:20] at a rate of once every 14 days at a concentration of g / liter .

The experiment included the following treatments:

1 -Ordinary control treatment / Verox and Humbid, a preventive and curative product from the American company Marvel. Its active substance is a group of environmentally friendly bacteria Rhizobacteria. It was used at a concentration of 2000ppm and on Phase and another 15 days

-2 Treatment of the control with nanomaterials / zinc oxide nanoparticles and it was used at a concentration of 4000ppm and in three stages between each stage and another 15 days

3-Chemical control treatment / Velum Prime pesticide, which is a chemical pesticide that contains the active substance Fluopyram, produced by the German company BAYER, and it was used at a concentration of 400 ppm / liter according to the recommendation of the producing company and in two phases Each stage and the other 20 days .

4-Chemical control treatment Velum Prime pesticide I used a concentration of [300 ppm] less than the recommended dose and in two stages between each stage and another 20 days .

5-The integrative treatment / included the use of the chemical pesticide Velum Prime [300ppm] + the biocidal [2000ppm] Verox + zinc oxide nanoparticles [4000ppm] ZnO-NPs].

6-Contaminated comparison treatment / inoculum use only without adding any pesticide or nanomaterial .

7 -Uncontaminated comparison treatment / without any additives.

The biocidal, chemical, and nanomaterial were added 3 days after adding the inoculum to the treatments, except for the uncontaminated control treatment, at a rate of 100 ml for each plant by immersion in the soil, and left for two months to take the readings, according to the calculation of and it consists of five degrees.

Results and Discussion

laboratory experiments

The results of [Table 1] showed that there was an effective influence of the biocidal Verox and the zinc oxide nanomaterial ZnO-NPs in inhibiting the hatching of eggs of root-knot nematodes RKN after three days of treatment. Verox showed the most

efficient effect at 2000ppm concentration. The eggs hatched were inhibited with 94.67% and while at a concentration of 1000ppm the inhibition rate was 75.34%. As for the application of ZnO-NPs, the percentage of inhibition ranged higher at 4000ppm with a percentage of 87.34%, and the lowest at 1000ppm was 51%. However, the control for the chemical pesticide Velum Prime amounted to 100%. On the other hand the inhibition rate in the control treatment containing distilled water was zero. This is consistent with what [15] found that the pesticide Velum Prime has the ability to inhibit the hatching of root-knot nematodes RKN eggs and stop the development of the embryo inside the egg by killing the embryo or young adults of the first stage before their hatching and the second egg explodes. The pesticide contains a triple fluorine atom, which enables it to penetrate the egg membrane and kill the embryos and prevent their development.

The treatment of the biocidal Verox and ZnO-NPs showed a significant effect in inhibiting egg hatching, and this effect was increased by increasing the concentrations in each treatment as well as increasing the exposure period. These results agree with what[16] and [17] found about the effect of bacterial isolates on preventing the hatching of root-knot nematodes eggs. Meloidogyne spp. Under laboratory conditions, the highest decrease in egg hatching was recorded after 24, 48, 72 hours, respectively, which activates the enzyme-specific enzymes. Chitinase and Chitin enzymes and their breakdown of chitin in the eggshell. Or the reason may be attributed to the bacteria production of toxins, hydrogen cyanide and DAPG 2,4-DiAcetylPhloroGlucinol-2,4], which affect the reduction of egg hatching [18]. Zinc oxide nanoparticles, ZnO-NPs, explanation in reducing the hatching eggs of root-knot nematodes. It agrees with results of[19]. The cause may be approved to the toxicity of the consequence [20]

As noted from the results of [Table 2], there was a significant effect of the biocidal Verox and ZnO-NPs on the death of second-stage juveniles 12. Root-knot nematodes RKN effect increased by increasing thier concentrations as well as the length of the coefficient. The biocidal Verox was the most efficient at 2000ppm concentration, as the percentage of adolescence death was 94.33%, When using the nano-material, the percentage of zinc oxide-ZnO-NPs ranged above 33%, such as at a concentration of 1000ppm was 48.33% percentage, and this result is compared to the control treatment of the chemical pesticide Velum Prime, which reached 100%, However, the comparison treatment containing distilled water, zero percentage, and this is in agreement with what was found by[21] that the chemical pesticide Velum Prime with a concentration of 400ppm significantly affected the destruction of the laboratory, where the concentration increased by 51%, with an increase of 51% hours later 72.

