Comparative Efficacy of Chitosan-Silver Nanoparticles and Bioproducts as Alternative Strategies Against Root-Knot Nematode *Meloidogyne incognita*, and Their Impact on The Non-Target Organisms

M.S. Hamed, H.M. Nasr, M.A. Abbassy, E.I. Rabea [1]

ABSTRACT

Nematodes with diversified parasitism are deliberated an significant pest all over the world, producing substantial damage to agricultural crops and decreasing their yield, valued at over $175 to 200 billion worldwide. The current research was performed to evaluate the potentials of four growth promoters, chitosan, chitosan-silver nanoparticles (Ch-AgNPs), bacterial biopesticide (*Pseudomonas fluorescens*) and phosphite for the control of the nematode *Meloidogyne incognita* root-knot nematode. The capacity of chitosan to synthesize silver nanoparticle Ch-AgNPs was investigated in this work. Scanning electron microscopy (SEM) was employed to characterize the Ch-AgNP. The mean particle size of Ch-AgNPs ranged between 81.08 and 216.22 nm. Ch-AgNPs demonstrated notable nematicidal activity with an impressively low LC_{50} value of 39.19 and 26.12 mg/L against J_2 larvae of *M. incognita* after 24 and 48 hours of treatment, respectively. Honeybee mortality increased with increasing concentrations of Ch-AgNPs. At the highest concentration tested (500 mg/L), mortality rates reached 20% after 72 hours, indicating a moderate impact on honeybee survival compared to other compounds. Earthworm mortality increased with higher concentrations of Ch-AgNPs. Mortality rates ranged from 10% to 46.67% after 4 weeks, indicating a moderate to high impact on earthworm populations. The results for Ch-AgNPs and phosphite even at the highest concentrations tested, showed these materials displayed very low toxicity towards earthworms, with weight loss similar to the control group.

Keywords: Nematicidal effect, chitosan-silver nanoparticles, *Pseudomonas fluorescens*, phosphite, *Meloidogyne incognita*.

INTRODUCTION

Plant-parasitic nematodes cause important damage to utmost agricultural crops (Sikora and Fernandez, 2005) with yearly losses expected to be $100 billion worldwide (El-Ghareeb *et al.,* 2020). The root-knot nematode (*Meloidogyne* spp.) is communal and affects a widespread range of crops cause grave injury to utmost agricultural crops around the world (Ghada *et al.,* 2022). *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne arenaria*, and *Meloidogyne hapla* are the most destructive nematodes for crops, infecting above 3000 host species (Shilpa, 2022). Plant-pathogenic nematodes decrease crop yield by 8.8% in developed countries and up to 14.6% in tropical and subtropical regions. Root-knot nematodes pose a serious threat as they infest plant roots, causing in substantial damage as they interfere with plant physiology, leading to condensed crop yield and negotiated product quality (Niu and Xu, 2020). Infestation of these nematode species results in the development of galls or root-knots on infected plants. Other symptoms include hindered growth, wilting, and condensed fruit production (Tapia-Vázquez *et al.,* 2022). *M. incognita* can cause crop failure in the lack of effective control. Avoidance and control of such pests will continue a significant objective of most investigates. Nowadays, they are controlled by cultural practices, chemical nematicides, and by the increasing of resistant cultivars (Curto *et al.,* 2012). Chemical control is expensive and is economically viable only for high-value crops and creates a potential hazard to the environment and human health. Hence, alternative nematode control methods must to be advanced (Desaeger *et al.,* 2020). One way of searching for such nematicidal compounds is to screen eco-friendly nematicides (Singh, 2023).

Chitosan, a highly biocompatible biological macromolecule with unique physiological and biological properties, warrants significant research in pest control. In agriculture, it's widely used as an antifungal agent, biostimulant, soil conditioner, seed treatment, and fertilizer. As a biostimulant, chitosan boosts crop disease resistance by stimulating the secretion of immune enzymes (Riseh *et al.,* 2022).

It has been described as an elicitor to influence the local and systemic resistance against *M. incognita* in tomato (Radwan *et al.,* 2012). Therefore, there is a growing interest in exploring the potential of nanotechnology in various fields, including agriculture, the food industry, pharmaceuticals, and medicine (Usman *et al.,* 2020). Chitosan nanoparticles are natural, environmentally friendly materials. Their exceptional physicochemical and biological properties make them highly bioactive and very safe for humans (Divya and Jisha, 2018). Therefore, chitosan is an excellent biopolymer for coating the anionic surfaces of emulsion droplets. This coating enhances colloidal stability in

DOI: 10.21608/asejaiqjsae.2025.437933
[1] Department of Plant Protection, Faculty of Agriculture, Damanhour University, Damanhour, Egypt.
Received, May 25, 2025, Accepted, June 27, 2025.

acidic conditions and improves the nematicidal activity of natural pesticides.

Phosphite, a reduced form of phosphate, promotes growth in nutrient-rich conditions for various plants like wheat, oilseed rape, oranges, and celery. It also effectively controls several phytopathogenic organisms, including nematodes, acting as a potent pesticide (Yáñez-Juárez *et al.,* 2018). Manganese phosphite effectively deterred *M. javanica* in *Glycine max* (soybean). When applied seven days before nematode infection in a pest-resistant cultivar, it reduced the number of eggs per gram of root (Puerari *et al.,* 2015). Likewise, Li *et al.* (2025) establish that the phosphite usage has the potential to advance crop yield and health, alleviating the challenges posed by the need of feed a growing world population, while reducing the agricultural impression on human health and the environment.

Pseudomonas fluorescens CHA0 as plant growth-promoting rhizobacteria, have been found to be highly effective in controlling plant pathogens like root-knot nematodes (Norabadi *et al.,* 2014). It is known for its high solubilization capacity of soil phosphorus (Galindo-Castañeda *et al.,* 2018). A mixture of *P. fluorescens* and *Azospirillum brasilense* was found to have a positive influence on the yield of three potato varieties (Reed and Glick, 2023). Several *Pseudomonas* species were also found to be effective in managing root-knot nematode under pot experiments (Gowda *et al.,* 2022).

Nanoparticles are small particles ranging between 1 and 100 nm (10^{-9} m) in size. According to Fabiyi *et al.* (2020), nanoparticles with a high surface-to-volume ratio have higher reactivity and biochemical activity. Nanotechnology is now widely used in various sectors, including agriculture and food, with numerous element nanostructures being produced. These nanostructures positively impact both the environment and human well-being. Nanotechnology offers many benefits, such as extending shelf life, mitigating toxicity, and increasing the solubility of low-water-soluble pesticides (Vishnu *et al.,* 2024).

Consequently, the present investigation was conducted with the aim of testing the potential of four growth promoters, chitosan, chitosan-silver nanoparticles, *Pseudomonas fluorescens* and phosphite against root-knot nematode *Meloidogyne incognita* infestation and growth. In addition, safety assessment studies were performed.

MATERIALS AND METHODS

Chemicals and pesticides:

Low molecular weight chitosan (made from coarsely ground crab, 89% degree of deacetylation) was obtained from Sigma-Aldrich Chemical Co. Sodium tripolyphosphate (STPP) was obtained from El-Gomhoria Company, Alexandria, Egypt and used without further purification. Phosphite (Cropper Guard® 33%) was obtained from Cropper Agra agency, Spain. *Pseudomonas fluorescens* (bio pesticide) (Agra Guard®) was obtained from Cropper Agra agency, Spain. Fenamiphos [ethyl 3-methyl- 4-(methylthio) phenyl isopropyl phosphoramidate] (Nemacur® 40 % EC) was obtained from Bridge Trade agency.

Nematodes extraction:

Egg masses separation:

M. incognita egg masses, sourced from *Solanum nigrum* plants in the El-Nubaria region, Behera Governorate, Egypt, were used for inoculum preparation. Eggs were extracted from eggplant roots by stirring them in 0.05% NaOCl for 2 to 3 min (Hussain *et al.,* 2024). The eggs were collected and washed using nested 150- and 25-µm-pore sieves. To obtain second-stage juveniles (J_2) for inoculum, infected eggplant roots were placed in hatching dishes and incubated in a mist chamber. The J_2 were then collected daily for 3 to 5 days using 150- and 25-µm-pore sieves. During this collection period, J_2 were stored in a 1-cm aqueous suspension at 5°C before being used to inoculate peanut plants.

Nematode larvae separation:

A 250 g soil sample was successively wet-sieved through 100, 200, and 325 mesh-sieves. The active nematodes from the fine sieve fraction were then extracted using the Baermann-plate technique (Knapp-Lawitzke *et al.,* 2014). To quantify *M. incognita* second-stage juveniles (J_2), the nematode extract solution was adjusted to a specific known volume. J_2 in each sample were then counted under a microscope using a counting slide.

