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EVALUATION OF THE EFFICIENCY OF SOME NANOCOMPOSITES IN ROOT-KNOT NEMATODES (MELOIDOGYNE SPP.) UNDER LABORATORY CONDITIONS

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Abstract

Root-knot nematode (Meloidogyne spp.) is the most important plant parasitic nematode that seriously affects the majority of agricultural crops, where the vessels are targeted inside the roots of the plants, which leads to the lack of water and nutrients from reaching the plants, and thus leads to wilting, yellowing, and stunting in the vegetative section, knots and swellings in the root.

The aim of the study was to evaluate the ability of some nanocomposites (silver Ag-NPs, aluminum oxide nanoparticles, ALO-NPs, ZnO-NPs nanoparticles and Si-NPs silicon nanoparticles) at concentrations of (1000,2000 and 4000) for each one of them to inhibit root-knot nematode egg hatching. These nanocomposites have also been used to evaluate their ability to destroy second-stage juveniles of root-knot nematodes. The study was conducted on the roots of cucumber plants from the greenhouse in the College of Agriculture / University of Karbala. The results of the study showed a significant effect of nanocomposites on the inhibition of egg hatching of root-knot worms and the destruction of second-stage juveniles of these worms. Aluminum oxide nanoparticles (ALO-NPs) parameter was the most efficient and effective in both inhibiting the hatching of nematode eggs and destroying second-stage juveniles of root-knot nematodes. It was found that with increasing concentration, there was a corresponding increase in hatching inhibition and mortality of second-phase juveniles of root-knot worms. We conclude from the current study that the nanocomposites possess streptococcal nematicidal activity and can serve as an alternative to high-risk synthetic nematicides or irregular biological control agents without inducing any phytotoxicity.

Keywords: Root-Knot Nematode; Nanocomposites; Ag-Nps; Aluminum Oxide Nanoparticles; ALO- NPs; ZnO-NPs; Hatching; Second-Stage Juveniles.

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Introduction

Root-knot nematode (*Meloidogyne spp.*) Parasites that settle inside the roots of the plant target the vessels, which leads to depriving the host plant of access to water and food, causing wilting, yellowing, and dwarfing on the vegetative system, and knots and swelling in the root system. They are obligate parasites that feed on hundreds of different plant species, including herbaceous and woody plants as well as monocotyledons and dicotyledons, it is dealt with by combating it with chemical pesticides to eliminate it (Tapia et al., 2022).

Due to environmental and human health concerns, alternative methods have been used to control these worms, including the control of nanomaterials. Nanotechnology has revolutionized the agricultural field because it is safe for the environment and enhances the ability of plants to absorb nutrients. These materials are expressed in nanometers (1-100 nm or 1.5 × 109 m) (Alamriet al., 2022). One of the previous technologies did not receive much attention, such as nanotechnology, which is the technology of the twenty-first century and is the magic key to progress and economic growth. The word nanotechnology was first used in Tokyo by researcher Norio Taniguchi, although it was not widespread at the time, and it affects the genetic material. al., 2016, Sanjay and Pandey, 2017).

Silicon oxide nanoparticles are known for their beneficial effects on plant growth and physiological activities, relieving biological stress in plants and stimulating defense in plants against pathogens, such as fungi, bacteria, viruses and nematodes, and can be effective due to their induction of defense (1) Gene expression is responsible for shoots The genetic characteristics of the plant and its connection at the level of the nitrogenous base is the basis for determining the amino acid and the genetic code. Therefore, according to the nitrogenous base, the genetic code differs, for example, the starting code is AUG. When the nitrogen base U uracil is replaced and replaced, for example, C cytosin, the code becomes ACG, which is a radically different code fromthe first. Hence the effect on proteins, which is associated with pathogen-related proteins, and hypersensitivity responses, is also used in the agricultural sector to develop nano-fertilizers and improve seed germination efficiency while protecting against several types of plant pathogens (Wang et al., 2017, Rajput et al., 2021).). Silicon oxide particles show different physical and chemical properties than bulk materials, which also had a positive effect on plant growth and development and SiONPs can be used as nano-insecticides, nano-herbicides and nano-fertilizers (Adhikari et al., 2020).

Zinc oxide is an inorganic compound in the form of a white powder with the composition ZnO. Zinc oxide is one of the most common nanoparticles in the world - after silver, carbon nanotubes, titanium dioxide and gold. It is an essential mineral for plant growth, is involved in many physiological and enzymatic activities, and contributes to protein and carbohydrate synthesis, DNA and chlorophyll biosynthesis, energy production, and macromolecule metabolism, serving as an enzyme component, a catalyst, or a structural cofactor. Aluminum oxide nanoparticles are among the most preferred nanoparticles. As an element, aluminum has been studied for many years, and its toxicity is relatively well known. However, the toxicity of Al2O3 nanoparticles is still not fully known, and some researchers have suggested that oxidative stress and DNA damage are responsible for their micro-toxic effects (2). It is often widely used in industries, and its use has recently expanded in the agricultural field (Ingle and Gupta, 2021, Dağlioğlu et al., 2022).

