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Research Article
Comparison Study Between Silver Nanoparticles and Two Nematicides Against *Meloidogyne incognita* on Tomato Seedlings

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Abstract

Background: Root-knot nematode *Meloidogyne incognita* is one of the most important genera of plant-parasitic nematodes on different crops, distributed all over the world and attacks threat of thousands of plant species. **Objective:** Comparison between silver nanoparticles, nematicides and silver nanoparticles in combination with nematicides against root knot nematode *Meloidogyne incognita* had been studied. **Methodology:** Evaluation depends on the results of silver nanoparticles, nematicides fenamiphos and oxamyl with different concentrations from 25-90% and different exposure periods from 24-72 h. **Results:** The results indicated that the highest concentration 90% achieved the highest percentage of juveniles mortality after 72 h, which were 95, 87, 98 and 100% for silver nanoparticles, polyvinylpyrrolidone (PVP), fenamiphos and oxamyl, respectively, while mortality of the control was 1.5%. The study included the malformed shape of juveniles, showed paralysis when treated with fenamiphos, oxamyl and PVP, while silver nano-particles showed degradation in cell wall under laboratory conditions. Evaluation included root of tomato seedlings and it showed positive effect for silver nanoparticles. The pathogen signs such as galls, egg-masses, developmental stages, rate of build up and nematode in 250 g soil had been studied. The combination of fenamiphos and silver nanoparticles (1:1) gave significant decrease in number of galls, egg-masses, developmental stages, rate of build up and number of nematode in soil. Evaluating growth parameters showed significant increase in root length which reached to (40 cm) in the combination of oxamyl and nanoparticles compared with the root length of infested tomato seedlings (18 cm) under greenhouse conditions. **Conclusion:** The results clarified the positive effect of silver nanoparticles alone and in combination with the nematicides on *M. incognita* and the enhancement on growth parameters of tomato seedlings. More research needs to determine the long run effect.

Key words: *Meloidogyne incognita*, nematicides, silver nanoparticles, tomato seedlings

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Root-knot nematode *Meloidogyne incognita* is one of the most important genera of plant-parasitic nematodes on different crops, distributed all over the world and attacks threat of thousands of plant species.

Nanotechnology is a new promising technique in field of plant pathology by providing controlled delivery of functional molecules or as diagnostic tool for disease detection by produce new materials[1].

Nanoparticles have strong inhibitory and antimicrobial activities by a lot of studied and utilized nanoparticles in bio-system. Antifungal effectiveness of colloidal nanoparticles, against rose powdery mildew caused by *Sphaerotheca pannosa* var rosae. Nanoparticles colloid is a well dispersed and stabilized[2]. Silver nanoparticles have adhesive on bacteria and fungus. Nanoparticles classified as pesticide by Anderson[3]. Silver is now an accepted agrochemical replacement, eliminates unwanted microorganisms in planter soils and hydroponics systems. Silver nanoparticles are also an excellent plant-growth stimulator. Silver nanoparticles can be used as an effective antibacterial, antifungal, insictcidal and nematicidal activity[4-10].

The aim of the present study is to prepare silver nanoparticles so effective against tomato root-knot nematode *Meloidogyne incognita*.

MATERIALS AND METHODS

Laboratory experiment

Preparation of silver nanoparticles: Silver nanoparticles were prepared according to microwave method[11] at Plant Pathology Research Institute, Agricultural Research Center, Egypt.

Polyvinylpyrrolidone (PVP) and ethanol is poured in 100 mL flask. The solution was mixed until PVP is dissolved. Then silver nitrate was added to the solution and stirring until silver nitrate dissolved. Evaluate different concentration from both PVP and silver nitrate until getting the suitable concentration and size which investigated by Transmission Electron Microscope (TEM) 5-10 nm (Faculty of Agriculture Cairo University, Egypt).

