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Assessment of nematicidal efficacy of some biomaterials against Meloidogyne incognita on eggplant (Solanum melongena L.)

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Abstract

Plant Parasitic Nematodes (PPN) are regarded as one of the major challenges of sustainable Eggplant production in the world. It is the most significant pests of vegetable crops and is known a root-knot nematode (*Meloidogyne* spp.). Eggplant (*Solanum melongena*) is one of the utmost common vegetable crops in Egypt. The present investigation was performed under greenhouse conditions to evaluate the potentials of four biomaterials, LMW Chitosan, Ch-AgNPs, bio pesticide (*Pseudomonas fluorescens*) ,and phosphite for the control of the nematode *M. incognita* root-knot disease of eggplant. All treatments reduced ($p \leq 0.05$) the nematode population in soil and roots as well as enhanced the plant growth parameters of eggplant remarkably than the control. The applied treatments varied in efficacy against the plant nematode infection in correspondence to the time of application. The recorded results demonstrated that maximum reduction in second stage Juvenile (J_2) in soil, egg mass/root, and egg/egg masses were obtained by treating the soil with Ch-AgNPs followed by biopesticide and LMW Chitosan. In addition, Ch-AgNPs resulted in a high reduction in root galls (77.55%) compared to control treatment (0.0%). The use of plant growth-promoting LMW Chitosan, Ch-AgNPs, biopesticide (*Pseudomonas fluorescens*), and phosphite achieved efficient control to *M. incognita* and consequently increased the eggplant growth parameters under greenhouse conditions. The highest PPO activity and total soluble phenol contents values were achieved by phosphite compared with other treatments. The present results suggested introducing such Ch-AgNPs in an integrated nematode management program.

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تقييم فعالية بعض المواد الحيوية ضد نيماتودا تعقد الجذور *Meloidogyne incognita* على نبات الباذنجان (*Solanum melongena* L.)

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الملخص العربى:

تعتبر النيماتودا الطفيلية النباتية (PPN) واحدة من التحديات الرئيسية لإنتاج الباذنجان المستدام في العالم. وهي من أهم الآفات التي تصيب محاصيل الخضر وتعرف بنيماتودا تعقد الجذور (Meloidogyne spp.). ويعتبر نبات الباذنجان (*Solanum melongena*) أحد محاصيل الخضر الأكثر شيوعًا في مصر. يتأثر هذا النبات بمجموعة واسعة من الآفات ومن أبرزها نيماتودا تعقد الجذور *Meloidogyne incognita*. والتي تشكل مجموعة كبيرة من الديدان الخيطية المتطفلة على النباتات وتسبب خسائر اقتصادية كبيرة على مستوى العالم، وخاصة في مصر. تم إجراء الدراسة الحالية في ظل ظروف الصوب الزراعية لتقييم كفاءة أربع مركبات معززة للنمو LMW Chitosan وCh-AgNPs والمبيد الحيوي(*Pseudomonas Fluorescens*) ، والفوسفيت لمكافحة مرض تعقد الجذور الخيطي *M. incognita* في نبات الباذنجان. أظهرت النتائج ان جميع المعاملات أدت إلى خفض ($p \geq 0.05$) تعداد النيماتودا في التربة والجذور وعززت مؤشرات نمو نبات الباذنجان بشكل ملحوظ أكثر من معاملة الكنترول. وتباينت فعالية المركبات المطبقة ضد الإصابة بالديدان الخيطية في النبات حسب وقت التطبيق. أظهرت النتائج المسجلة أنه تم الحصول على الحد الأقصى من الخفض في J_2 في التربة، وكتلة البيض/الجذر، وكتل البيض/البيض عن طريق معاملة التربة باستخدام Ch-AgNPs متبوعة بالمبيد الحيوي و LMW Chitosan. بالإضافة إلى ذلك، أدى Ch-AgNPs إلى انخفاض كبير في العقد الجذرية (77.55٪) مقارنة بمعاملة الكنترول (0.0٪). أدى استخدام LMW Chitosan المعزز لنمو النبات، Ch-AgNPs، والمبيد الحيوي(*Pseudomonas Fluorescens*) ، والفوسفيت إلى تحقيق مكافحة فعالة على *M. incognita* وبالتالي زيادة معدلات نمو نبات الباذنجان في ظل ظروف الصوب الزراعية. تم تحقيق أعلى نشاط PPO وقيم محتوى الفينول الذائب الكلي بواسطة الفوسفيت مقارنة بالمعاملات الأخرى. تقترح النتائج الحالية إدخال Ch-AgNPs في برنامج المكافحة المتكاملة لنيماتودا تعقد الجذور.

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الكلمات المفتاحية: نيماتودا تعقد الجذر، المواد الحيوية، تأثير مبيد النيماتودا، شيتوزان *LMW*، *Ch-AgNPs*، المبيدات الحيوية (الزائفة المتألقة)، الفوسفيت، بوليفينول أوكسيديز (*PPO*) ومحتويات الفينول الكلية القابلة للذوبان.

INTRODUCTION

Plant-parasitic nematodes cause significant damage to most crops (Sikora and Fernandez, 2005) with annual losses estimated to be $100 billion worldwide (Ghareeb et al. 2020). Plant-pathogenic nematodes (including root-knot nematodes) reduce crop yield by 8.8% in developed countries and up to 14.6% in tropical and subtropical regions. Root-knot nematodes pose a grave threat as they infest plant roots, resulting in substantial damage as they interfere with plant physiology, leading to reduced crop yield and compromised product quality (Niu et al. 2020). Infestation of these nematode species results in the formation of galls or root-knots on infected plants. Additional symptoms include hindered growth, wilting, and reduced fruit production (Tapia-Vázquez et al. 2022). *M. incognita* can cause crop failure in the absence of effective control. Prevention and control of such pests will remain an important objective of most researche. Today, plant-parasitic nematodes are controlled by cultural practices, chemical nematicides, and by the growing of resistant cultivars (Curto et al. 2006). Chemical control is expensive and is economically viable only for high-value crops and creates a potential hazard to the environment and human health. Therefore, alternative nematode control methods or less toxic nematicides need to be developed (Ploeg, 2007). One way of searching for such nematicidal compounds is to screen eco-friendly nematicides (Wiratno et al. 2009).