Nematicidal activity assay:

Nematicidal activity of tested compounds against egg hatching:

A 1 ml suspension, containing roughly 5000 eggs, was added to a labeled, sterilized 9 cm Petri dish that held 2 ml of filtrate from various treatment dilutions (El-Habashy *et al.,* 2020). Water served as the control. Each treatment was replicated three times and arranged in a completely randomized design. The Petri dishes were maintained at room temperature (22-26°C) in darkness, and hatching was observed after 7 days. The total number of hatched juveniles and the percentage of hatching inhibition were calculated using the following formula:

Hatched juveniles (%) = $(\frac{No.hatched\ juveniles}{Total\ no.of\ eggs})x100$

Inhibition (%) = $100\ -percentage\ of\ hatching$

Nematicidal activity of second stage juveniles (J_2):

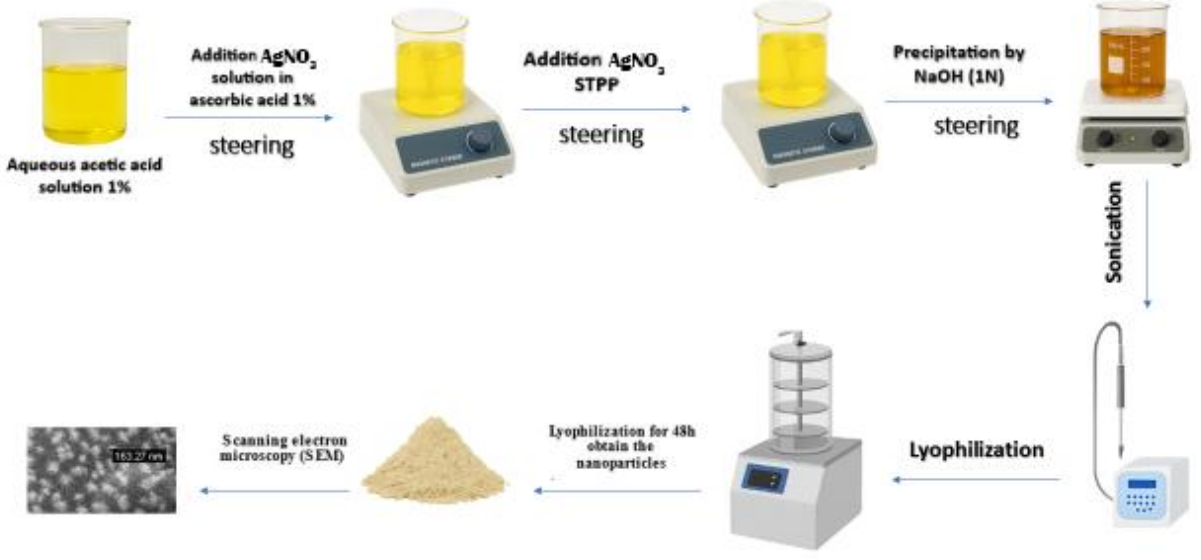
To assess nematode mortality, 1 ml of each treatment dilution (each containing approximately 5000 juveniles, as previously prepared) was combined with 1 ml of *M. incognita* J_2 nematode suspension in individual 50 ml glass capsules. A control was established by mixing 2 ml of distilled water with 2 ml of the nematode suspension. After incubation at 25±1ºC for 24, 48, and 72 hours, the number of viable and dead nematodes was counted under a light microscope. Nematode mortality for each treatment was then calculated, defining "dead" nematodes as those incapable of regaining motility. The % mortality was calculated using Abbott's Formula (Abbott, 1925) as follows:

$$\text{Juvenile mortality (\%)} = \frac{(m-n)}{(100-n)} \times 100$$

Whereas: m a percentage of mortality in the treated sample and n a percentage of mortality in the control. Each treatment was replicated three times.

Preparation of chitosan silver nanoparticle:

Chitosan- silver nanoparticle (Ch-AgNPs) were prepared by direct reduction of $AgNO_3$ with vitamin C as shown in Scheme (1) according to Cao *et al.* (2010) with some modifications. Silver nitrate (0.3% w/v) was dissolved in distilled water then added dropwise under magnetic stirring onto 50 mL of chitosan solution (2%, w/v) (dissolved in 1% acetic acid). 5 mL of vitamin C solution (1%) was added dropwise into the mixture after magnetic stirring for 30 min and the pH was adjusted to 6. The reaction continued stirring for 60 min at 50°C, then STPP solution (1%) was added dropwise to the previous mixture. The suspension was sonicated for 15 min (Ultrasonic Homogenizers HD 2070 with HF generator (G 2070), ultrasonic converter UW2070, booster horn SH 213 G and probe microtip MS 73, Ø 3 mm). The tip of the horn was symmetrically placed in the coarse suspension and the process was carried out at 15 min, power 50 kHz and pulses or cycles of 5 cycle /sec controlled by the software of the device to produce the nanoparticles. Then lyophilized (Alph 1-2 LD plus, Martin Christ Gefriertrocknung sanlagen Gb An der Unteren Söse 50, 37520 Osterode Harz, Germany) for 48h to obtain nanoparticles.



Scheme 1. Preparation of Ch-AgNPs

Characterizations of nanoparticle:

Scanning electron microscopy (SEM):

Morphological characterization of the Ch-AgNPs samples was performed using a JEOL JCM-7000 Benchtop Scanning Electron Microscope (SEM). For analysis, dry particles were dispersed in ethyl alcohol through sonication to ensure individual particle observation. These dispersed particles were then mounted on metal stubs using double-sided tape, sputter-coated with gold, and examined at 10,000x magnification with a 20 kV acceleration voltage. The SEM also facilitated the measurement of the product's particle size.

***In vitro* experiments:**

Chitosan, phosphite, and biopesticide at concentrations ranged from 100 to 1000 mg/L and from Ch-AgNPs ranged from 10 to 500 mg/L in sterile distilled water was tested against *M. incognita*. For the egg hatching test, 1 ml of water suspension containing 100 nematode eggs was transferred to a glass vial containing 1 ml of double concentrations of tested compound solutions and incubated at 25 ± 2°C for 7 days. Hatched juveniles were counted under a light microscope, and egg hatching percentage was estimated. The mortality of J_2 was estimated by mixing 1 ml of water suspension containing 100 newly hatched J_2 with 1 ml of double concentrations of the tested compound on a glass vial and incubated at 25 ± 2°C for 24 and 48 h (Khan *et al.*, 2016). After incubation, J_2 was transferred in distilled water for 24 h, and active and dead nematodes were counted by the microscope.

Safety assessment studies:

Honeybees (*A. meliffera* L.):

Laboratory experiments utilized colonies of *Apis mellifera* L. (Hymenoptera: Apidae). Adult worker bees were obtained from the El-Sabahia Research Station, Agriculture Research Center, Ministry of Agriculture, Alexandria, Egypt. This particular strain was developed through crosses between Carniolan and Egyptian honeybee strains. The foraging bees used were nearing the end of their typical worker lifespan (Picard-Nizou *et al.*, 1995), and extensive literature confirms that foragers are higher than 20-day bees in typical colonies reviewed by Winston (1987). At the time of collection, the bee hives showed no obvious signs of disease that would typically be noticed during routine colony maintenance. The bees were transported to the laboratory in containers with solid plastic lids. Immediately upon arrival from the field, bees were transferred to experimental cages in groups of 50. They were maintained at 25±2°C and 65±5% relative humidity and fed a 50% (w/v) sucrose solution.

Acute toxicity assay:

The efficacy of chitosan, Ch-AgNPs, phosphite, and a biopesticide, at concentrations ranging from 100 to 1000 mg/L, was evaluated on *Apis mellifera* L. worker honeybees via oral administration of spiked syrup under controlled laboratory conditions. Stock solutions of the test compounds were formulated in a 50% (w/v) sucrose vehicle. Prior to treatment application, worker bees were briefly anesthetized using carbon dioxide gas for a duration not exceeding 3 minutes. Each experimental group for a given concentration comprised three plastic cups, each containing 30 honeybees (10 workers per cup), covered with a nylon mesh. The respective treatment solutions were presented on a cotton substrate affixed to the inner surface of the cup lid, allowing for a 24-hour feeding period. A control treatment, consisting solely of 50% sucrose solution, was included for comparison. Experimental conditions were maintained at 25±2°C, 65±5% relative humidity, and a 12:12 (light: dark) photoperiod. Bee mortality was defined as the absence of mobility (inability to walk or fly). Mortality percentages were subsequently recorded at 24, 48, and 72 hours following treatment initiation.

Earthworms (*Lumbricus terrestris*):

Earthworms (*Lumbricus terrestris*) were gathered from dunghills around El-Bahara Governorate. These earthworms were then reared in an artificial soil medium within large plastic containers (38×60×10 cm) covered with muslin cloth. Rearing conditions were maintained at 23±2°C according to Heimbach (1984). We used adult earthworms with well-developed clitella for this study. Before use, adults were removed from the artificial soil 24 hours prior and stored in Petri dishes on damp filter paper in the dark at 23±2°C to allow them to void their gut contents. The artificial soil consisted of 70% industrial sand, 20% kaolin clay, and 10% sphagnum peat moss, with the pH adjusted to 6.0.

Growth of earthworms:

We determined the weights of each earthworm after 1, 2, 3, and 4 weeks of exposure, comparing them with controls. Before weighing, the worms were sorted, washed with tap water, and blotted dry with filter paper. This weight determination was performed using four replicates.

Acute toxicological tests.

To prepare the treatments, compounds (chitosan, Ch-AgNPs, phosphite, and a biopesticide) were dissolved in distilled water and then thoroughly blended into artificial soil at concentrations from 100 to 1000 mg (a.i)/Kg soil. Fenamiphos was applied at 0.25, 0.5, 1, and 2 fold rates per Kg soil. Control groups received only distilled water mixed into their artificial soil. We

maintained the soil moisture content at 35% of the final weight, with bi-weekly monitoring. All experiments were conducted at 23±2°C with continuous light. We investigated four exposure periods (1, 2, 3, and 4 weeks). For each period and concentration, we used four replicates, each containing 10 earthworms, for analysis. Mortality was determined weekly by washing the soil; earthworms that failed to respond to a gentle mechanical stimulus were classified as dead (OECD, 1984).

Statistical analysis:

Statistical analysis was performed using the SPSS 27.0 software program (Statistical Package for Social Sciences, Chicago, USA). The mortalities were plotted against the concentrations and fitted using SPSS software to determine the LC_{50} according to the probit analysis (Finney, 1971). The 95% confidence limits for the range of LC_{50} values were determined by the least-square regression analysis. The data from experiments were analyzed by one-way ANOVA. Mean separations were performed by the student-Newman-Keuls (SNK) test and the significant differences between means at a probability level of ≤ 0.05.