As for the effect of the biocidal Verox on the mortality of second-stage juveniles 12. It is in agreement with finding of [22] about the effect of bacteria on the mortality rate of instars of the total 4, the second J2 from the root-knotting nematode Meloidogyne incognita was in vitro. 212 of the 662 strains of the plant species caused very high mortality, compared to the other races in the two races, Bac.It was attributed the efficiency of the pesticide bio-active role to produce hydrogen cyanide Hydrogen Cyanide was also detected many of the enzymes and hormones of them Chitinase, Siderophores, Dissolve phosphorus, Indole Acetic Acid, Protease barcinonensis led these bacteria to the destruction of imagines the second phase of the laboratory by 100% [23] mentioned that the secondary receptors produced by bacterial strains are responsible for the biological control activity and the effect of these strains on Bacillus aryabhatustai, bacce firm1 bacillus. laboratory. another study indicated by[24] from the use of four isolates of bacteria and test their impact on the nematodes hold the roots Meloidogyne javanica to imagines the second phase and J in the laboratory and gave all isolates the results of effective at higher concentrations. showed the treatment of zinc nanoscale ZnO oxide - NPs effect Moral in doom It imagines the second phase and this is in line with [25]and attributed the cause to the toxicity of nanoparticles dissolved zinc [26] or because of the negative effect of zinc oxide on the nematodes through the effect on fat and glycogen multiple sugars external wall of the nematodes [27]

Experiment field

The results showed that the usage of chemical pesticides and bio-nanomaterials have had a positive effect and reduced the incidence of nematodes hold the roots and have reduced the number of nodes on the roots of varieties of tomatoes used in the experiment and hence reduce her severity of nematodes. It outperformed all treatments in reducing the number of nodes on the roots and the severity of the injury and differed significantly from the treatment of contaminated comparison nematode which only reached the average number of contract in which 41.66 and the degree of injury 2:25 by scale [28]and with respect to the rate of species observed that more varieties efficient .

Maysaloun cultivar recorded the lowest number of knots with an average of 0.14] and the infection degree reached 0.09, followed by the Samar and Meyameya grade with 0.42, 3.47 and the infection degree was 0.33 and 0.71], respectively, and these results compared to the sensitive cultivar treatments with a mean of 76 and Super marmand.42 grades of infection 1.Evident from the results referred to in tables [3, 4] the important role of the factor Biological control using the pesticide Verox under study, which reduce the incidence of nematodes hold the roots RKN varieties of tomato studied and

cultivar Maysaloun the rest of the items did not score any number held, followed by class Samar Meyameya reached The number of knots was 0.66 knots for both cultivars and the degree of infestation was 0.66, 0.33], respectively, compared to the sensitive variety Super Marmand, as the average number of knots on the roots was 7.33 and the infestation score was 2.00, and these two contaminations recorded significant differences with any treatment - Significantly than the contaminated control treatment, the degree of infection was 2.25, and the number of nodes was 41.66 knots . This study is in agreement with a previous studies [29] .[30]When using bacteria Bacillus subtilis to control root-knot nematodes in two varieties of tomato One of them is sensitive and the other is resistant, and inoculation after planting reduced the number of the nematode in the roots of the tomato is handed, thus reducing the number of nodes on the roots. The reason for reducing the number of nodules on the roots of tomato cultivars inoculated with root-knot nematodes is attributed to the biological factor, which is the inhibition of nematode egg hatching, and this in turn stimulates the stimulation of the enzyme, Chinase [31]The treatment of the effect of zinc oxide nano-ZnO-NPs [under study] on root-knot nematodes RKN showed that Maysaloun cultivar was the most effective for controlling nematodes through no infection was recorded, followed by Samar and Meyameya Supermand cultivar, with a score of 0.3, Mar3. The number of nodules on the roots was 16.00 and the degree of infection was 2.66, and they did not differ significantly from the uncontaminated control treatment as well as the control treatment with the chemical pesticide at the recommended dose [400ppm] and the integration treatment, and these results are in agrement with[9] results stated that nano-fertilizers play their role in discouraging harmful micro-organisms in the soil, including root-knot nematodes, to fertilize tomato cultivation under global warming conditions, while at the same time promoting plant growth and maintaining healthy plants. The results of using the chemical pesticide Velum Prime at the recommended concentration showed a clear consequence and did not differ significantly from the uncontaminated comparison treatment and the integrative treatment, and no infection was recorded for the cultivars, the nodes number was 1.33 and the infection degree was 1.00 on the effect of the Super Marmand compared to the chemical pesticide used

The results of the integrated control treatment using resistant cultivars, a biocidal and a chemical pesticide at a dose lower than the recommended one showed significant results in dropping the number of knots at a rate of 0.25 and an infection degree at a rate of 0.16 and did not significantly differ from the two treatments compared to the two treatments. As it was noted from the results, there are significant differences in the contaminated comparison treatment among the tomato cultivars, where the Meyameya class excelled by registering the highest number of root nodes at a mean rate of 22.00,

and the mean score was 0.30, and the infection was 0.6, respectively. , 1.00, respectively, compared to the cultivar Super Marmand, which is sensitive to infection, as the average number of nodes on the roots was 142.66 and the degree of infection was 4.66

Among the mechanisms that explain the effect of bacteria and their effective role in the fight is that they produce toxic compounds that prevent the growth and activities of nematodes, or competition for food and induced systemic resistance or the production of antibiotics, and its responsibility is to reduce bacterial infections called B. Proteins called delta-endotoxins that disrupt the digestive pathways of nematodes by the formation of pores in membranes [32] by the influence of nucleic influence on the nematodes. The primary target tissue is the intestines of nematodes. Zinc oxide toxicity is believed to be a combination of the effects of NPs and the cause of toxicity. Dissolved zinc. It is believed that zinc oxide generally affects the skin of nematodes and works to increase antioxidant activities. Zinc is considered a living factor .