Chitosan oligosaccharide is an alternative biological macromolecule worthy of deep research in pest control. Due to its excellent biocompatibility and unique physiological and biological activity, Chitosan has been widely used as a biostimulant, antifungal agent, seed treatment agent, soil conditioner, and fertilizer in agriculture. As biostimulants, Chitosan can enhance the crop disease resistance by stimulating the secretion of immune enzymes and compounds (such as salicylic acid and jasmonic acid) (Khalil and Badawy, (2012); Yuan et al. 2019).

Chitosan has been reported as an elicitor to influence the local and systemic resistance against *M. incognita* in tomatoes (Radwan et al. 2012). As a result, there is a growing interest in exploring the potential of nanotechnology in various fields, including agriculture, the food industry, pharmaceuticals, and medicine (Alfy et al. 2020). Chitosan nanoparticles are natural materials with exceptional physicochemical, antibacterial, and biological properties, making them ideal environmentally friendly materials with bioactivity that is very safe for humans (Divya and Jisha, 2018). Therefore, chitosan is well-suited as a biopolymer for coating the anionic surfaces of emulsion droplets to enhance colloidal stability in acidic conditions and improve nematicidal activity of natural pesticides.

Phosphite is a reduced form of phosphate and has been shown to have growth-promoting properties in nutrient-replete conditions in oranges, celery, satsuma, wheat, oilseed rape and several other plants (Rossall et al. 2016). Phosphite is an effective pesticide for controlling various phytopathogenic organisms, including nematodes (Puerari et al. 2015).

Manganese phosphite was also effective against *Meloidogyne javanica* prevention in Glycine max and decreased the number of eggs/grams of

root when applied 7 days before infection of nematodes in pest-resistant cultivar (Puerari et al. 2015). Similarly, Oka et al. (2007) found that the use of Phi in wheat and oats significantly suppressed *Heterodera avenae* and *Meloidogyne marylandi*, which stimulated Phi's ability to confirm the synthesis of phytoalexins in plants (Han et al. 2021).

Plant growth-promoting rhizobacteria, such as *Pseudomonas fluorescens* CHA0, have found to be highly effective in controlling plant pathogens like root-knot nematodes (Tavakol Norabadi et al. 2014). *P. fluorescens* is known for its high solubilisation capacity of soil phosphorous (Galindo 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 (Trdan et al. 2018). Several *Pseudomonas* species were also found to be effective in managing root-knot nematode under pot experiments (Kavitha et al. 2011). Therefore, the present investigation was conducted with the aim of testing the potential of four growth promoting, LMW Chitosan, Chitosan-Ag nanoparticles (Ch-AgNPs), biopesticide (*Pseudomonas fluorescens*), and phosphite against root-knot nematode Meloidogyne incognita infestation and growth, as well as growth yielding attributes of eggplant under greenhouse conditions. In addition, the biochemical parameters were performed.

MATERIALS AND METHODS:

Chemicals and pesticides:

Low molecular weight chitosan (made from coarse ground crab, 89% degree of deacetylation) was obtained from Sigma-Aldrich Chemical Co. Sodium tripolyphosphate (STPP), Pyrogallol, catechol, polyvinyl pyrrolidone (PVPP) were obtained from El- Gomhoria Company, Alexandria, Egypt and used without further purification. Phosphite (Cropper guard® 33%) was obtained from Cropper Agra agency, Spain. *Pseudomonas fluoresce* (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 the Bridge Trade agency.

Source of root knot-nematodes:

The eggs of the root-knot nematode, *M. incognita*, were isolated from infested roots of eggplant (*Solanum melongena L.*) which is collected from El Behera Governorate fields. Sodium hypochlorite (NaOCl) was used for the isolation of nematode eggs from root galls according to Hussey and Barker (1973). They passed through 200 and 400 mesh sieves to obtain free eggs directly before carrying out the experiments and then put in the incubator at 27°C for 72 hours. The suspension of two-days-old second-stage larvae (J_2) of *M. incognita* was passed through a 325-mesh sieve and then the retained larvae were collected on the sieve by backwashing into a 250 ml beaker. The larvae were counted under a microscope, which showed 200 larvae/1 ml.

Plant materials:

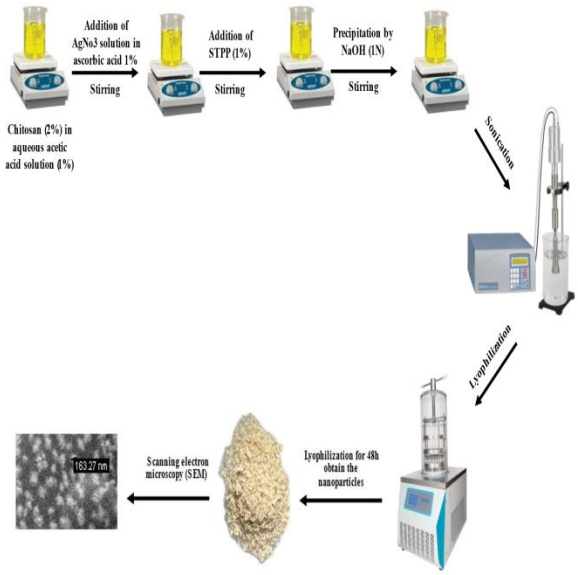
Surface-sterilized seeds of eggplant (*Solanum melongena* L.) were germinated in seedling trays filled with a sterilized peat-moss and allowed to grow up in the greenhouse for 3 weeks. Healthy and uniform 3-week-old seedlings were transplanted into plastic pots (one seedling per pot) of 20 cm diameter and filled with a 2.5 kg mixture of autoclaved sand and clay (3:1, v:v). Plants were kept in the greenhouse at 30±2 °C

under natural daylight length conditions, watered once every 2, days and fertilized once a week with commercial fertilizer with N (18%), P (18%), K (18%) and S (1.26%) (Pharma 18-18-18, Farmers for Agriculture Development, Egypt) at the rate of 2.5 g/L of water until the end of the experiment.