RESULTS AND DISCUSSION

Characterization of Ch-AgNPs:

Ch-AgNPs prepared in the experiment exhibited a yellow powder shape. The physiochemical characteristics of Ch-AgNPs were analysed using scanning electron microscopy (SEM). SEM showed that the nanoparticles were nearly uniform in shape and size. Fig. (1) shows the morphology of lyophilized Ch-AgNPs by SEM. The mean particle size of Ch-AgNPs ranged between 81.08 and 216.22 nm (Fig. 1).

A straightforward chemical method to synthesize chitosan-silver (CS-Ag) nanocomposite materials, with the resultant composite comprising 20 wt% silver was employed (Govindan *et al*., 2020). Silver nanoparticles were separately synthesized using a chemical reduction approach. Characterization of the CS-Ag nanocomposite involved techniques such as Field Emission Scanning Electron Microscopy (FESEM), X-ray Diffraction (XRD), and FTIR. The XRD analysis revealed silver and chitosan within the nanocomposite, with the silver exhibiting a cubic crystal structure. The spherical shape of the silver nanoparticles was confirmed through FESEM imaging. FTIR spectroscopy aided in structural elucidation.

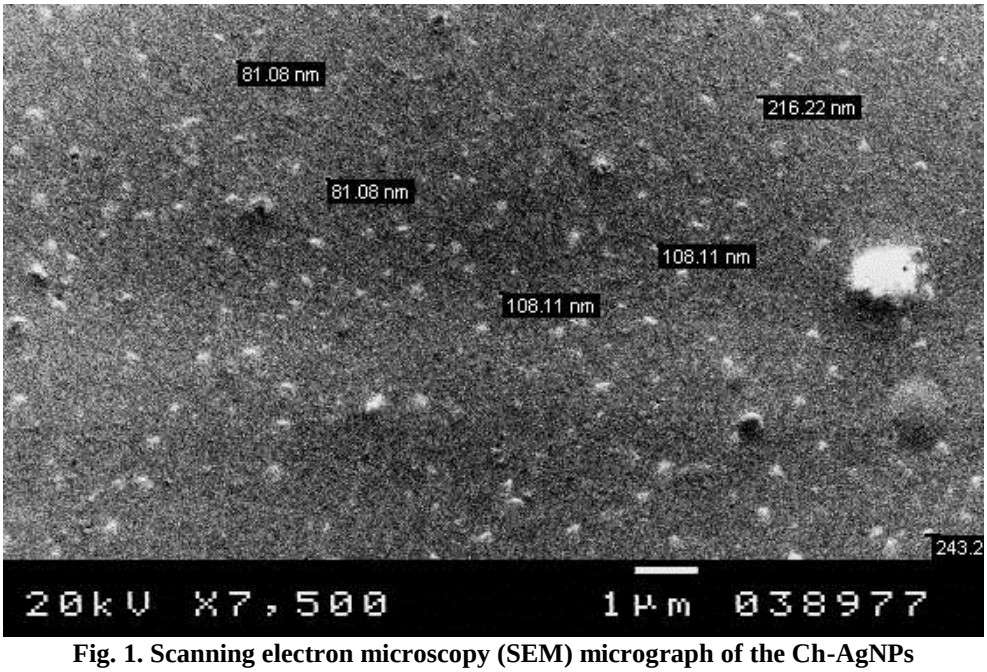


Fig. 1. Scanning electron microscopy (SEM) micrograph of the Ch-AgNPs

Chitosan-mediated silver nanoparticles (AgNPs) were synthesized and characterized using UV-Vis spectroscopy, FTIR spectroscopy, XRD, and TEM (Kalaivani and Mathubala, 2024). The UV-Vis absorption spectrum revealed enhanced AgNPs formation efficiency with increased chitosan concentration, leading to a noticeable size reduction of the nanoparticles. XRD analysis indicated the spherical, crystalline nature of the AgNPs. Surface morphology and nanoparticle size were further elucidated through AFM and TEM imaging, while the DLS image confirmed the size distribution and range of the AgNPs.

Chitosan-silver nanoparticles (Ch-AgNPs), a subset of Ag-based nanocomposites, are recognized for their biocompatibility and biodegradability (Badawy *et al*., 2019). The reduction study of silver ions in the presence of chitosan, resulting in Ch-AgNPs. The researchers characterized these hybrid materials through scanning electron microscopy (SEM), X-ray diffraction (XRD), and zeta potential analysis.

The focus was on exploring the potential applications of Chi-AgNPs composites was done by Dara *et al*. (2020). The researchers synthesized Chi-AgNPs through the chemical reduction of silver nitrate salts and the biodegradable chitosan. Characterization of the obtained Chi-AgNPs involved analyses such as Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), Transmission electron microscopy (TEM), and X-ray powder diffraction. UV-visible spectra highlighted the efficient configuration of AgNPs and chitosan, while FTIR spectra indicated specific interactions between the functional groups of chitosan and AgNPs. Particle size and TEM analyses revealed that Chi-AgNPs were uniformly dispersed in the matrix, with an average size ranging from 10 to 230 nm. The surface morphology of chitosan played a crucial role in influencing the adsorption of AgNPs.

Silver nanoparticle-chitosan composite particles while utilizing various characterization techniques like UV-Vis spectroscopy, FTIR spectroscopy, XRD, SEM, and zetasizer nano were successfully synthesized (Mirda *et al*., 2021). The UV-Vis spectroscopy results demonstrated optimal absorption at 410 nm within the AgNPs-chi-spheres. Through XRD analysis, the crystalline and spherical structure of these particles was verified. SEM characterization revealed that the addition of 20% NaOH led to the smallest average particle size of 46.91 nm in the AgNPs-chi-spheres. EDX analysis further supported these findings, illustrating the production of regularly spherical and relatively nonporous particles under these conditions. Meanwhile, zetasizer nano analysis indicated a trend of increasing zeta potential and polydispersity index values with higher NaOH concentrations.

Nematicidal activity of chitosan different molecular weights, biopesticide, phosphite, Ch-AgNPs and fenamiphos against egg filtrate of *M. incognita* after 7 days of treatment:

The nematicidal activity of various compounds against *M. incognita* eggs after seven days of treatment was evaluated, with key data summarized in Table (1). The tested compounds included chitosan of different molecular weights (low, medium, and high), a biopesticide, phosphite, Ch-AgNPs, and fenamiphos. LC_{50} values and 95% confidence limits were determined to assess treatment efficacy and statistical significance.

Chitosan compounds exhibited differential nematicidal activity against *M. incognita* eggs, with LMW chitosan demonstrating the highest efficacy (LC_{50} = 181.02 mg/L). This suggests that lower molecular weight chitosan has a greater impact on egg-hatching inhibition compared to its higher molecular weight counterparts. MMW and HMW chitosan showed lower efficacy, with LC_{50} values of 510.62 mg/L and 699.62 mg/L, respectively, indicating the importance of molecular weight in determining chitosan's nematicidal activity. The biopesticide treatment also displayed significant nematicidal activity, with an LC_{50} value of 215.41 mg/L, underscoring its potential as an environmentally friendly alternative nematicide. Phosphite exhibited moderate nematicidal activity against *M. incognita* eggs, with an LC_{50} value of 470.30 mg/L. Further optimization of concentration may be necessary to enhance its efficacy as a nematicidal agent. Ch-AgNPs demonstrated potent nematicidal activity, with an exceptionally low LC_{50} value of 41.79 mg/L. The synergistic effect of combining chitosan with silver nanoparticles likely contributed to the enhanced efficacy against *M. incognita* eggs, possibly due to increased surface area and improved dispersibility.

Fenamiphos exhibited the highest nematicidal activity among all tested compounds, with an LC_{50} value of 24.35 mg/L. While fenamiphos is primarily known for its insecticidal properties, its efficacy against *M. incognita* eggs highlights its potential as a dual-purpose pest control agent. However, further investigation into safety and application methods is warranted before considering its use in agricultural practices.

Table 1. Nematicidal activity of chitosan different molecular weights, biopesticide, phosphite, Ch-AgNPs and fenamiphos against egg filtrate of *M. incognita* after 7 days of treatment

Compound	LC_{50} [a] (mg/L)	95% Confidence limits		Slope[b] ± S.E.	Chi[c] (x^2)	*P*[d]
		Lower	Upper			
LMW chitosan	181.02	134.76	225.27	1.43 ± 0.17	2.64	0.44
MMW chitosan	510.62	433.68	610.02	1.65 ± 0.18	6.34	0.09
HMW chitosan	699.62	586.31	873.46	1.62 ± 0.19	3.64	0.30
Ch-AgNPs	41.79	9.80	81.56	1.04 ± 0.11	10.08	0.01
Phosphite	470.30	395.77	564.38	1.55 ± 0.17	6.23	0.10
Biopesticide	215.41	73.24	317.99	1.52 ± 0.17	9.36	0.02
Fenamiphos	24.35	6.66	40.19	1.2 ± 0.11	9.69	0.02

[a] LC_{50} concentration causing 50% non-hatched egg.
[b] Slope of the concentration – mortality regression line
[c] Chi-square value
[d] Probability value

Statistical analysis, represented by the chi-square (χ^2) value and probability values (*P*), indicated the goodness of fit between concentration-mortality regression lines and observed data. Both the biopesticide and Ch-AgNPs treatments yielded low probability values (*P*=0.02 and P=0.01, respectively), signifying statistically significant effects on *M. incognita* egg hatching inhibition.