Tested compounds

Silver nanoparticles: Nanoparticles were used by different concentrations:

- Original concentration (0.1 g/100 mL) from $AgNO_3$
- 25, 50 and 90% (*in vitro*)
- Nanoparticles original concentration combined with the rate of application of nematicides (1:1)

Polyvinylpyrrolidone (PVP): The PVP used in preparation of silver nanoparticles (1 g/100 mL ethanol). It was used as control and its effect was separately evaluated. Two nematicides, fenamiphos and oxamyl are given in Table 1.

Screening of compounds on nematode juveniles *in vitro*

Nematode stock culture: Nematode populations were maintained on tomato plants cv. Castle rock under greenhouse conditions. Plants were infected at 2-3 leaves stage by adding egg-masses to roots (one egg-mass per one plant for making pure culture from *M. incognita* in plastic cups) then covered with soil. After 60 days nematode egg-masses collected from each root by a needle, put in petri dishes and put it in incubator for hatching at 25±2°C. The hatched juveniles were collected daily.

To study the efficacy of tested compounds on *M. incognita* under laboratory conditions, 1 mL from each tested compound (Ag-nanoparticles, fenamiphos, oxamyl and PVP) were added to second stage nematode juveniles (500 individuals) at different concentrations (25, 50 and 90% for each treatment) in sterilized glass bottles in three replicates and non-treated bottles, containing 5 mL distilled water and 500 sec stage juveniles, served as control. The nematode percent mortality was recorded after 24, 48 and 72 h with different concentrations using a stereoscopic microscope where the juveniles mortality was counted. Morphological changes in juveniles were observed.

Greenhouse experiment: Two greenhouse experiments were done in 2014. The first to determine the efficiency of nano material and suitable concentration on healthy and infested tomato seedlings with *M. incognita*. The second was to control *M. incognita* by recommended nematicides as a traditional method.

Table 1: Common and trade names, formulations and rates of applications of tested nematicides

Common names	Trade names	Formulations used (%)	Rates of application per kilogram soil (mL)	Oral LD (50 mg kg^{-1})	Hazard** class
Fenamiphos	Dento	EC40	0.003	15	Ib*
Oxamyl	Vydate	SL24	0.003	6	Ib

*Highly hazardous, **WHO recommended classification of pesticides

Tomato seedlings were planted in 25 diameter pots filled with sterilized loamy sand soil. After 1 week suspension of second stage juveniles (about 1000 fresh hatched juveniles) and treatments of nematicides (0.003 mL kg^{-1} soil), PVP 1 mL kg^{-1} soil, silver nanoparticles 1 mL kg^{-1} soil and mixed half dose of recommended nematicides with half dose of silver nanoparticles were added to pots in three halls surrounding roots. Pots were watered regularly. After two months pots were uprooted and data were recorded.

Statistical analysis: The obtained data were statistically analyzed using the complete random design according to Snedecor and Cochran[12]. The LSD was used for the comparison between means at 5% level of probability according to Fisher[13].

RESULTS AND DISCUSSION

Preparing Ag-nanoparticles were succeeded as shown in Fig. 1, which determines the size and shape of the silver nano-particles, showed the different size and total percentage of silver nanoparticles prepared. The size and percentage of silver nanoparticles were determined by particle size analyzer equipment in Agricultural Research Center, Regional Center for Food and Feed. Nanotechnology Laboratory characterization were 5.258 nm and 62.5%, respectively. While, the sizes of silver nanoparticles 538.0 nm were the total percentage 37.5%.

Meloidogyne incognita mortality as affected by the four treatments were shown in Table 2. Obviously, the juvenile mortality was affected by the treatment type. The maximum percentage of mortality was mostly achieved by using the oxamyl at 90% concentration and exposure period 72 h, as it reached 100%, followed by fenamiphos with 98% mortality at the some concentration and period.

The juvenile's mortality was increased with the increase of concentration and exposure time. The highest concentration 90% of Ag-nanoparticles showed increasing of mortality with exposure time. This observation of Ag-nanoparticles was similar to the behavior of both nematicides fenamiphos and oxamyl on mortality for the highest concentration 90% and biggest exposure time 72 h.