Preparation of silver nanoparticles:

Chitosan-Ag nanoparticles (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 and then added dropwise under magnetic stirring onto 50 mL of chitosan solution (2%, w/v) to obtain a homogeneous solution. After magnetic stirring for 30 min, 5 mL of vitamin C solution (1%) was added dropwise into the mixture and the pH was adjusted to 6. The reaction continued stirring for 60 min at 50 °C. STPP solution (1%) was added dropwise to the previous mixture. The resultant 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 5 cycles /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, 37520Osterode Harz, Germany) for 48h to obtain nanoparticles.

Morphological properties of the Ch-AgNPs samples were investigated with a JEOL(JCM-7000 Benchtop Scanning Electron Microscope) with a magnification of 10000x and acceleration voltage 20 kV. The dry particles were suspended in ethyl alcohol by sonication to dismantle the assembled particles. After that, the particles were mounted on metal stubs with double-sided tape, sputtered with gold, and viewed in a SEM. In addition, the SEM also measured the particle size of the products.



Scheme 1: Preparation of Ch-AgNPs

Characterizations of nanoparticles

Scanning electron microscopy (SEM):

Pot experiment:

Ten millilitres of water suspension of eggs and J_2 were pipetted into four holes of 5 cm depth around the plant roots zone after ten days after transplanting, each pot was inoculated with 5000 eggs and freshly hatched J_2 and the holes were covered immediately with soil. Treatments included chitosan, Ch-AgNPs, phosphite, biopesticide and fenamiphos 40% EC at the recommended rate were applied a soil drench in 150 ml water per pot. Untreated un-inoculated and untreated inoculated (nematode alone) pots served as controls. The experiment was arranged in a randomized block design with 20 replicates

(pots) per treatment. Five replicates were used for nematode assay and the remaining replicates were used for enzymes analysis. Plants were uprooted after 60 days of inoculation and gently washed with running tap water, then shoot and root lengths (cm) and fresh weights (g) was recorded, and the increase percentage over untreated inoculated control were estimated. Roots were stained in 0.015% Phloxine B solution for 15–20 min (Holbrook *et al*. 1983) and numbers of nematode galls, egg masses, and eggs/plant root were counted and recorded (Taylor and Sasser 1978). (J_2) were extracted and counted from 250 cm^3 soil per pot using the bucket sieving technique (Cobb 1918). The reduction percentage in nematode parameters over untreated inoculated control was calculated as follows:

$$\text{Reduction (\%)} = \frac{\text{Numbers in the control} - \text{Numbers in the treated plants}}{\text{Number in control}} x100$$

Biochemical studies:

Polyphenol oxidase (PPO) activity:

Polyphenol oxidase (PPO) was determined in eggplant roots after 0, 2, 7, 12, and 20 days post inoculation (dpi). Three plants (replicates) per treatment were randomly collected at each specific sampling time, rinsed with demineralized water, and stored at − 80 °C for further analysis. One gram of root was homogenized in 5 ml extraction buffer (50 mM phosphate buffer, pH 7.0, 1 mM EDTA, and 2% PVPP) in an ice-cold mortar. The homogenate was centrifuged at 12,000 ×g for 20 min at 4 °C and the supernatant was used for enzyme activity assays (Jakovljevicet et al. 2017). PPO activity assays were performed as described by Hussey *et al*. (1972). The mixture contained 200 µl of enzyme extract to 1.5 ml of 0.1 M phosphate buffer (pH 6.5) and 200 µl of 10 mM catechol. The changes in absorbance were recorded for 1 min at 495 nm. Enzyme activity was presented as ΔOD_{495} min /g FW (Mayer et al. 1965).

Total soluble phenol contents:

The total soluble phenol content of eggplant leaves was extracted as described by Slinkard and Singleton (1997). The absorbance was carried out on 765 nm by using a spectrophotometer (Tuner, model 390). Total soluble phenol content was standardized against tannic acid and absorbance values were converted to µg of phenols per gram fresh weight of eggplant leaves. Each value reported was the average of three replicates. The results were expressed as tannic equivalents according to the following equation:

$$\mu g \text{ tannic } acid \,/\, g \text{ fresh weight} = \frac{OD}{K} \times 100$$

Where: OD = absorbance at 765 nm, K= the extension coefficient = 0.016898 μg^{-1}

Statistical analysis:

Statistical analysis was performed using the SPSS 27.0 software program (Statistical Package for Social Sciences, Chicago, USA). The mortality rates of the nematodes in the exposure groups were corrected using Abbott's formula (Abbot 1925). The corrected 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 of the relative growth rate (% control) against the logarithm of the compound concentration. The data from in vivo 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:

1. Characterization of Ch-AgNPs:

Scanning electron microscopy (SEM):