Nematicidal activity of chitosan different molecular weights, biopesticide, phosphite, Ch-AgNPs and fenamiphos against second-stage juveniles (J_2) of *M. incognita* after 24 hrs:

Table (2) presents essential data on the nematicidal activity of various compounds against second-stage juveniles (J_2) of *M. incognita* after 24 hours of exposure. This data is crucial for assessing the effectiveness of these compounds against the mobile and infective stages of the nematode life cycle. Among the chitosan compounds, LMW chitosan demonstrated potent nematicidal activity, with an LC_{50} value of 257.55 mg/L, indicating moderate effectiveness against *M. incognita* J_2 larvae. In contrast, MMW chitosan and HMW chitosan displayed higher LC_{50} values of 488.14 mg/L and 706.32 mg/L, respectively, suggesting reduced efficacy against the targeted nematode stage. These results imply that the molecular weight of chitosan influences its nematicidal activity, with lower molecular weight chitosan exhibiting relatively stronger effects.

Chitosan nanoparticles, specifically Ch-AgNPs, exhibited significant nematicidal activity against J_2 larvae, with an LC_{50} value of 39.19 mg/L. This finding suggests a synergistic enhancement of nematicidal effects by combining chitosan and silver nanoparticles against *M. incognita* J_2 larvae, making it a promising treatment option. The incorporation of silver nitrate ($AgNO_3$) into the chitosan nanoparticle matrix likely contributed to the enhanced nematicidal activity, as reported by Ahmed *et al*. (2019).

Table 2. Nematicidal activity of chitosan different molecular weights, biopesticide, phosphite, Ch-AgNPs and fenamiphos against second-stage juveniles (J_2) of *M. incognita* after 24 h

Compound	LC_{50} [a] (mg/L)	95% Confidence limits		Slope [b] ± S.E	Chi[c] (x^2)	*P*[d]
		Lower	Upper			
LMW chitosan	257.55	203.11	312.57	1.42 ± 0.16	7.18	0.06
MMW chitosan	488.14	389.86	628.60	1.16 ± 0.16	3.11	0.37
HMW chitosan	706.32	555.41	985.08	1.15 ± 0.17	3.97	0.26
Ch-AgNPs	39.19	28.29	51.28	1.07 ± 0.11	3.25	3.25
Phosphite	603.42	455.62	886.62	0.92 ± 0.16	3.46	0.34
Biopesticide	157.28	103.73	207.57	1.17 ± 0.16	6.76	0.07
Fenamiphos	4.64	1.58	8.80	0.85 ± 0.12	4.90	4.90

[a] LC_{50} concentration causing 50% death for nematode larvae.
[b] Slope of the concentration – mortality regression line
[c] Chi-square value
[d] Probability value

The biopesticide treatment demonstrated promising nematicidal activity against *M. incognita* (J_2) larvae, with an LC_{50} value of 157.28 mg/L. This suggests the potential of the biopesticide to disrupt the development and survival of the nematode at this specific stage. Moreover, these findings align with previous research highlighting the efficacy of natural products in sustainable nematode management (Muigai *et al*., 2023).

Phosphite, a phosphorus-based compound, exhibited lower nematicidal activity against *M. incognita* juveniles, with an LC_{50} value of 603.42 mg/L, indicating relatively less effectiveness. This observation is consistent with earlier studies emphasizing the utility of phosphite as a nematicide for controlling root-knot nematodes (Benabdallah *et al*., 2018). However, its efficacy may vary depending on application methods and environmental conditions.

In contrast, fenamiphos, a conventional synthetic nematicide, demonstrated the highest nematicidal activity among all treatments, with a low LC_{50} value of 4.64 mg/L. While fenamiphos effectively reduced nematode populations, its use raises concerns regarding environmental contamination and non-target toxicity (Abdel-Rahman *et al.,* 2023).

Statistical analysis, represented by chi-square (χ^2) values and probability values (*P*), indicates the goodness of fit between concentration-mortality regression lines and observed data. Most compounds exhibited non-significant chi-square values, indicating a satisfactory fit between the model and observed mortality data.

Results presented in Table (3) revealed variations in the nematicidal activity of different chitosan compounds based on their molecular weight. LMW chitosan displayed moderate nematicidal activity against *M. incognita* J_2 larvae, with an LC_{50} value of 200.82 mg/L after 48 hours of treatment. Similarly, MMW chitosan and HMW chitosan exhibited LC_{50} values of 390.41 mg/L and 546.98 mg/L, respectively, suggesting lower efficacy compared to LMW chitosan. These findings underscore the importance of considering chitosan's molecular weight when evaluating its nematicidal potential.

Khalil and Badawy (2012) investigated the efficacy of chitosan with varying molecular weights against *M. incognita* through *in vitro* and pot experiments. Their results demonstrated that nematode mortality was significantly influenced by exposure duration and chitosan molecular weight. Specifically, LMW chitosan exhibited the highest effectiveness in nematode control, with notable reductions in nematode populations, egg masses, and root galling of tomato seedlings compared to the untreated control. Overall, the nematicidal potency of chitosan compounds increased notably as their molecular weights decreased.

Nematicidal activity of chitosan different molecular weights, biopesticide, phosphite, Ch-AgNPs and fenamiphos against second-stage juveniles (J_2) of *M. incognita* after 48 hrs:

The biopesticide treatment also demonstrated moderate nematicidal activity against *M. incognita* J_2 larvae, with an LC_{50} value of 122.22 mg/L after 48 hours. This finding suggests that the biopesticide remains effective in reducing nematode populations within this timeframe.

Phosphite, however, exhibited a higher LC_{50} value of 422.52 mg/L, indicating lower efficacy against *M. incognita* J_2 larvae after 48 hours compared to other tested compounds. Further optimization of the concentration may be necessary to enhance its effectiveness in controlling nematode populations. Ch-AgNPs demonstrated notable nematicidal activity, with an LC_{50} value of 26.12 mg/L after 48 hours of treatment. This result suggests that the use of chitosan nanoparticles enhances the nematicidal effects against *M. incognita* J_2 larvae, offering potential advantages over conventional chitosan-based treatments.

Fenamiphos continued to exhibit the highest nematicidal activity among all tested compounds, with an extremely low LC_{50} value of 1.65 mg/L after 48 hours of treatment. This reaffirms its potency as a potent nematicide against *M. incognita* J_2 larvae. Nonetheless, the use of fenamiphos should be carefully considered due to potential safety concerns and environmental impacts associated with its use.

The statistical analysis, represented by the chi-square (χ^2) values and probability values (*P*), provided insights into the goodness of fit between the concentration-mortality regression line and the observed data. Most compounds displayed non-significant chi-square values, indicating a good fit between the model and the observed mortality data, further supporting the reliability of the obtained results. In the provided results, the *P*-values ranged from 0.05 to 0.90. A *P*-value of 0.05 suggests a significance level of 5%, indicating relatively strong evidence against the null hypothesis for the corresponding compound. Conversely, higher *P*-values such as 0.90 suggest a significance level of 90%, indicating weaker evidence against the null hypothesis. The combination of the slope ± S.E, Chi (χ^2) value, and *P*-value provides valuable information about the relationship between the concentration of each compound and its effect on the measured parameter. These statistical measures help assess the significance and strength of the observed

effects, contributing to the interpretation and understanding of the study results. Further discussion and interpretation of these results would depend on the specific objectives, context, and implications of the research study.

Overall, the findings from this study emphasize the varying degrees of nematicidal activity among different compounds. Ch-AgNPs, LMW chitosan, biopesticide, and fenamiphos exhibited notable efficacy, while MMW chitosan, HMW chitosan, and phosphite showed moderate to lower activity. These results contribute to our understanding of the potential applications and effectiveness of these compounds in targeted nematode control strategies.

Bernardo *et al.* (2022) investigated the efficacy of silver nanoparticles (AgNPs) as a biopesticide *in vitro*. AgNPs were synthesized using chitosan, a natural biopolymer, as a reducing agent through microwave irradiation. Exposure of J_2-stage nematodes to low concentrations of AgNPs resulted in significant mortality rates, with nearly 100% nematode inactivation within 24 to 48 hours.

Sahel *et al.* (2020) conducted two experiments under screen house conditions to assess specific *P. fluorescens* isolates concerning their impact on the reproductive potential of *M. incognita*, infecting tomato and eggplant. Results from the tomato experiment indicated that *P. fluorescens* isolate Pf_2 exhibited the most remarkable nematode reduction, with a significant average reduction of 61.3% and a substantial decrease in female nematodes by 77% per plant.

Hassan *et al.* (2021) conducted a bioassay using botanically synthesized silver nanoparticles (AgNPs) against *M. javanica* J_2 nematodes. The data demonstrated a correlation between increased concentrations of the materials and higher mortality rates of *M. javanica* J_2. For instance, AgNPs derived from oleander extract caused mortality rates ranging from 37.1% to 93.25%, with an LC_{50} of 15.31 µl/50 ml. Chamomile-synthesized silver nanoparticles resulted in mortality rates of 34.30% to 91.33%, with an LC_{50} of 18.72 µl/50 ml. Thyme-synthesized AgNPs caused mortality rates of 14.3% to 91.0%, with an LC_{50} of 37.71 µl/50 ml. Ginger-synthesized AgNPs had mortality rates of 52.0% to 92.0%, with an LC_{50} of 11.11.

Toxicity of tested nematicidal compounds on non-target organisms:

Assessing the impact of nematicidal compounds on non-target organisms is crucial for evaluating their overall safety and environmental sustainability. In this study, honeybees and earthworms were selected as representative non-target organisms due to their ecological importance and susceptibility to pesticide exposure.