For plant growth and development, zinc also plays a major role in plant metabolism by regulating the activities of key plant enzymes such as Anhydrase Carbonic [33]. ZnO-NPs reduce UV-induced stress as well as protect against a wide range of biotic and abiotic stresses. ZnO-NPs have been shown to protect the photosynthesis system from oxidative stresses [34]. The effective role of the chemical pesticide Velum Prime is due to it inhibiting the respiratory chain in the mitochondria and stopping the production of Adenosine triphoate, which is the main substance for providing energy in the living cell, leading to short-term death of the digestive system and its death. Selective [35]. Several studies indicated the effectiveness of Velum Prime in suppressing nematode species and reducing their damage to various plants such as tomato, cotton, and soybean, whether in the field or greenhouse, and leading to low concentrations of nematodes [36]. Previous studies indicated that the use of nanoparticles with the biological control agent Verox plays an important role in the control and management of crops. Plant growth and stress management as well as providing a suitable environment for the synthesis and trapping of nanoparticles. A study indicated carried out b y[37] The combined application of chemical pesticide Biological Brassinolide Velum and Neemheyoupo led to improved plant growth of tomatoes and increase the activity of protective enzymes growth and all treatments have had a positive effect but is considered Velum and Brassinolide is optimized to control nematodes.

Table [1] The effect of the Verox, ZnO-NPs and Velum Prime on the egg hatching of RKN

Treatment	concentration	Number of rotten eggs			Inhibition %
		24 hours	48 hours	72 hours	
Verox	1000 ppm	18.00	20.66	24.66	75.34
	1500 ppm	10.00	13.00	13.66	86.34
	2000 ppm	3.66	5.33	5.33	94.67
ZnO-NPs	1000 ppm	36.00	45.66	49.00	51
	2000 ppm	23.33	36.00	40.00	60
	4000 ppm	10.00	11.00	12.66	87.34
	400 ppm	0	0	0	100
Velum Prime		78	92	100	0.00
Control		6.86	10.99	7.17	

Table [2] The effect of different concentration of the Verox and ZnO-NPs on the vitro J2 root- Knot nematode

Treatment	concentration	Number of dead juveniles			Inhibition %
		24 hours	48 hours	72 hours	
verox	1000 ppm	60.66	63.33	72.33	72.33
	1500 ppm	52.33	81.00	86.66	86.66
	2000 ppm	66.00	83.00	94.33	94.33
ZnO-NPs	1000 ppm	32.66	40.66	48.33	48.33
	2000 ppm	39.33	54.33	66.33	66.33

	4000 ppm	64.00	76.00	88.33	88.33
Velum Prime	400 ppm	92	100	100	100
Control		0	0	0	0.00
[L.S.D[0.05		8.26	14.83	8.16	

Table [3] The effect of the use of pesticides on the number of root nodules resulting from infection with root knot nematodes of the studied tomato cultivars

Treatment	Number of root nodes				rate
	Masaloun	Meyameya	Samar	Super marmand	
[Verox [2000ppm	0.00	0.66	0.66	7.33	2.16
[ZnO-NPs [4000PPM	0.00	1.33	0.33	16.00	4.41
[Velum Prime[400ppm	0.00	0.00	0.00	1.33	0.33
[Velum Prime[300ppm	0. 33	0.33	0.66	2.66	1.00
Complete [2000+4000+300] ppm	0.00	0.00	0.00	1.00	0.25
Polluted comparison	0. 66	22.00	1.33	142.66	41.66
Proper compassion	0.00	0.00	0.00	0.00	0.00
Items rate	0.14	3.47	0.42	24.42	
[L.S.D[0.05	Treatments	Categories	overlap		
	9.8773	7.4666	19.755		

Table [4] Effect of pesticide use on the degree of infestation of root knot nematodes of the studied tomato cultivars

Treatment	Number of root nodes				rate
	Masaloun	Meyameya	Samar	Super marmand	
[Verox [2000ppm	0.00	0.66	0.33	2.00	0.75
[ZnO-NPs [4000PPM	0.00	1.00	0.33	2.66	1.00
[Velum Prime[400ppm	0.00	0.00	0.00	1.00	0.25
[Velum Prime[300ppm	0.33	0.33	0.66	1.33	0.66
[Complete 2000+4000+300 ppm	0.00	0.00	0.00	0.66	0.16
Polluted comparison	0.33	3.00	1.00	4.66	2.25
Proper compassion	0.00	0.00	0.00	0.00	0.00
Items rate	0.09	0.09	0.71	0.33	
[L.S.D[0.05	Treatments	Categories	overlap		

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