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

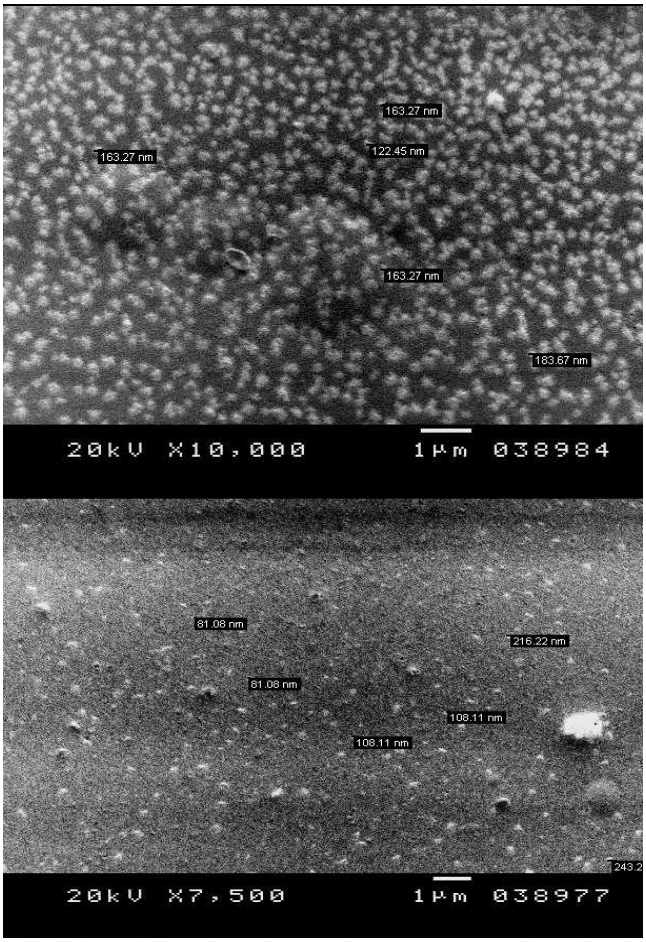


Fig. 1: Scanning electron microscopy (SEM) micrograph of the Ch-AgNPs

2. Pot experiments:

In pot experiments, the four compounds, LMW chitosan, Ch-AgNPs, phosphite and biopesticide at 0.5, 1, 2, and 4fold of LC_{50} value were significantly effective in reducing the nematode population, root galling and egg mass compared with the control after two months of a single application (Table 1). The experiments revealed that the total number of live nematodes on roots of eggplant treated with Ch-AgNPs was significantly the lowest when it reduced the population to 911.67 larvae/250 g soil (75.60 % reduction) at the high concentration compared with 3736.67 larvae/250 g soil in the control. Following in the descending order with LMW chitosan, with a population 961.67 larvae/250 g soil (74.26 % reduction) at the same fold of LC_{50}. Therefore, there was no significant difference between phosphite and biopesticide at 0.5, 1, 2 and 4fold of LC_{50} values on the nematode population (ranging from 1054-1477 larvae/250 g soil) as shown in Table 1.

Roots of eggplant grown in the soil treated with Ch-AgNPs had very few galls especially at the four-fold LC_{50} value (68.33 galls/plant, 77.55 % reduction). While control plants were heavily galled (304.33 galls/root). The effect of biopesticide treatment on eggplant roots on gall was found to be 66.05 % at four-fold LC_{50} value (103.33 galls/plant) and the reduction (%) is concentration dependent. Following in the descending order with LMW chitosan, with 123.0 galls/plant, 59.58 % reduction at the four-fold LC_{50} value. However, the highest gall (223.0 and 207.0) was found in phosphite treatment at the 0.5 and 1.0-fold LC_{50} value with 26.72 and 31.98 % reduction, respectively.

Table (1): Nematicidal effect of LMW chitosan, Ch-AgNPs, phosphite and biopesticide on eggplant roots infested with the root-knot nematode *M. incognita* under greenhouse conditions.

Compounds	Fold of LC_{50}	Population (larvae/250 g soil) ± SE	Reduction (%)	Number of galls ± SE	Reduction (%)	Number of egg mass/root ± SE	Reduction (%)
LMW Chitosan	0.5	1527.33^{b}	59.13	162.67^{d}	46.55	125.33^{e}	56.63
	1	1308.67^{bcde}	64.98	157.67^{d}	48.19	112.67^{ef}	61.01
	2	1089.33^{efg}	70.85	143.00^{e}	53.01	96.00^{g}	66.78
	4	961.67^{fg}	74.26	123.00^{f}	59.58	79.33^{h}	72.55
Ch-AgNPs	0.5	1477.00^{b}	60.47	104.33^{g}	65.72	94.33^{g}	67.36
	1	1221.33^{cdef}	67.31	80.00^{h}	73.71	79.67^{h}	72.43
	2	985.33^{fg}	73.63	74.67^{h}	75.47	61.33^{i}	78.78
	4	911.67^{g}	75.60	68.33^{h}	77.55	51.67^{j}	82.12
Phosphite	0.5	1477.00^{b}	60.47	223.00^{b}	26.72	213.67^{b}	26.07
	1	1377.67^{bcd}	63.13	207.00^{c}	31.98	194.67^{c}	32.64
	2	1206.00^{cdef}	67.73	163.00^{d}	46.44	154.67^{d}	46.48
	4	1054.00^{efg}	71.79	128.67^{f}	57.72	114.00^{ef}	60.55
Bio pesticide	0.5	1398.00^{bc}	62.59	143.33^{e}	52.90	119.00^{e}	58.82
	1	1282.33^{bcde}	65.68	126.00^{f}	58.60	113.00^{ef}	60.90
	2	1141.33^{defg}	69.46	114.67^{fg}	62.32	102.00^{fg}	64.71
	4	1006.00^{fg}	73.08	103.33^{g}	66.05	92.67^{g}	67.94
Fenamiphos	0.5	317.67^{h}	91.50	56.00^{i}	81.60	26.67^{k}	90.77
	1	284.00^{h}	92.40	42.33^{j}	86.09	17.33^{kl}	94.00
	2	222.00^{h}	94.06	36.67^{j}	87.95	10.67^{lm}	96.31
	4	195.00^{h}	94.78	33.33^{j}	89.05	6.00^{m}	97.92
Infection control	-	3736.67^{a}	0.00	304.33^{a}	0.00	289.00^{a}	0.00

In addition, all the treatments were significantly effective in the reduction of egg mass compared with the untreated plants and the reduction (%) is concentration-dependent. Ch-AgNPs were the most active ones (51.67 egg mass/plant at the 4.0-fold LC_{50} value compared with 289.0 egg mass/plant in the control). Moreover, moderate effects were detected with LMW chitosan and biopesticide treatments (79.33 and 92.67 egg mass/plant compared at high concentrations, respectively). However, the highest egg mass (213.67 egg mass/plant) were found in phosphite treatment at the 0.5 fold of LC_{50} value with a 26.07 % reduction. Generally, it can be noticed that the nematicidal activity was increased dramatically with an increase in the concentration and there was no plant mortality during the experiment.

Results presented in Table 2 demonstrated different significant ($P \leq 0.05$) stimulatory effects of the evaluated agents on vegetative eggplant growth parameters in terms of shoot, root lengths, fresh shoot and root weights, relative to different concentrations. Plant heights, root lengths and plant biomass had the highest values in Phosphite treatment (shoot length 56.00 and 36.33 cm/plant and 138.67 g/plant, respectively at high concentration) and followed by biopesticide and Ch-AgNPs (as shown in Table 2). Wet root weight was found to be lower in the positive control

(42.33 g/plant) than the nematode-free control (20.33 g/plant), and a statistical difference was determined between them (P≤0.05). Generally, there was no significant difference between Ch-AgNPs and LMW chitosan treatments in terms of the shoot system of plant growth parameters. Plant growth parameters of 0.5-fold of LC_{50} concentration of all treatments were determined lower than that of 4.0-fold of LC_{50}, and it was noticed that the Plant growth parameters were concentrations dependent (Table 2).