Effects of chitosan different molecular weights, biopesticide, phosphite, Ch-AgNPs and fenamiphos on the percentage of mortality of the honeybee after 24, 48 and 72 hours of application:

In the context of ecosystem preservation, pollinators, especially honeybees (*Apis mellifera)*, play a pivotal role by facilitating the pollination of diverse plant species, including numerous crops. However, the use of pesticides and other agrochemicals presents potential risks to non-target organisms like honeybees. It is crucial to evaluate the toxicity of chemical compounds on honeybees to mitigate adverse effects on these essential pollinators. In this regard, Table (4) provides data on the effect of various compounds, such as chitosan with different molecular weights, biopesticide, phosphite, Ch-AgNPs, and fenamiphos, on the mortality percentage of honeybees post-application for 24, 48, and 72 hours. Understanding the impact of these compounds on honeybee mortality can guide the development of sustainable agricultural practices that prioritize both crop protection and pollinator well-being.

Table 3. Nematicidal activity of chitosan different molecular weights, biopesticide, phosphite, Ch-AgNPs and fenamiphos against *M. incognita* (J_2) second-stage juveniles after 48 hrs

Compound	LC_{50} [a] (mg/L)	95% Confidence limits		Slope [b] ± S.E.	Chi [c] (x^2)	*P* [d]
		Lower	Upper			
LMW chitosan	200.82	150.07	249.76	1.36 ±0.16	6.71	0.08
MMW chitosan	390.41	310.52	488.13	1.20 ±0.16	4.12	0.24
HMW chitosan	546.98	434.67	722.69	1.13 ±0.16	3.26	0.35
Ch-AgNPs	26.12	17.51	35.52	1.03 ±0.11	3.72	0.29
Phosphite	422.52	295.39	618.36	0.76 ±0.16	0.57	0.90
Biopesticide	122.22	73.47	168.24	1.14 ±0.16	5.69	0.12
Fenamiphos	1.65	0.21	4.44	0.74 ±0.13	7.47	0.05

[a] LC_{50} concentration causing 50% death for nematode larvae.
[b] Slope of the concentration – mortality regression line
[c] Chi-square value
[d] Probability value

Table 4. Effects of chitosan different molecular weights, biopesticide, phosphite, Ch-AgNPs and fenamiphos on the percentage of mortality of the honeybee after 24, 48 and 72 hours of application

Compound	Conc.	Mortality (%) ± SE		
Time (hour)	(mg/L)	24 hrs	48 hrs	72 hrs
LMW Chitosan	100	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	250	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	500	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	750	0.00^{f}±0.00	3.33^{f}±0.27	6.67^{fg}±0.27
	1000	0.00^{f}±0.00	6.67^{f}±0.27	13.33^{def}±0.27
MMW Chitosan	100	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	250	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	500	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	750	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	1000	0.00^{f}±0.00	3.33^{f}±0.27	6.67^{fg}±0.27
HMW Chitosan	100	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	250	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	500	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	750	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	1000	0.00^{f}±0.00	3.33^{f}±0.27	6.67^{fg}±0.27
Ch-AgNPs	10	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	50	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	100	0.00^{f}±0.00	0.00^{f}±0.00	3.33^{g}±0.27
	200	0.00^{f}±0.00	3.33^{f}±0.27	13.33^{def}±0.27
	500	0.00^{f}±0.00	10.00^{f}±0.00	20.00^{d}±0.00
Phosphite	100	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	250	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	500	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	750	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	1000	3.33^{f}±0.27	6.67^{f}±0.27	10.00^{efg}±0.00
Biopesticide	100	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	250	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	500	0.00^{f}±0.00	0.00^{f}±0.00	0.00^{g}±0.00
	750	3.33^{f}±0.27	3.33^{f}±0.27	6.67^{fg}±0.54
	1000	6.67^{de} ±0.27	6.67±0.27	16.67^{de}±0.27
Fenamiphos	1.5	6.67^{de}±0.27	46.67^{e}±0.27	63.33^{c}±0.27
	7.5	10.00^{d}±0.00	56.67^{d}±0.27	66.67^{c}±0.27
	15	20.00^{c}±0.00	63.33^{c}±0.27	83.33^{b}±0.27
	30	36.67^{b}±0.27	76.67^{b}±0.27	100.00^{a}±0.00
	60	46.67^{a}±0.27	90.00^{a}±0.00	100.00^{a}±0.00
control	-	0.00^{f}±0.00	0.00^{f}±0.00	3.33^{g}±0.27

Data are means ± SE of three replicates; values within a column bearing the same superscript are not significantly different ($P \leq 0.05$) according to the Student-Newman-Keuls (SNK).

Across all molecular weights (LMW, MMW, and HMW) and concentrations, chitosan demonstrated consistently low honeybee mortality rates of 0% up to 72 hours. This indicates that chitosan, at the tested concentrations, did not exert significant acute toxic effects on honeybees within the study's timeframe. In contrast, the biopesticide showed an increase in honeybee mortality with higher concentrations over time. At 1000 mg/L, mortality rates reached up to 16.67% after 72 hours, suggesting a moderate impact on honeybee populations compared to chitosan treatments.

Similarly, to chitosan, phosphite did not induce significant mortality in honeybees at any of the tested concentrations within 72 hours; with mortality, rates remaining at 0% at all time points. However, honeybee mortality increased with increasing concentrations of Ch-AgNPs. At the highest concentration tested (500 mg/L), mortality rates reached 20% after 72 hours, indicating a moderate impact on honeybee survival compared with other treatments. Fenamiphos displayed a concentration-dependent increase in honeybee mortality, with rates ranging from 6.67% to 100% across different concentrations after 72 hours. Higher concentrations led to higher mortality rates, highlighting the potential acute toxicity of fenamiphos on honeybees.

The control group showed minimal mortality rates, with 3.33% mortality observed after 72 hours, validating the experimental setup and indicating that mortality in the experimental groups likely resulted from exposure to the tested compounds rather than external factors. Overall, chitosan, phosphite, and the biopesticide demonstrated low to no acute toxicity toward honeybees at the tested concentrations and time points.

In contrast, Ch-AgNPs and fenamiphos exhibited concentration-dependent effects on honeybee mortality, with higher concentrations leading to increased mortality rates. These findings underscore the importance of assessing the potential risks of agrochemicals on non-target organisms like honeybees and emphasize the need for sustainable agricultural practices to safeguard pollinator health and biodiversity.

Abou-Shaara (2022) explored the beneficial effects of chitosan on bee health, particularly focusing on chitosan nanoparticles (CHNPs) in honeybee colonies. Survival experiments indicated the safety of these CHNPs concentrations for bees. CHNPs 30 and CHNPs 60 exhibited superior effects on parameters such as comb building, hygienic behavior, stored food, and colony strength compared to CHNPs 10 and the control group. Observations of dead bee numbers at hive entrances supported the absence of adverse effects of CHNPs on bee colonies.

Effects of chitosan different molecular weights, biopesticide, phosphite, Ch-AgNPs and fenamiphos on the percentage of mortality of the earthworm *L. terrestris* after 1, 2, 3, and 4 weeks of application:

Earthworms, such as *L. terrestris*, are essential soil-dwelling organisms that play a crucial role in maintaining soil health and ecosystem functions. These organisms contribute to soil aeration, nutrient cycling, and overall soil structure improvement. However, the presence of various agrochemicals and pesticides in the environment can pose risks to non-target organisms like earthworms. Table (5) presents data on the effects of different compounds, including chitosan of different molecular weights, biopesticide, phosphite, Ch-AgNPs, and fenamiphos, on the percentage of mortality of the earthworm *L. terrestris* following application for 1, 2, 3, and 4 weeks. Understanding the impact of these compounds on earthworm mortality is crucial for assessing their potential risks to soil ecosystems and highlighting the importance of sustainable agricultural practices that preserve soil biodiversity and ecosystem services provided by earthworms. Across all molecular weights (LMW, MMW, and HMW) and concentrations of chitosan, there was a gradual increase in earthworm mortality over time. Mortality rates ranged from 0% to 16.67% after 4 weeks of exposure, indicating a moderate impact that was dependent on concentration and molecular weight. Higher concentrations and longer exposure periods led to increased mortality. The biopesticide displayed concentration-dependent effects on earthworm mortality, with mortality rates ranging from 10% to 50% after 4 weeks. Higher concentrations resulted in higher mortality rates, highlighting the potential toxicity of the biopesticide on earthworms over time. Phosphite showed a similar concentration-dependent pattern of toxicity, with mortality rates ranging from 10% to 30% after 4 weeks. The data suggest that higher concentrations of phosphite can have adverse effects on earthworm survival over time. Earthworm mortality increased with higher concentrations of Ch-AgNPs. Mortality rates ranged from 10% to 46.67% after 4 weeks, indicating a moderate to high impact on earthworm populations. The concentration of Ch-AgNPs played a significant role in determining the level of toxicity.

Fenamiphos exhibited a strong concentration-dependent effect on earthworm mortality. Mortality rates ranged from 20% to 100% after 4 weeks, with higher concentrations leading to near-complete mortality.