Materials and Methods

Preparation of hanging root knot worm eggs

A quantity of cucumber plant roots were brought from the greenhouse of the College of Agriculture / University of Karbala after noticing the symptoms that appear on them with wiltingand yellowing of the vegetative system. The roots were cut with sterile scissors into small pieces (1-2 cm), then 100 grams of them were taken and placed in an electric mixer, 250 ml of sodium hypochlorite solution (NAOCL) at a concentration of 0.01 was added to it to separate the eggs andthe mixer rotated for 30 seconds, then the solution was passed through Through four sieves of different sizes (100, 250, 300, 400 mesh) where the first and second are done to isolate the impurities and cut the large roots and impurities. A final sieve to collect the eggs released from the

roots and get rid of the remnants of sodium hypochlorite, pass the last sieve with water for 1 minuteand then collect the eggs into a glass beaker according to Javid's method (Javid et al., 2007).

Prepare hanging eggs

After obtaining nematode eggs, the number of eggs was estimated using the counting method described by Quinn (2007). The suspension (autoclaved sterile distilled water) containing nematode eggs is shaken for homogenization and egg distribution, then 1 ml of the solution is withdrawn through a sterile pipette and transferred to a counting slide. The number of eggs in 1 ml was then counted using an optical microscope with 40X magnification. The method was repeated 5 times to confirm the number of eggs in 1 ml containing 100 eggs.

Vaccine preparation for second stage adolescents

Suspended eggs were placed in large 20 cm petri dishes. Then the dishes are placed in the incubator at a temperature of 28 ± 2 °C for three days to incubate the eggs in the juvenile. Featuring100 little girls (House 1973).

Effect of nanocomposites in inhibiting root-knot nematode hatching

Petri dishes with a diameter of 9 cm and a height of 2 cm were prepared, and 6 ml of each of (Ag-NPs, ALO-NPs, ZnO-NPs and Si-NPs) was added at three concentrations for each treatment,namely (1000, 2000, 4000) parts per The millionth. Volume was completed to 7ml by adding 100eggs/dish. This experiment was carried out according to a complete randomized design (CRD) with three replications for each concentration with comparison work using sterile distilled water. Hatched eggs were counted under a microscope at x40 magnification and tripled at 24 h, 72 h and7 days. The corrected percentage of inactivated eggs was calculated using the Abbott equation (1925):

$$Corrected\ hatching\ inhibition\ percentage = \frac{\text{Eggs inhibiting } parameter - \text{Eggs damper in comparison}}{Eggs\ damper\ in\ comparison - 100} \times 100$$

Effect of nanocomposites on the destruction of the second stage of root-knot nematodes

Petri dishes with a diameter of 9 cm and a height of 2 cm were prepared, and 10 ml of each of(Ag-NPs, ALO-NPs, ZnO-NPs, and Si-NPs) was added in three concentrations for each parameter,which are (1000, 2000, 4000) ppm. This experiment was carried out according to a Complete Randomized Design (CRD) with three replications for each concentration with comparison work using sterile distilled water, and placed in the incubator at a temperature of 28 ± 2 °C, then the readings were taken at three times 24 hours, 72 hours, 7 days through Count the immobile larvae (adults) in the plate. The percentage of adolescent death was calculated using the following equation:

$$\text{Percentage of adolescent death} = \frac{number\ of\ immobile\ juveniles\ in\ the\ plate}{\text{Total number of tested juveniles in the dish}} \times 100$$

Results

Effect of nanocomposites in inhibiting root-knot nematode hatching

The results showed a significant effect of nanocomposites on inhibiting the hatching of RKN worm's eggs, and this effect increases with increasing concentration as well as increasing the exposure period. At a concentration of 1000ppm (1g), aluminum oxide nanoparticles ALO-NPs parameter at a concentration of 4000ppm showed the most efficient and effective in inhibitingnematode eggs hatching by 91.7% and this percentage decreased by decreasing the concentration

used to record its lowest level at a concentration of 1000ppm with an inhibition rate of 58.0%. Thelowest percentage was Si-NPs at a concentration of 1000 ppm, which amounted to 41.4%, and thepercentage of inhibition of nematode eggs hatching was increased for the same parameter with an increase in the concentration used, which reached 56.0%. At a concentration of 2000 ppm and 74.7% at a concentration of 4000 ppm. ZnO-NPs parameter of nanoparticles showed a variation depending on the type of concentration, the maximum was at a concentration of 4000 ppm with an inhibition rate of 78.7% and the lowest at a concentration of 1000 ppm with a percentage of 51.4%,these results differed significantly from the control parameter, which had inhibition of 2% (Table 1).

parameters	concentration	number of hatched eggs			Inhibition percentage
		24 hours	72 hours	7 days	
ZnO-NPs	1000ppm	17.7	36.0	48.6	51.4%
	2000ppm	23.3	39.3	48.0	52.0%
	4000ppm	10.3	16.0	21.3	78.7%
ALO-NPs	1000ppm	22.7	36.0	42.0	58.0%
	2000ppm	10.3	13.7	20.3	79.7%
	4000ppm	4.3	6.7	8.3	91.7%
Si-NPs	1000ppm	32.7	52.7	58.6	41.4%
	2000ppm	20.7	34.7	44.0	56.0%
	4000ppm	15.3	20.3	25.3	74.7%
Ag-NPs	1000ppm	17.0	34.3	47.3	52.7%
	2000ppm	21.0	27.3	30.6	69.4%
	4000ppm	8.7	14.0	17.0	83.0%
Control	___	41	89	98	2.0%
L.S.D 0.05	___	6.58	5.69	11.39	—