Table (2): Plant growth in eggplant infected with *M. incognita* in which LMW chitosan, Ch-AgNPs, phosphite and biopesticide concentrations were applied under greenhouse conditions.

Compounds	Fold of LC_{50}	Shoot system		Root system	
		Shoot length (cm/plant± SE)	Fresh Shoot weight (g/plant± SE)	Root length (cm/plant± SE)	Fresh root weight (g/plant± SE)
LMW Chitosan	0.5	26.00^{e}±0.67	124.33^{fg}±0.58	28.00^{efghi} ±1.33	40.67^{k}±1.33
	1	33.67^{d}±0.33	130.67^{de}±1.00	30.00^{cdefg} ±1.13	50.00^{fghi}±1.15
	2	44.00^{b}±0.88	134.00^{bcd}±0.33	30.33^{bcdef}±0.88	54.00^{efgh}±0.88
	4	44.67^{b}±0.58	135.67^{abc}±0.88	31.67^{abcdef}±1.20	55.67^{defg}±1.20
Ch-AgNPs	0.5	28.00^{e}±1.20	124.00^{fg}±1.20	27.67^{efghi}±0.58	54.67^{defg}±0.58
	1	34.67^{d}±1.20	128.33^{ef}±0.33	31.00^{abcdef}±1.20	56.33^{cdefg}±1.20
	2	43.67^{b}±1.00	137.67^{ab}±1.20	33.67^{abcde}±0.58	59.33^{bcde}±0.58
	4	46.67^{b}±0.67	136.00^{abc}±1.20	35.33^{abc}±0.88	66.00^{ab}±0.88
Phosphite	0.5	35.33^{d}±0.58	130.33^{de}±1.20	32.00^{abcd}±1.00	48.33^{ghij}±1.00
	1	42.33^{bc}±0.67	132.33^{cde}±0.67	34.33^{abcd}±0.58	54.33^{efgh}±0.58
	2	54.33^{a}±0.33	136.67^{abc}±1.00	36.00^{ab}±0.67	63.33^{bc}±0.67
	4	56.00^{a}±0.58	138.67^{a}±0.88	36.33^{a}±0.67	70.67^{a}±0.67
Bio pesticide	0.5	27.67^{e}±2.85	123.00^{g}±0.58	26.00^{fghij}±0.67	53.00^{efghi}±0.67
	1	33.67^{d}±0.58	124.00^{fg}±1.00	26.67^{fghij}±1.00	54.67^{defg}±1.00
	2	43.67^{b}±0.88	129.33^{de}±0.58	29.33^{defgh}±0.88	57.33^{cdef}±0.88
	4	46.00^{b}±3.38	130.67^{de}±1.15	32.33^{abcd}±1.20	62.33^{bcd}±1.20
Fenamiphos	0.5	28.67^{e}±1.00	120.00^{g}±1.15	24.33^{ij}±1.00	43.67^{jk}±1.00
	1	33.00^{d}±0.33	124.00^{fg}±0.67	25.00^{hij}±2.33	44.00^{jk}±2.33
	2	35.67^{d}±0.88	131.00^{de}±0.67	25.67^{ghij}±1.45	46.33^{ijk}±0.67
	4	38.67^{cd}±0.33	131.00^{de}±1.53	25.33^{hij}±0.67	47.00^{hijk}±1.45
Infection control	-	22.00^{f}±0.58	97.67^{h}±2.85	14.33^{k}±1.45	20.33^{l}±1.45
Unification control	-	36.00^{d}±2.52	129.67^{de}±0.67	23.33^{j}±0.33	42.33^{jk}±0.33

Results presented in Tables 3 and 4 demonstrated different significant (P ≤ 0.05) stimulatory effects of the evaluated agents on biochemical parameters. The highest PPO value (30.0 OD/mg protein) at high concentration after 12 days, was recorded in Phosphite followed in descending order by biopesticide (22.44 OD/mg protein) compared with the control (9.33 OD/mg protein) as shown in Table 3. Treatment with Ch-AgNPs increased the activity of PPO by 15.89 OD/mg proteins at high concentrations after 12 days.

The total soluble phenolic content increased significantly in the leaves of eggplant (as seen in Table 4). Among the treated plants, the maximum total soluble phenolic content of 44.07 ug gallic acid equivalents (GAE)/g leave was displayed by Phosphite at high concentration (4-fold of LC_{50} value) after 12 days. The least effect was recorded by LMW-chitosan with 27.0 ug gallic acid equivalents (GAE)/g leave at high concentration (4-fold of LC_{50} value) after 12 days (Table 4).

Table (3): Effects of LMW-chitosan, Ch-AgNPs, Phosphite and biopesticide on PPO activity of eggplant in relation to root-knot development caused by *M. incognita.*