Table 5. Effects of chitosan different molecular weights, biopesticide, phosphite, Ch-AgNPs and fenamiphos on the percentage of mortality of the earthworm *L. terrestris* after 1, 2, 3, and 4 weeks application

Compound	Conc. mg (a.i)/Kg soil	Mortality (%) ± SE			
Time (week)		1 week	2 weeks	3 weeks	4 weeks
LMW Chitosan	100	$0.00^{i} \pm 0.00$	$3.33^{hi} \pm 0.27$	$10.00^{hij} \pm 0.00$	$13.33^{hij} \pm 0.27$
	250	$3.33^{hi} \pm 0.27$	$6.67^{ghi} \pm 0.27$	$10.00^{hij} \pm 0.00$	$13.33^{hij} \pm 0.27$
	500	$6.67^{ghi} \pm 0.27$	$10.00^{fghi} \pm 0.47$	$10.00^{hij} \pm 0.47$	$16.67^{ghij} \pm 0.27$
	750	$6.67^{ghi} \pm 0.27$	$10.00^{fghi} \pm 0.47$	$10.00^{hij} \pm 0.47$	$16.67^{ghij} \pm 0.27$
	1000	$6.67^{ghi} \pm 0.27$	$10.00^{fghi} \pm 0.47$	$10.00^{hij} \pm 0.47$	$16.67^{ghij} \pm 0.27$
MMW Chitosan	100	$0.00^{i} \pm 0.00$	$0.00^{i} \pm 0.00$	$6.67^{ij} \pm 0.27$	$10.00^{ij} \pm 0.27$
	250	$0.00^{i} \pm 0.00$	$0.00^{i} \pm 0.00$	$6.67^{ij} \pm 0.27$	$10.00^{ij} \pm 0.47$
	500	$3.33^{hi} \pm 0.27$	$6.67^{ghi} \pm 0.27$	$10.00^{hij} \pm 0.00$	$13.33^{hij} \pm 0.47$
	750	$6.67^{ghi} \pm 0.27$	$6.67^{ghi} \pm 0.27$	$10.00^{hij} \pm 0.00$	$13.33^{hij} \pm 0.27$
	1000	$6.67^{ghi} \pm 0.27$	$6.67^{ghi} \pm 0.27$	$10.00^{hij} \pm 0.00$	$13.33^{hij} \pm 0.27$
HMW Chitosan	100	$0.00^{i} \pm 0.00$	$0.00^{i} \pm 0.00$	$6.67^{ij} \pm 0.27$	$10.00^{ij} \pm 0.27$
	250	$0.00^{i} \pm 0.00$	$0.00^{i} \pm 0.00$	$6.67^{ij} \pm 0.27$	$10.00^{ij} \pm 0.00$
	500	$3.33^{hi} \pm 0.27$	$3.33^{hi} \pm 0.27$	$6.67^{ij} \pm 0.27$	$10.00^{ij} \pm 0.47$
	750	$6.67^{ghi} \pm 0.27$	$6.67^{ghi} \pm 0.27$	$6.67^{ij} \pm 0.27$	$10.00^{ij} \pm 0.47$
	1000	$6.67^{ghi} \pm 0.27$	$6.67^{ghi} \pm 0.27$	$6.67^{ij} \pm 0.27$	$16.67^{ghij} \pm 0.47$
Ch-AgNPs	10	$10.00^{fghi} \pm 0.00$	$13.33^{efghi} \pm 0.27$	$23.33^{fghi} \pm 0.54$	$33.33^{efg} \pm 0.54$
	50	$16.67^{efgh} \pm 0.27$	$20.00^{efgh} \pm 0.47$	$30.00^{defg} \pm 0.47$	$36.67^{def} \pm 0.27$
	100	$20.00^{efg} \pm 0.00$	$23.33^{efg} \pm 0.27$	$30.00^{defg} \pm 0.00$	$36.67^{def} \pm 0.27$
	200	$26.67^{de} \pm 0.54$	$30.00^{de} \pm 0.47$	$36.67^{def} \pm 0.27$	$43.33^{def} \pm 0.27$
	500	$26.67^{de} \pm 0.54$	$30.00^{de} \pm 0.47$	$40.00^{de} \pm 0.00$	$46.67^{de} \pm 0.27$
Phosphite	100	$10.00^{fghi} \pm 0.00$	$13.33^{efghi} \pm 0.27$	$20.00^{ghij} \pm 0.00$	$26.67^{fghi} \pm 0.00$
	250	$13.33^{efghi} \pm 0.27$	$16.67^{efghi} \pm 0.27$	$20.00^{ghij} \pm 0.47$	$26.67^{fghi} \pm 0.27$
	500	$16.67^{efgh} \pm 0.27$	$20.00^{efgh} \pm 0.47$	$23.33^{fghi} \pm 0.27$	$26.67^{fghi} \pm 0.27$
	750	$20.00^{efg} \pm 0.00$	$23.33^{efg} \pm 0.27$	$26.67^{efgh} \pm 0.27$	$30.00^{efgh} \pm 0.27$
	1000	$20.00^{efg} \pm 0.00$	$23.33^{efg} \pm 0.27$	$30.00^{defg} \pm 0.00$	$30.00^{efgh} \pm 0.00$
Biopesticide	100	$10.00^{fghi} \pm 0.00$	$16.67^{efghi} \pm 0.27$	$23.33^{fghi} \pm 0.27$	$33.33^{efg} \pm 0.27$
	250	$23.33^{ef} \pm 0.27$	$26.67^{def} \pm 0.27$	$33.33^{defg} \pm 0.27$	$46.67^{de} \pm 0.27$
	500	$23.33^{ef} \pm 0.27$	$26.67^{def} \pm 0.27$	$40.00^{de} \pm 0.00$	$46.67^{de} \pm 0.00$
	750	$23.33^{ef} \pm 0.27$	$26.67^{def} \pm 0.27$	$43.33^{d} \pm 0.27$	$46.67^{de} \pm 0.27$
	1000	$26.67^{de} \pm 0.27$	$30.00^{de} \pm 0.00$	$43.33^{d} \pm 0.27$	$50.00^{d} \pm 0.27$
Fenamiphos	1.5	$20.00^{efg} \pm 0.00$	$40.00^{d} \pm 0.00$	$56.67^{c} \pm 0.27$	$73.33^{c} \pm 0.27$
	7.5	$36.67^{d} \pm 0.27$	$56.67^{c} \pm 0.27$	$70.00^{b} \pm 0.47$	$83.33^{bc} \pm 0.27$
	15	$60.00^{c} \pm 0.47$	$73.33^{b} \pm 0.27$	$90.00^{a} \pm 0.00$	$93.33^{ab} \pm 0.27$
	30	$76.67^{b} \pm 0.27$	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$
	60	$90.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$
Control	-	$0.00^{i} \pm$ ٠.00	$0.00^{i} \pm 0.00$	$3.33^{i} \pm 0.27$	$3.33^{j} \pm 0.47$

Data are means ± SE of three replicates; values within a column bearing the same superscript are not significantly different ($P \leq 0.05$) according to the Student-Newman-Keuls (SNK).

The control group exhibited minimal mortality rates over the 4 weeks, ranging from 0% to 3.33%. This validates the experimental setup and indicates that mortality in the experimental groups can be attributed to exposure to the tested compounds.

Chitosan, phosphite, and Ch-AgNPs – exhibited minimal toxicity on earthworms, with negative effects only increasing at higher concentrations. This low toxicity profile starkly contrasts the conventional insecticide fenamiphos, which displayed significantly higher toxicity levels. These findings suggest that bio-based materials offer a promising avenue for sustainable pest control. Their minimal impact on earthworms positions them as potential control methods that promote soil biodiversity and contribute to a healthier ecosystem.

Effects of chitosan different molecular weights, biopesticide, phosphite, Ch-AgNPs and fenamiphos on the weight of earthworm *L. terrestris* in artificial soil after 1, 2, 3, and 4 weeks of application:

Table (6) presents the effects of different treatments, including chitosan of varying molecular weights, biopesticide, phosphite, and Ch-AgNPs, on the weight of *L. terrestris* in artificial soil throughout 1, 2, 3, and 4 weeks after application. By examining how these treatments influence the weight of earthworms over time, valuable insights can be gained into the potential impacts of these treatments on soil-dwelling organisms and, ultimately, on the overall health and functioning of terrestrial ecosystems. Understanding these effects is crucial for sustainable soil management practices and for maintaining biodiversity and ecosystem services in natural environments. The weights of *L. terrestris* exhibit diverse responses to LMW, MMW, and HMW chitosan treatments.

Table 6. Effects of chitosan different molecular weights, biopesticide, phosphite, Ch-AgNPs and fenamiphos on the weight of earthworm *L. terrestris* in artificial soil after 1, 2, 3, and 4 weeks of application

Compound	Conc.mg(a.i)/Kg soil	Weight (g) ± S.E			
Time (week)		1 week	2 weeks	3 weeks	4 weeks
LMW Chitosan	100	19.33±0.62	18.33±0.27	14.33±0.27	12.33±0.27
	250	18.67±0.27	18.33±0.72	14.00±0.27	11.33±0.27
	500	18.00±0.72	18.00±0.47	13.67±0.47	11.13±0.27
	750	18.33±0.47	18.33±0.27	13.33±0.27	10.17±0.38
	1000	18.53±0.27	18.33±0.72	13.33±0.27	10.17±0.10
MMW Chitosan	100	19.67±0.61	19.67±0.54	17.00±0.27	11.77±0.03
	250	19.33±0.54	19.33±0.27	16.33±0.47	11.13±0.32
	500	18.33±0.27	18.00±0.47	16.00±0.27	10.47±0.38
	750	18.00±0.27	18.00±0.00	15.00±0.82	9.25±0.05
	1000	18.00±0.00	18.00±0.47	14.00±0.82	9.07±2.52
HMW Chitosan	100	19.67±0.47	19.67±0.27	16.67±0.00	11.50±0.23
	250	19.33±0.27	19.33±0.27	15.00±0.54	10.63±0.21
	500	18.00±0.27	18.00±0.47	14.33±0.00	10.17±0.23
	750	18.00±0.47	18.00±0.47	13.33±0.27	9.20±0.10
	1000	18.00±0.47	17.33±0.27	19.00±0.27	8.40±0.09
Ch-AgNPs	10	16.33±0.82	15.33±0.27	10.33±0.90	5.80±0.25
	50	16.00±0.27	14.67±0.54	9.00±0.27	4.53±0.22
	100	16.00±0.54	13.67±0.27	9.00±0.72	4.33±0.22
	200	15.67±0.27	13.33±0.47	7.67±0.00	4.00±0.03
	500	15.67±0.47	13.00±0.47	7.67±0.27	3.67±0.07
Phosphite	100	18.00±0.27	16.67±0.27	12.67±0.27	11.07±0.31
	250	17.33±0.00	16.00±0.47	11.33±0.27	9.50±0.43
	500	17.33±0.27	16.33±0.72	10.00±0.27	8.10±0.61
	750	17.00±0.27	16.33±0.27	10.00±0.00	7.17±0.05
	1000	16.67±0.00	16.00±0.47	9.00±0.47	6.13±0.10
Biopesticide	100	17.33±0.27	17.33±0.54	12.33±0.00	8.47±0.38
	250	16.33±0.27	16.33±0.54	11.33±0.98	7.83±0.16
	500	16.33±0.47	16.33±0.27	10.00±0.82	7.33±0.03
	750	16.00±0.00	16.00±0.27	9.33±0.47	6.13±0.27
	1000	15.33±0.27	14.00±0.00	9.33±0.27	5.77±0.00
Fenamiphos	1.5	14.67±0.27	11.00±0.82	4.33±0.27	3.33±0.27
	7.5	13.67±0.27	8.33±0.27	1.67±0.27	1.23±0.15
	15	8.67±0.27	7.33±0.27	0.27±0.27	0.13±0.02
	30	6.33±0.54	0.43±0.05	0.00±0.05	0.00±0.00
	60	3.00±0.72	0.30±0.08	0.00±0.00	0.00±0.00
Control	-	20.00±0.00	18.67±0.00	18.67±0.00	16.33±0.00