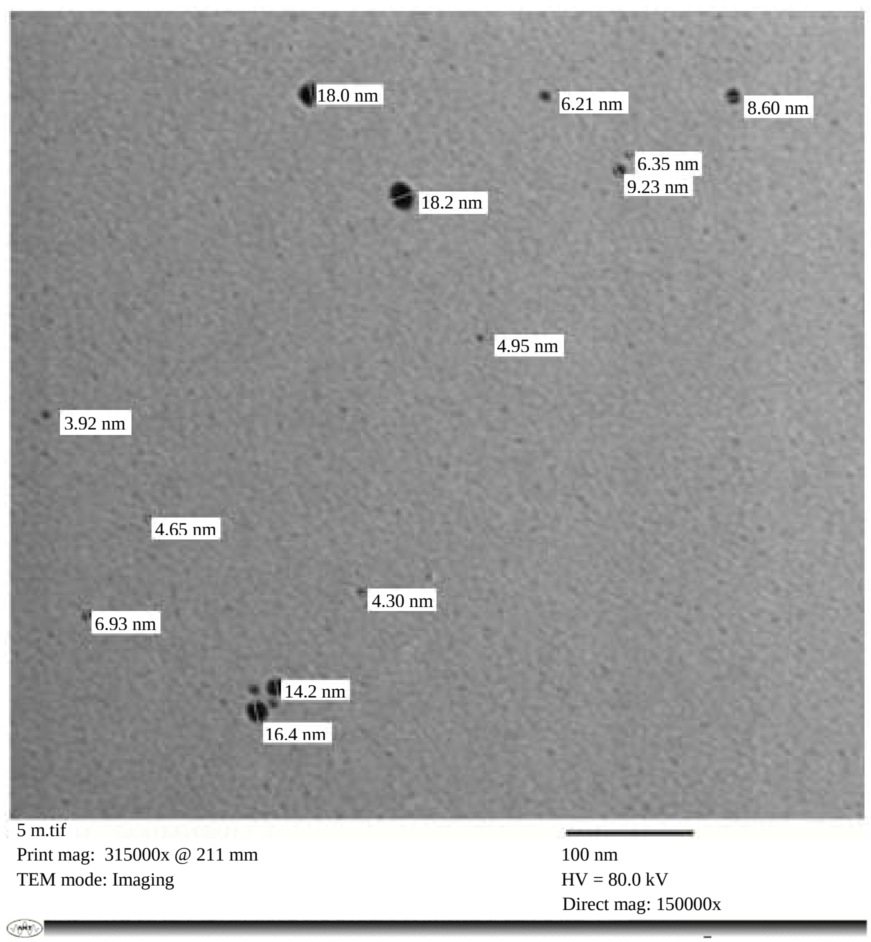

Fig. 1: Different size of silver nanoparticles

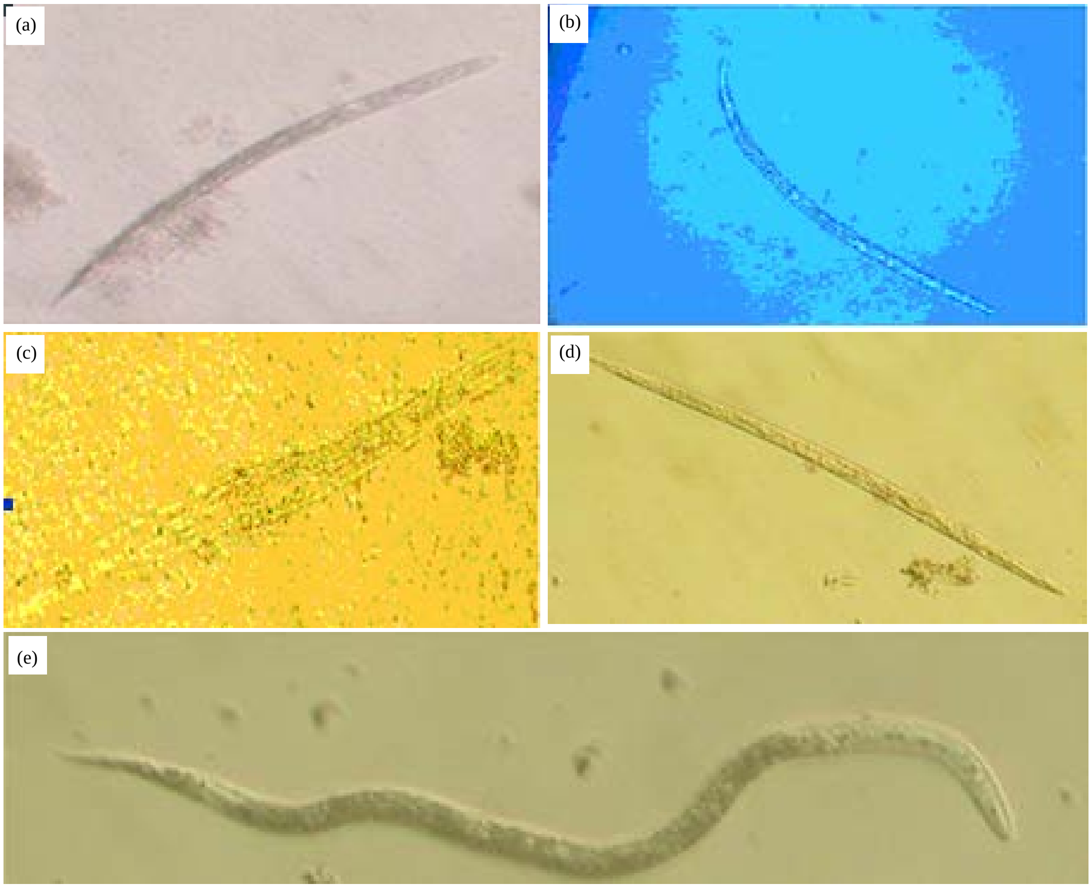

Fig. 2(a-e): Different images of treated nematode with (a) Fenamiphos, (b) Oxamyl, (c) Ag-nanoparticles, (d) Polyvinylpyrrolidone (PVP) and (e) Control

Table 2: Effect of different incubation periods and different concentrations of silver nanoparticles (Ag-nanoparticles) and nematicides on percentege mortality of *Meloidogyne incognita* under laboratory conditions

		Mortality (%)		
		Exposure period (h)		
Treatments	Concentrations (%)	24	48	72
Ag-nanoparticles	90	88	92	95
	50	76	82	85
	25	57	64	73
Fenamiphos	90	94	95	98
	50	88	90	92
	25	75	76	77
Oxamyl	90	83	98	100
	50	66	85	96
	25	47	87	88
PVP[1]	90	76	85	87
	50	61	68	75
	25	46	52	66
Control[2] (nematodes only)		0.5	1.2	1.5

Treatments LSD ($\leq$0.05) 12.65, concentrations LSD ($\leq$0.05) 8.15, exposure period LSD ($\leq$0.05) 9.77, [1]Use in preparation of silver nanoparticles, [2]Soil was nematodes only

All treatments used were completely positive compared with control which reaches to 1.5% after 72 h. All treatments had various degrees of effectiveness towards the juveniles. Moreover, the percentage of mortality with most treatments increased with increasing the concentration from 25-90% and exposure period from 24-72 h.

After 24 h, the highest mortality percent by fenamiphos was 94 with 90% concentration compared with the percent mortality in control (0.5%). Also, after 48 h oxamyl showed the highest percent mortality 98% using the concentration 90% compared with the percent mortality in control (1.2%).

The highest percentage of mortality were recorded by oxamyl, fenamiphos, Ag-nanoparticles and PVP as 100, 98, 95 and 87%, respectively for the concentration 90% and exposure period 72 h, compared with the ercent mortality in control (1.5%).