Compound	Fold of LC_{50}	Specific activity (OD/mg protein) ± SE				
After / Day		0	2	7	12	20
LMW Chitosan	0.5	3.78^{a}±0.97	4.67^{e}±0.33	6.89^{ghi}±0.11	12.22^{hi}± 0.48	9.22^{ijk} ± 0.44
	1	3.56^{a}±0.29	4.56^{e}±0.29	8.11^{fghi}±0.40	12.44^{hi}± 0.40	10.00^{hij} ± 0.19
	2	4.22^{a}±0.97	6.11^{de}±0.56	8.56^{fgh}±0.29	16.33^{f}± 0.67	10.22^{hij} ± 0.29
	4	5.11^{a}±0.87	7.00^{cde}±0.19	8.78^{fg}±0.73	20.44^{e}± 0.95	11.11^{ghij} ± 0.62
Ch-AgNPs	0.5	3.78±0.97	6.44^{cde}±0.56	9.78^{ef}±0.11	14.67^{fgh}±0.19	11.56^{ghi} ± 0.29
	1	3.56^{a}±0.29	6.89^{cde}±0.29	10.22^{def}±0.48	13.44^{ghi}±0.44	10.56^{ghij}± 0.40
	2	4.22^{a}±0.97	6.89^{cde}±0.29	10.22^{def}±0.11	15.89^{fg}± 0.62	12.22^{fgh} ± 0.29
	4	5.11^{a}±0.87	7.00^{cde}±0.84	11.22^{cde}±0.73	15.89^{fg}± 0.80	12.56^{fg} ± 0.29
Phosphite	0.5	3.78^{a}±0.97	8.22^{cd}±0.59	14.22^{b}±2.12	24.67^{c}± 0.67	14.78^{de} ± 0.87
	1	3.56^{a}±0.29	8.11^{cd}±0.95	19.22^{a}±0.44	23.67^{cd}± 1.07	17.22^{c} ± 0.56
	2	4.22^{a}±0.97	8.00^{cd}±2.01	21.22^{a}±0.40	27.44^{b}± 0.97	20.11^{b} ± 0.29
	4	5.11^{a}±0.87	12.67^{a}±0.84	21.11^{a}±0.62	30.00^{a}± 0.38	21.89^{a} ± 0.95
Bio pesticide	0.5	3.78^{a}±0.97	8.44^{cd}±0.91	12.44^{bcd}±0.22	16.00^{fg}± 1.00	13.56^{ef} ± 0.29
	1	3.56^{a}±0.29	8.33^{bc}±0.58	12.56^{bcd}±0.44	16.67^{f}± 0.00	15.22^{de} ± 0.40
	2	4.22^{a}±0.97	9.67^{ab}±0.00	13.33^{bc}±0.19	21.22^{e}± 0.80	14.67^{de} ± 0.88
	4	5.11^{a}±0.87	11.44^{a}±0.62	14.11^{b}±0.29	22.44^{de}± 0.59	16.11^{cd} ± 0.59
Fenamiphos	0.5	3.78^{a}±0.97	4.22^{e}±0.48	6.00^{hi}±0.33	12.11^{hi}± 0.73	7.56^{k} ± 0.11
	1	3.56^{a}±0.29	4.22^{e}±0.11	5.78^{i}±0.22	10.78^{ij}± 0.11	8.89^{jk} ± 0.78
	2	4.22^{a}±0.97	5.22^{de}±0.56	6.67^{ghi} ±0.19	11.33^{ij}± 0.67	9.89^{hij} ± 0.11
	4	5.11^{a}±0.87	6.00^{de}±0.33	6.89^{ghi}±0.29	12.78^{hi}± 0.22	9.78^{ij} ± 0.59
Infection control	-	4.11^{a}±0.29	5.89^{de}±0.29	8.56^{fgh}±0.59	9.33^{j}±0.67	10.44^{ghij}±0.40
Un infection control	-	4.11^{a}±0.59	4.22^{e}±0.59	3.89^{j}±0.40	4.00^{k}±0.00	4.00^{l} ± 0.51

The nematicidal activity of four molecular weights (2.27×10^5, 3.60×10^5, 5.97×10^5, and 9.47×10^5 g/mol) of chitosan was assayed against *M. incognita*, *in vitro* and in pot experiments (Khalil & Badawy, 2012).

In laboratory assays, the nematode mortality was significantly influenced by exposure times and chitosan molecular weight. Low molecular weight chitosan (2.27 × 105 g/mol) was the most effective in killing the nematode with EC_{50} of 283.47 and 124.90 mg/l after 24 and 48 h of treatment, respectively. In a greenhouse bioassay, all the compounds mixed in soil at one- and five-fold concentrations of the LC_{50} value significantly reduced the population, egg mass, and root galling of tomato seedlings compared with the untreated control.

Two different chitosan with low and high molecular weight at different dilutions were evaluated against, *M. javanica in vitro* and in *vivo* under greenhouse conditions (El-Sayed & Mahdy, 2015). *In vitro* results revealed that both chitosan with all used concentrations significantly reduced the egg hatching when compared to control. No hatched eggs were shown with the standard concentration of both chitosan. The high molecular weight chitosan gave no significant results in egg hatching with the most evaluated concentrations i.e. standard, 1:1, 1:5 and 1:10 compared to the control. Both low and high molecular weight chitosan significantly affected the larvae mortality of *M. javanica* compared to the control. Results found that standard, 1:1, and 1:2 dilutions were the most effective concentrations of the high molecular weight

chitosan as they prevent completely the larvae to live. In vivo results revealed that standard concentration of the high molecular weight chitosan was the highest effective one on nematode parameters as the reduction percentage was 92, 97, 92, 88, and 79%, respectively. Both chitosan was encouraged the contents of enzymes compared to the treated plants with nematode alone. Results found also that both chitosan markedly enhanced the plant growth parameters compared to the plants treated with nematode alone.

Table (4): Efficacy of LMW-chitosan, Ch-AgNPs, Phosphite, and biopesticide the on total soluble phenol eggplant plants to root-knot development caused by *M. incognita*