Data are means ± SE of three replicates.

Over varying concentrations and time intervals, the earthworm weights display fluctuations, suggesting distinct reactions to the chitosan treatments.

The biopesticide and phosphite treatments also affect the weight of earthworms over the experimental period. Changes in weight trends suggest varying degrees of influence on the growth and development of *L. terrestris*, highlighting the importance of considering these treatments' consequences on soil-dwelling organisms. The results for Ch-AgNPs and phosphite are particularly encouraging. Even at the highest concentrations tested, these materials displayed very low toxicity towards earthworms, with weight loss similar to the control group. Fenamiphos exposure resulted in a significant and concentration-dependent decrease in earthworm weight. At the highest concentration (60 mg/L), all earthworms died within the first week. This highlights the detrimental effects of conventional insecticides on soil biodiversity and emphasizes the need for alternative, more sustainable pest control strategies.

The control group's weight values remained relatively stable over the 4 weeks, providing a reference point for assessing the effects of the experimental substances. Contrasting the control group data with the treated groups enables a clearer understanding of how each substance influences the weight of earthworms in artificial soil conditions. The observed changes in earthworm weight in response to the different treatments underscore the importance of evaluating the effects of agricultural inputs on soil organisms. Understanding how these substances impact earthworm populations is crucial for maintaining soil health and ecosystem balance, given the essential role of earthworms in soil processes.

Akat and Arman (2016) investigated the harmful effects of fenamiphos on earthworms, specifically focusing on *Eisenia fetida*. Earthworms were exposed to different concentrations of fenamiphos (5, 10, and 20 mg/kg) over a 96-hour period. Afterward, the earthworms were euthanized and preserved for analysis. The study observed histopathological changes in the body wall, chloragogen us tissue, and intestinal epithelium of the earthworms. The medium (10 mg/kg) and high-concentration (20 mg/kg) groups exhibited more significant histopathological changes compared to the low-concentration (5 mg/kg) group.

Conclusion

The results concluded that using the plant growth promoters, LMW chitosan, Ch-AgNPs, phosphite and biopesticide achieved efficient control to the *M. incognita*. The biological manufacturing of silver nanoparticles using renewable sources may be safe, harmless, and compatible with the environment. The capacity of chitosan to synthesize silver nanoparticles (Ch-AgNPs) was investigated in this work. SEM was employed to characterize the Ch-AgNP. The mean particle size of spherical Ch-AgNPs ranged between 81.08 and 216.22 nm. Ch-AgNPs demonstrated notable nematicidal activity with an impressively low LC_{50} value of 39.19 and 26.12 mg/L against J_2 larvae of *M. incognita* after 24 and 48 hours of treatment, respectively. Honeybee mortality increased with increasing concentrations of Ch-AgNPs. At the highest concentration tested (500 mg/L), mortality rates reached 20% after 72 hours, indicating a moderate impact on honeybee survival compared to other compounds. Earthworm mortality increased with higher concentrations of Ch-AgNPs. Mortality rates ranged from 10% to 46.67% after 4 weeks, indicating a moderate to high impact on earthworm populations. The results for Ch-AgNPs and phosphite even at the highest concentrations tested, showed these materials displayed very low toxicity towards earthworms, with weight loss similar to the control group.

REFERENCES

Abbott, WS. 1925. A method of computing the effectiveness of an insecticide. J. Econ Entomol 18: 265– 267. https://doi.org/10.1093/jee/18.2.265a.

Abdel-Rahman, A. A., H. H. Kesba, H. G. Mohamed, D. F. Kamel and F. S. Ahmed. 2023. Sublethal concentrations of conventional nematicides alter the physiological activities of *Meloidogyne incognita* and suppress parasitism. Scientific Reports, 13(1). 229.

Abou-Shaara, H. F. 2022. Testing the ability of chitosan nanoparticles to improve development and performance of honey bee colonies during early spring. *Entomological Research*, *52*(4): 214-222.

Ahmed, S. A., H. S. El-Mahallawy and P. Karanis. 2019. Inhibitory activity of chitosan nanoparticles against Cryptosporidium parvum oocysts. *Parasitology Research*, *118*:2053-2063.

Akat, E. and S. Arman. 2016. Toxic Effect of Fenamiphos on the Earthworm, Eisenia fetida Annelida: Oligochaeta . Hacettepe Journal of Biology and Chemistry, 44(2): 133-137.

Badawy, M. E., T. M. Lotfy and S. Shawir. 2019. Preparation and antibacterial activity of chitosan-silver nanoparticles for application in preservation of minced meat. *Bulletin of the National Research Centre*, *43*(1): 1-14.

Benabdallah, M., O. Talhi, F. Nouali, N. Choukchou-Braham, K. Bachar and A. Silva. 2018. Advances in spirocyclic hybrids: chemistry and medicinal actions. *Current Medicinal Chemistry*, *25*(31): 3748-3767.

Bernardo, W. L. D. C., M. F. G. Boriollo, C. C. Tonon, J. J. da Silva, M. C. Oliveira, F. C.de Moraes and D. M. P. Spolidorio. 2022. Biosynthesis of silver nanoparticles from Syzygium cumini leaves and their potential effects on odontogenic pathogens and biofilms. *Frontiers in microbiology*, *13*.995521.

Cao, X. L., C. Cheng, Y. L. Ma and C. S. Zhao. 2010.Preparation of silver nanoparticles with antimicrobial activities and the researches of their biocompatibilities. *Journal of Materials Science: Materials in Medicine*, *21*: 2861-2868.

Curto, G., E. Dallavalle, G. R. De Nicola and L. Lazzeri. 2012. Evaluation of the activity of dhurrin and sorghum towards *Meloidogyne incognita*. *Nematology*, *14*(6): 759-769.

Dara, P. K., R. Mahadevan, P. A. Digita, S. Visnuvinayagam, L. R. Kumar, S. Mathew and R. J. S. A. S. Anandan. 2020. Synthesis and biochemical characterization of silver nanoparticles grafted chitosan (Chi-Ag-NPs): In vitro studies on antioxidant and antibacterial applications. *SN Applied Sciences*, *2*: 1-12.

Desaeger, J., C. Wram and I. Zasada. 2020. New reduced-risk agricultural nematicides-rationale and review. *Journal of Nematology*, *52*: e2020-91.

Divya, K. and M. S. Jisha. 2018. Chitosan nanoparticles preparation and applications. *Environmental chemistry letters*, *16*:101-112.

El-Ghareeb, W. R., S. M. Abdel-Raheem, T. M. Al-Marri, F. A. Alaql and M. M. Fayez. 2020. Isolation and identification of extended spectrum β-lactamases (ESBLs) Escherichia colifrom minced camel meat in Eastern province, Saudi Arabia. *The Thai Journal of Veterinary Medicine*, *50*(2):155-161.

El-Habashy, D. E., M. A. Abdel Rasoul and S. A. Abdelgaleil. 2020. Nematicidal activity of phytochemicals and their potential use for the control of Meloidogyne javanica infected eggplant in the greenhouse. *European Journal of Plant Pathology*, *158*: 381-390.

Fabiyi, O. A., R. O. Alabi and R. A. Ansari. 2020.Nanoparticles' synthesis and their application in the management of phytonematodes: An overview. *Management of phytonematodes: recent advances and future challenges*:125-140.

Finney, DJ, 1971: Probit Analysis, 3rd ed. Cambridge University Press, Cambridge.

Galindo-Castañeda, T., K. M. Brown and J. P. Lynch. 2018. Reduced root cortical burden improves growth and grain yield under low phosphorus availability in maize. Plant, Cell & Environment, 41(7):1579-1592.

Ghada, W., E. Casellas, J. Herbinger, A.Garcia-Benadí, L. Bothmann, N. Estrella and A. Menzel. 2022. Stratiform and convective rain classification using machine learning models and micro rain radar. Remote Sensing, 14(18). 4563.

Govindan, L., S. Anbazhagan, A. B. Altemimi, K. Lakshminarayanan, S. Kuppan, A. Pratap-Singh and M. Kandasamy. 2020. Efficacy of antimicrobial and larvicidal activities of green synthesized silver nanoparticles using leaf extract of Plumbago auriculata lam. Plants, 9(11): 1577.