Studying the effect of different treatments under stereoscopic microscope (Fig. 2) showed the difference of juveniles malformations between using Ag-nanoparticles

Fig. 3(a-e): Root treatments with (a) Control infested with nematode, (b) Orgin Ag-nanoparticles was free nematode, (c) Half Ag-nanoparticles was free nematode, (d) Ag-nanoparticles after infested with nema and (e) PVP

and the nematicides. The morphological change in juvenile's showed paralysis with fenamiphos and oxamyl, while silver nano-particles gave degradation in cell wall.

Testing these Ag-nanoparticles were done on tomato seedlings root under green house and observed the positive effect of this new material (Fig. 3). The root treatments with control with infested nematode, orgin Ag-nanoparticles was free nematode, half Ag-nanoparticles was free nematode, Ag-nanoparticles after infested nematode and PVP. To determine the potential of Ag-nanoparticles material and suitable concentration on healthy and infested seedling tomatoes with *M. incognita*.

The results indicated that silver nanoparticles either alone or combined with nematicides fenamiphos or oxamyl had positive effect on controlling *M. incognita.* All treatments decreased the nematode parameters as shown in Table 3. The most effective treatment was Ag-nanoparticles which showed the same results of control. While, the combined treatment fenamiphos: Ag-nanoparticles (1:1) recorded the lowest number of galls (13), egg-masses (5.33) developmental stages (9), rate of build up (0.133) and number of nematode in soil 250 g (53.33) compared with control infested with nematode treatment which recorded 684, 459, 517, 50.36 and 8400, respectively while the rest of treatments were varied in their effects and this reflected on the rate of build up. The treatment with fenamiphos recorded 3.59 rate of build up followed by oxamyl, PVP and oxamyl: Ag-nanoparticles (1:1) which recorded 2.21, 0.4 and 0.21 rate of build up, respectively.

Table 4 and Fig. 4 and 5 showed the efficiency of using silver nanoparticles alone or combined with nematicides on growth parameters such as root height which achieved 30, 30

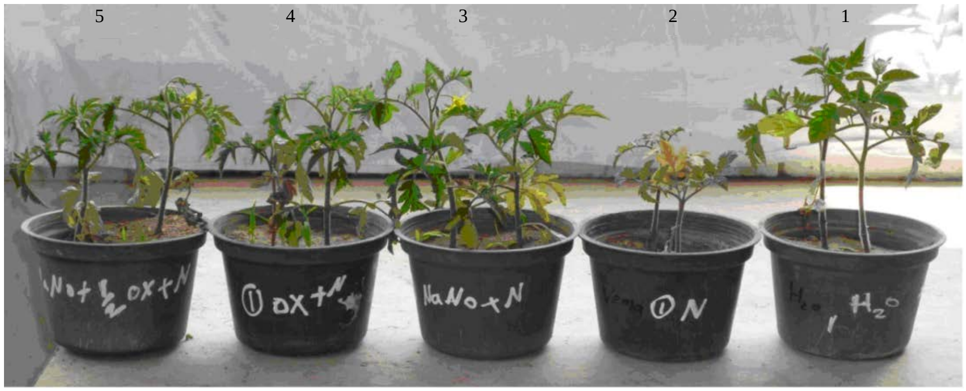

Fig. 4: Effect of using different treatments such as 1: Control was free nematode, 2: Control with infested nematode, 3: Ag-nanoparticles, 4: Fenamiphos and 5: Oxamyl: Ag-nanoparticles (1:1) on tomato seedlings infested with *Meloidogyne incognita* under greenhouse conditions