Compound	Fold of LC_{50}	ug gallic acid equivalents (GAE)/g leave				
Time (day)		0	2	7	12	20
LMW Chitosan	0.5	11.17^{a}± 0.54	11.48^{f} ± 0.31	18.00^{f}±0.82	22.97^{e}± 0.31	30.11^{a}±0.31
	1	11.17^{a}± 0.54	13.35^{def} ± 0.62	17.69^{f}±0.54	25.45^{de}± 0.62	31.66^{a}±0.00
	2	10.86^{a}± 1.35	14.28^{def} ± 0.31	18.93^{f}±0.82	26.69^{cde}± 0.31	32.28^{a}±0.62
	4	8.69^{a}± 0.82	16.45^{def}± 0.31	20.79^{ef}±0.31	27.00^{cde}± 0.00	34.14^{a}±0.62
Ch-AgNPs	0.5	11.17^{a}± 0.54	14.59^{def} ± 1.64	20.17^{f}±0.82	25.45^{de}± 1.24	30.11^{a}±0.31
	1	11.17^{a}± 0.54	14.59^{def}± 0.31	20.79^{ef}±1.12	29.49^{bcd}± 1.12	31.66^{a}±0.00
	2	10.86^{a}± 1.35	15.21^{def}± 0.31	21.42^{ef}± 0.93	31.04^{b} ± 0.62	32.28^{a}±0.62
	4	8.69^{a}± 0.82	18.00^{cd}± 0.62	26.07^{d}±0.93	30.42^{bc} ± 1.24	30.73^{a}±0.54
Phosphite	0.5	11.17^{a} ±0.54	23.59^{b} ± 0.82	31.97^{c}±0.31	41.59^{a} ± 0.31	31.97^{a}±1.35
	1	11.17^{a} ±0.54	24.21^{b} ± 0.93	33.83^{c}±1.12	42.21^{a}± 0.31	33.52^{a}±1.42
	2	10.86^{a} ±1.35	31.97^{a} ± 0.82	37.56^{b}±0.31	43.76^{a} ± 0.00	31.97^{a}±1.12
	4	19.55^{a} ±0.93	33.52^{a} ± 0.54	40.66^{a}±0.62	44.07^{a} ± 1.55	30.42^{a}±0.82
Bio pesticide	0.5	11.17^{a}± 0.54	15.52^{def}± 1.55	20.79^{ef}±1.35	26.38^{cde} ± 1.12	30.11±0.31
	1	11.17^{a} ±0.54	17.07^{de} ± 1.12	20.79^{ef}±1.12	31.04^{b} ± 0.62	31.66^{a}±0.00
	2	10.86^{a} ±1.35	16.45^{def} ± 1.55	24.21^{de}±1.42	32.59^{b} ± 0.93	32.28^{a}±0.62
	4	8.69^{a} ± 0.82	20.48^{c} ± 1.86	26.07^{d}±0.93	32.59^{b} ± 2.46	30.73^{a}±0.54
Fenamiphos	0.5	11.17^{a} ±0.54	11.48^{f} ± 0.62	17.69^{f}±0.54	23.28e ± 0.00	29.80^{a}±0.54
	1	11.17^{a} ±0.54	11.79^{f} ± 1.12	18.00^{f}±0.82	25.45^{de} ± 0.62	31.35^{a}±0.31
	2	10.86^{a} ±1.35	13.04^{ef} ± 0.93	19.24^{f}±0.82	26.38^{cde} ± 0.31	31.97^{a}±0.82
	4	8.69^{a} ± 0.82	14.59^{def} ± 1.35	21.11^{ef}±0.62	26.69^{cde} ±0.31	32.90^{a}±1.64
Infection control	-	11.17^{a} ±0.54	12.41^{ef} ± 0.62	11.17^{g}±1.08	18.00^{f} ± 0.62	16.14^{b}±1.35
Un infection control	-	11.17^{a}± 0.54	12.41^{ef} ± 0.62	11.17^{g}±1.08	12.73^{g}± 1.35	16.14^{b}± 1.35

Asif et al., (2017) evaluated the potential of chitosan alone and in combination with various agricultural wastes for the management of *M. incognita* on eggplant under greenhouse conditions. The maximum reduction in eggmass/root, eggs/eggmasses, nematode population and root-knot indices, was acquired by the treatments: chitosan + onion and chitosan + mentha. It was followed by chitosan + Brassica, chitosan + urad and chitosan + coconut whereas, chitosan combined with corn cob waste was found to be the least effective when compared to the control. Compared to the control applications of all the treatments significantly increased plant growth in terms of length, fresh and dry weights, pollen fertility, yield and biochemical parameters such as chlorophyll, carotenoid content and antioxidant enzymes. This may have been due to the eliciting activity of chitosan, causing systemic resistance in the plant and the release of various toxic chemical compounds during decomposition,

which have lethal effects against the J_2 of M. *incognita* and nematode multiplication.

The nematicidal efficacy of abamectin, boron, chitosan, hydrogen peroxide, *Bacillus thuringiensis* and oxamyl 24% SL against citrus nematode, *Tylenchulus semipenetrans* were examined on Valencia orange trees under field condition for two successive seasons (2017 and 2018) (Mam et al., 2019). The obtained results showed that all the tested treatments reduced nematode final population (Pf) and reproduction factor (Rf) compared with that obtained from the untreated trees. The highest percentages of Pf reductions (74.5-83.4 %) and (70%-82%) were recorded with oxamyl, boron, abamectin, chitosan and H_2 O_2 in the 1st and the 2nd tested seasons, respectively. Whereas, *B. thuringiensis* had the least nematode Pf reduction with 60.7 and 55.8% in the 1st and the 2nd seasons, respectively. Additionally, all treatments significantly improved orange yield (30.9-83.2% increase), physical fruit parameters and orange juice properties. The highest orange yield increase (83.2%) was recorded with boron treatment followed by oxamyl (70.3%). In addition, boron increased total soluble solids (TSS) by 13.6%, the volume of orange juice (36.4%) and vitamin C (19.7%) and decreased juice acidity (A) by (16.7%). Experiments were conducted to assess the efficacy of chitosan at different concentrations against *M. incognita* (Mouniga et al., 2022). Exposure of *M. incognita* egg mass to 5000 ppm and 10,000 ppm chitosan decreased the hatchability of eggs by 96.19% to 100% within 24 hrs time intervals, compared to the control. Similarly, chitosan at 5000 ppm and 10,000 ppm concentration caused 100% infective juvenile mortality. Under pot culture conditions, the application of chitosan at 10,000 ppm concentration decreased the number nematode population and enhanced plant growth. There was a 51.72% reduction in the number of *M. incognita* adult females in roots of treated plants and a 61.84% reduction in eggs over untreated control. Chitosan enhances the physiological response and mitigates the adverse effect of abiotic stresses through the stress transduction pathway via secondary messenger(s) (Hidangmayum et al., 2019). Chitosan treatment stimulates photosynthetic rate, and stomatal closure through ABA synthesis; enhances antioxidant enzymes via nitric oxide and hydrogen peroxide signaling pathways, and induces production of organic acids, sugars, amino acids and other metabolites which are required for the osmotic adjustment, stress signaling, and energy metabolism under stresses.