Gowda, M. T., B. R. Meena, N. Krishnan, M. Manjunath, C. Sellaperumal, A. B. Rai and J.Singh. 2022. Antimicrobial peptides producing native Bacillus spp. for the management of root-knot nematode *Meloidogyne incognita* infecting okra (Abelmoschus esculentus L. Moench). *Biological Control*, *171*:104951.

Hassan, H. M., A. S. M. H. El Roby and W. S. Mohamed. 2021. Effectiveness of Silver Nanoparticles Botanically and Chemically Synthesized as well as Chitosan against Root-Knot Nematode Meloidogyne javanica in Vitro. Journal of Plant Protection and Pathology, 12(12): 849-854.

Heimbach, F. 1984. Correlations between three methods for determining the toxicity of chemicals to earthworms. Pesticide science, 15(6): 605-611.

Hussein, A. A., M. B. Abd El-latif, M. I. Saad El-Din, N. S. El-Shenawy, O. Hammam and A. M. Ibrahim. 2024. The molluscicidal activity of green synthesized copper oxide–based Annona squamosa seed extract nanoparticles on the feeding behavior, biochemical, molecular, and immunohistochemical alterations of Biomphalaria alexandrina snails. *Biological Trace Element Research*, *202*(5): 2327-2337.

Kalaivani, P. and G. Mathubala. 2024. Biosynthesis of Copper oxide Nanoparticles Using Azadirachta indica Seed Extract and Its Characterization. *Oriental Journal of Chemistry*, *40*(3).

Khalil, M. S. and M. E. Badawy. 2012. Nematicidal activity of a biopolymer chitosan at different molecular weights against root-knot nematode, *Meloidogyne incognita*. *Plant Protection Science*, *48*(4). 170.

Khan, A.M., S. Naz and M. Abid. 2016. Evaluation of marine red alga Melanothamnus afaqhusainii against *Meloidogyne incognita*, fungus and as fertilizing potential on okra. Pak J Nematol 34(1):91–100 (Pakistan Society of Nematologists).

Knapp-Lawitzke, F., G. von Samson-Himmelstjerna and J. Demeler. 2014.Rapid method for recovery of strongylid third stage larvae of parasitic nematodes from small soil samples. *Experimental Parasitology*, *142*: 91-94.

Li, Z., X. Kong, Z. Zhang, F. Tang, M. Wang, Y. Zhao and F. Shi. 2025. The functional mechanisms of phosphite and its applications in crop plants. *Frontiers in Plant Science*, *16*, 1538596.

Mirda, E., R. Idroes, K. Khairan, T. E. Tallei, M. Ramli, N. Earlia and Z. Jalil. 2021. Synthesis of chitosan-silver nanoparticle composite spheres and their antimicrobial activities. *Polymers*, *13*(22).3990.

Muigai, M., N. O. Odhiambo, J. K. Muli, J. Mugweru and R. Mwirichia. 2023. Use of bacterial biocontrol agents for the control of Fusarium oxysporum f. sp. Lycopersici, tomato wilt in Kenya.

Niu, Y. and F. Xu. 2020. Deciphering the power of isolation in controlling COVID-19 outbreaks. *The Lancet Global Health*, *8*(4):e452-e453.

Norabadi, M. T., N. Sahebani and H. R. Etebarian. 2014. Biological control of root-knot nematode (Meloidogyne javanica) disease by Pseudomonas fluorescens (Chao). *Archives of Phytopathology and Plant Protection*, *47*(5):615-621.

OECD. 1984. OECD guidelines for testing chemicals. Test 207: earthworm, acute toxicity test. Organization for Economic Co-operation and Development (OECD), Paris.

Picard-Nizou, A.L., M.H. Pham-Delegue, V. Keruelen, P. Doualt and R. Marilleau. 1995. Foraging behavior of honey bees (Apis mellifera L.) on transgenic oilseed rape (Brassica napus L. var. oleifera). Transgen. Res. 4:70-6

Puerari, H. H., C. R. Dias-Arieira, M. R. Cardoso, I. Hernandes and O. D. Costa Brito. 2015. Resistance inducers in the control of root lesion nematodes in resistant and susceptible cultivars of maize. Phytoparasitica, 43: 383-389.

Radwan, J., M. A. G. D. A. L. E. N. A. ZAGALSKA-NEUBAUER, M.Cichoń, J.Sendecka, K. Kulma, L. Gustafsson and W. Babik. 2012. MHC diversity, malaria and lifetime reproductive success in collared flycatchers. Molecular ecology, 21(10):2469-2479.

Reed, L. and B. R. Glick. 2023. The recent use of plant-growth-promoting bacteria to promote the growth of agricultural food crops. *Agriculture*, *13*(5). 1089.

Riseh, R. S., M. Hassanisaadi, M. Vatankhah, S. A. Babaki and E. Ait Barka. 2022. Chitosan as a potential natural compound to manage plant diseases. *International Journal of Biological Macromolecules*, *220*: 998-1009.

Sahel, N. R., E. H. Taha, W. M. El-Nagdi and M. M. Zayed. 2020. Effect of certain Pseudomonas Fluorescents isolated on the infection of ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* in tomato and eggplant and the plant growth [23]. In J. Agric. Sci., Ain Shams Univ (Vol. 82, Issue 1).

Shilpa, G. V. 2022. Emotional analysis using hybrid deep learning models. *International Journal of Signal Processing*, *7*.

Sikora, R. A., and E. Fernandez. 2005. Nematode parasites of vegetables. In *Plant parasitic nematodes in subtropical and tropical agriculture* (pp. 319-392). Wallingford UK: CABI publishing.

Singh, S. 2023. Natural Product Chemistry in Agriculture. *Green Chemistry in Agriculture and Food Production*, *1*.

Tapia-Vázquez, I., A. C. Montoya-Martínez, S. De los Santos-Villalobos, M. J. Ek-Ramos, R. Montesinos-Matías and C. Martínez-Anaya. 2022. Root-knot nematodes (Meloidogyne spp.) a threat to agriculture in Mexico: Biology, current control strategies, and perspectives. *World Journal of Microbiology and Biotechnology*, *38*(2). 26.

Usman, M., M. Farooq, A. Wakeel, A. Nawaz, S. A.Cheema, ur H.Rehman and M. Sanaullah. 2020. Nanotechnology in agriculture: Current status, challenges and future opportunities. *Science of the total environment*, *721*.137778.

Vishnu, M., K. Malaisamy, R. P. Soundararajan, A. Suganthi, A. Subramanian, M. Senthilkumar and G. Kasivelu. 2024. Nano-bioformulations: emerging trends and potential applications in next generation crop protection. *Environmental Science: Nano*.

Winston, ML.1987. The Biology of the Honey Bee. Harvard University Press, Cambridge, MA

Yáñez-Juárez, M. G., C. A. López-Orona, F. Ayala-Tafoya, L. Partida-Ruvalcaba, T. D. J. Velázquez-Alcaraz and R. Medina-López. 2018. Phosphites as alternative for the management of phytopathological problems. *Revista mexicana de fitopatología*, *36*(1): 79-94.

الملخص العربي

مقارنة فاعلية الحبيبات النانوية للفضة مع الكيتوزان والمنتجات الحيوية كاستراتيجيات بديلة لمكافحة نيماتودا تعقد الجزور وتأثيرها على الكائنات الغير مستهدفة

محمد سلامه حامد ، هدي متولي نصر ، مصطفي عبداللطيف عباسي ، انتصار إبراهيم ربيع

تُعد نيماتودا تعقد الجذور من الآفات الخطيرة التى تهدد المحاصيل الزراعية وتؤثر سلبًا على إنتاجيتها، مما تسبب خسائر عالمية تتجاوز ١٧٥ إلى٢٠٠ مليار دولار. فى هذا السياق أُجري هذا البحث لتقييم فاعلية أربعة مواد مُعززة للنمو، وهي الكيتوزان، وجزيئات الفضة النانوية مع الكيتوزان (Ch-AgNPs)، والمبيد الحيوي البكتيري (*Pseudomonas fluorescens*)، والفوسفيت، في مكافحة نيماتودا تعقد الجذور *M. incognita*. ركزت الدراسة بشكل خاص على Ch-AgNPs، حيث تم تحضير المستحضر ووُصفت خصائصه باستخدام المجهر الإلكتروني الماسح (SEM)، وأظهرت النتائج أن متوسط حجم جسيمات Ch-AgNPs تتراوح بين ٨١,٠٨ و٢١٦,٢٢ نانومتر. وأظهرت النتائج ايضا كفاءة Ch-AgNP العالية فى القضاء على يرقات (J_2) من النيماتودا، حيث سجلت قيم LC_{50} منخفضة بلغت ٣٩,١٩ ملجم/لتر بعد ٢٤ ساعة و ٢٦,١٢ ملجم/لتر بعد ٤٨ ساعة. ومن ناحية الأمان البيئى، تبين أن Ch-AgNPs يمتلك تاثيرًا معتدلا على نحل العسل عند التركيزات العالية (٢٠% موت عند ٥٠٠ ملجم/لتر بعد ٧٢ ساعة)، وتاثيرًا يتراوح بين معتدل إلى مرتفع على ديدان الأرض يتراوح بين ١٠٪ إلى ٤٦,٦٧٪ موت بعد ٤ أسابيع من التعرض. وكانت سميته تجاه ديدان الأرض أقل بكثير من الفوسفيت عند أعلى التركيزات، مع فقدان وزن يقارب الكنترول.

الكلمات المفتاحية: تأثير مبيد النيماتودا، جزيئات الفضة النانوية مع الكيتوزان، المبيد الحيوي البكتيري، الفوسفيت، نيماتودا تعقد الجذور.