Table1. Effect of different nanocomposites concentrations on the root-knot worms eggs hatching

* The numbers are averages of three replicates

Effect of nanocomposites on the destruction of the second stage of root-knot nematodes

The results showed a significant effect of nanocomposites on the destruction of the second-stage juveniles of root-knot worm's concentration in each parametert as well as increasing the exposure period to the nanomaterial. The nanocomposite ALO-NPs had a high effect at the concentration of 4000ppm, with a death rate of 94.3%, and the lowest death rate for the juvenilesfor the same parameter at the concentration of 1000ppm, and it reached 59.3% after 7 days of exposure of the juveniles to the nanocomposite. When using ZnO-NPs parameter, the highest killing rate of juveniles at the concentration of 4000ppm was 81.0%, and the lowest killing rate atthe concentration of 1000ppm was 47.6%. The lowest percentage among the parameters was SI- NPs with a concentration of 1000ppm and it amounted to 40.3%. The rate of killing of second stage juveniles of root-knot worms by the same factor increased by increasing the concentration (4000 ppm) and reached 75.0%. The parameter Ag-NPs at the concentration of 4000ppm showedthe most efficient and effective in killing the second-stage juveniles of root-knot worms with a rate

of 86.0% and this percentage decreased with a decrease in the concentration used to record thelowest level at the concentration of 1000ppm with a killing rate of 54.6% (Table 2).

parameters	concentration	number of dead juveniles			Inhibition percentage
		24 hours	72 hours	7 days	
ZnO-NPs	1000ppm	19.7	36.0	47.6	47.6%
	2000ppm	23.3	41.0	62.3	62.3%
	4000ppm	37.0	56.6	81.0	81.0%
ALO-NPs	1000ppm	22.3	43.0	59.3	59.3%
	2000ppm	28.0	58.0	85.6	85.6%
	4000ppm	42.3	82.6	94.3	94.3%
Si-NPs	1000ppm	12.3	23.6	40.3	40.3%
	2000ppm	20.3	39.3	54.6	54.6%
	4000ppm	26.0	45.3	75.0	75.0%
Ag-NPs	1000ppm	18.3	36.3	54.6	54.6%
	2000ppm	27.0	59.0	76.3	76.3%
	4000ppm	44.0	68.00	86.00	86.00%
Control	___	0	0	0	0.00
L.S.D 0.05	___	5.6	9.7	4.8	____

Table 2. Effect of nanocomposites concentrations on the death of root-knots juveniles

* The numbers are averages of three replicates

Conclusion

In the past few years, the use of NPs in agriculture has become more widespread, and thereis still a growing interest in using nanocomposites to reduce dependence on chemical fertilizers for agricultural development, environmental and food security, and to meet the nutritional needs of an ever-increasing world population (Rajput et al., 2021).

It was observed from the results that there is a positive effect of aluminum oxide nanoparticles on inhibiting root-knot worms hatching eggs as well as killing larvae outweighing the other nanocomposites used in the experiment. Many studies indicated that aluminum oxide nanoparticles are characterized by their hardness and are often used in the field of industry and minerals, but recently they have been introduced as an inhibitor of fungi and insects (Wang and Nowack, 2018).

ZnO-NPs have antimicrobial capabilities as they have been found to be toxic to lipids, glycogen and various polysaccharides of the outer wall of the worms (Kalaba et al., 2021). Several previous studies indicated the efficiency of silver nanoparticles and their role in inhibiting the eggsof root-knot worms as well as killing second-phase juveniles, represented by their effectiveness inmembrane permeability, response to oxidative stress and dysfunction of cellular mechanisms (Abdel-Raouf.2016, Heflish et al., 2021, El-Shafeey et al., 2021).

The results obtained from the use of silicon nanoparticles SiNPs showed that the rate of inhibition of egg hatching as well as killing of larvae increases with increasing concentration and exposure period through its effect on inhibition of egg hatching and reduced larval movement (Taha and Abo-Shady, 2016, El-Ashry et al., 2022).

A study also showed that smaller sizes of nanoparticles are more toxic than larger ones, and it was noted that black silver nanoparticles with a size of 20 nm are more toxic to root knot worms than larger nanoparticles; however, the results were not at the required level, with the exception of the aluminum oxide nanoparticle parameter. The reason may be attributed to a defectin the work of cellular mechanisms that allow penetration of the cell wall of nematode eggs (Sharonet al., 2010).

We conclude from this work that the use of nanoparticles is a promising tool even if it is used alone, and future work should focus on the physical and chemical properties about the toxicity of nanomaterials and their role in control as an alternative to harmful chemical pesticides, whetherin the laboratory or the field.

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