Chitosan nanospheres were obtained from chitosan using the ionic gelation technique (Mouniga et al., 2023). The efficacy of chitosan nanospheres in suppressing *M. incognita* was studied. The particle size of nanospheres was 380.2 nm, with a polydispersity index (PI) of 0.4 and Zeta potential of 45.7 or 50.9 mV at pH 5.2. In *In-vitro* studies, chitosan nanospheres showed significant nematicidal activity against *M. incognita*. Under pot culture conditions, chitosan nanospheres (1% chitosan) at 2ml/plant decreased the nematode population in roots or soil. Compared to the control, the number of galls was reduced by 83.68%, the number of egg masses by 83.85%, the number of adult females by 66.56%, and the number of second-stage juveniles by 73.20%. In a field experiment, the application of chitosan nanospheres (1%) was followed by an

18.75% increase in fruit yield compared to the non-treated control.

The efficacy of the phosphonate fertilizers, Calphos (a.i. calcium phosphonate), Magphos (a.i. magnesium phosphonate and potassium phosphonate) and Phosphoros (a.i. potassium phosphonate) against *M. javanica* and *M. incognita* is evaluated (Habash & Al-Banna, 2011). Laboratory experiments showed that Calphos, Magphos and their main components inhibited egg hatching and caused 100% mortality of the (J2s) of the two species; the hatching inhibition effects persisted after transferring the egg masses of both species to water. However, Phosphoros (0.5%) did not suppress egg hatching or the survival of J2s of both species. No hatching occurred when egg masses were treated for one week with the nematicide Vydate L (2 ml/l), however, J2s hatched when the Vydate L treated egg masses were moved to water. The glasshouse study indicated that Magphos, Calphos and Phosphoros reduced root galling caused by *M. javanica* by 98, 66 and 47%, respectively, in comparison to the untreated controls. Magphos resulted in the lowest number of root galls formed by *M. incognita,* and the reduction was 84%. In contrast, Calphos and Phosphoros reduced galling by 47 and 39%, respectively. Magphos treatment resulted in the lowest numbers of egg masses and the lowest reproductive factor (RF) of both nematode species. However, plants treated with Phosphoros resulted in higher foliage weights compared with the application of the other two fertilizers and the untreated plants.

Under screen house conditions, two experiments were carried out to evaluate certain bacterium, P. fluorescens isolates regarding the reproductive potential of *M. incognita*, infecting tomato or eggplant (Sahel et al., 2020). Results on tomato revealed that, based on average total percentages of nematode reduction, the over topped results were gained with *P. fluorescens* (Pf2) which recorded the highest significant (P≤0.05) average nematode reduction (61.3%) and higher percentage reduction of females (77%) per plant. The second rank was obtained by Pf3 which reduced all nematode numbers as an average of 56.9%. On the basis of average total percentages of plant growth and weight of fruit increases, four bacterial treatments can be ranked in a descending order as follows: Pf9 > Pf4>Pf1and Pf7, as they achieved the highest average total percentages increases of 96.0, 47.3, 38.2 and 29.8%, respectively compared to other treatments and untreated check. Regarding to eggplant, the over topped results observed was achieved by *P. fluorescens* (Pf10) which recorded the highest average total nematode reduction (66.2%) with a higher reduction of (J2s) in roots (89.9%) per plant and in soil (78.8%) per pot. The second rank was obtained by Pf9 and Pf2 where they reduced all nematode numbers as averages of 55.9% and 54.9%, respectively. Also, *Pseudomonas*.

Effects of *P. fluorescens* L. (Pf) isolate and the two plant extracts, *Datura stramonium* and *Myrtus communis*, were investigated on hatching and juvenile (J2s) mortality of *M. javanica* under laboratory conditions (Moazezikho et al., 2020). After determining the values of LC_{30}, LC_{50}, and LC_{70} of each extract, four leaf stage seedlings of tomato were treated with 20 ml of Pf suspension at a concentration of 108 CFU/ml, using a soil drenching method. After 1 week, the tested plants were inoculated by 4000 eggs and (J2s) of *M. javanica* and simultaneously were treated with 100 ml of the selected concentrations of *D.*

stramonium (1.1, 1.4, and 1.8%) and *M. communis* (1.8, 3 and 5.2%), as soil drench. Results showed that a combination of Pf and *D. stramonium* at the rate of 1.8% or *M. communis* at the rate of 5.2%, respectively, reduced the number of eggs per root system and the reproduction factor by 68 and 45%, the number of galls by 64 and 33%, and the number of egg masses by 65 and 43%, then the control. *Pseudomonas sp.* is a well-known rhizosphere growth-promoting bacterium. Abdelraouf et al., (2023) investigated the synergetic effect of using nano chitosan to deliver *P. fluorescens* on its stability in the soil and induction resistance against fusarium wilt in tomato plants. Results showed that nano-*Pf* treatment resulted in the highest growth percent (46.62%), and disease reduction percent (115.85%). This enhancement because of the significant increase in soil enzymes activity under the nano-*Pf* treatment, this increase percent was about 411% in urease, 488% in phosphatase, 765% in catalase, 194% in glucosidase and antifungal enzymes (1666% in chitinase and 89% in glucanase) in compared to non-treated infected soil. Also, nano-*Pf* treatment upregulated defense enzymes in tomato plants under infection, the increase percent was about 64% in superoxide dismutase, polyphenol oxidase, 80% in cell wall-bound peroxidase, and 180% in phenylalanine ammonia-lyase. Interestingly the increase percent of the antifungal enzymes chitinase and glucanase in the nano-*Pf* treated plants was about 231% and 260% respectively in compared to infected non-treated tomato plants. Nano-chitosan encapsulated *P. fluorescens* successfully mitigated Fusarium wilt infection and is a promising approach for a larger greenhouse study.

CONCLUSION:

The results concluded that using the plant growth-promoting, LMW chitosan, Ch-AgNPs, phosphite and bio pesticide achieved efficient control to the *M. incognita*, with the consequent increase in eggplant growth under greenhouse conditions. The results showed that Ch-AgNPs had a significant effect on nematode activity in eggplant root systems. This was evident in the reduced number of nematodes, galls, and egg masses. Phosphite was also effective in activating the enzymes PPO activity and total soluble phenol content values. Based on these results, we recommend the use of Ch-AgNPs and phosphite for nematode control in eggplant. These compounds have the potential to reduce nematode populations and improve plant defence mechanisms.

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