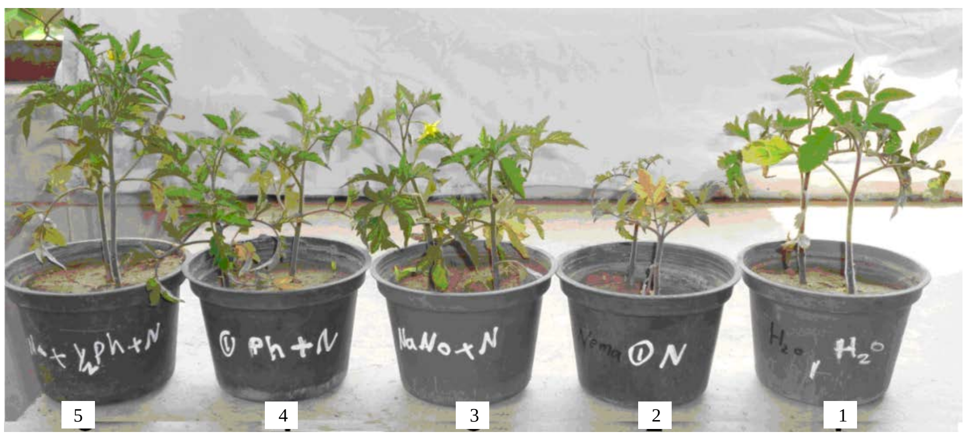

Fig. 5: Effect of using different treatments such as 1: Control was free nematode, 2: Control with infested nematode, 3: Ag-nanoparticles, 4: Fenamiphos and 5: Fenamiphos: Ag-nanoparticles (1:1) on tomato seedlings infested with *Meloidogyne incognita* under greenhouse conditions

Table 3: Effect of silver nanoparticles (Ag-nanoparticles) and nematicides treatments on different parameters of *Meloidogyne incognita* developed on tomato under greenhouse conditions

Treatments	Galls	Egg-masses	Developmental stages	Rate of build up	Nematode in soil (250 g)
Ag-nanoparticles	0	0	0	0	0
Fenamiphos	64	31	63	3.59	1320
Oxamyl	38	21	18	2.21	210
Fenamiphos: Ag-nanoprticles (1:1)	13	5.33	9	0.133	53.33
Oxamyl: Ag-nanoparticles (1:1)	36	9.33	24.66	0.21	70
PVP[1]	42	20.66	73.33	0.4	110
Control[2]	0	0	0	0	0
Control infested with nematode	684	459	517	50.36	8400
LSD (≤0.05)	14.64	13.91	14.09	13.56	87.82

[1]Use in preparation of silver nanoparticles, [2]Soil was free nematode

and 40 cm for treatments with silver nanoparticles alone or combined with nematicides fenamiphos and oxamyl, respectively compared with nematode treatment 18 cm. The same behavior can recognized with shoot height, fresh weight and dry weight on tomato seedlings. In some cases the treatment outperformed the untreated plants. The effect of different treatments on growth characterization parameters like root length, shoot height, fresh weight and dry weight of tomato seedlings infested with *M. incognita* under greenhouse conditions. The combined treatment oxamyl:

Table 4: Effect of Ag-nanoparticles and nematicides treatments on some parameters of tomato infested with *Meloidogyne incognita* under greenhouse conditions

Treatments	Root length (cm)	Shoot height (cm)	Fresh weight (cm)	Dry weight (g)
Ag-nanoparticles	30.00	26.00	47.44	12.10
Fenamiphos	30.00	19.00	39.03	13.68
Oxamyl	35.00	35.00	57.52	8.83
Fenamiphos: Ag-nanoparticles (1:1)	30.00	21.00	38.55	12.78
Oxamyl: Ag-nanoparticles (1:1)	40.00	30.00	43.82	11.09
PVP[1]	30.00	27.00	40.17	10.68
Control[2]	30.00	26.00	36.10	12.81
Control infested with nematode	18.00	18.00	26.89	3.27
LSD ($\leq$0.05)	4.08	4.37	3.57	3.55

[1]Use in preparation of silver nanoparticles, [2]Soil was free nematode

Ag-nanoparticles showed the highest root length which reach to (40 cm), compared with all treatments, while oxamyl treatment showed the highest shoot height reached to (35 cm). The order of different treatments depend on the biggest fresh weight were oxamyl, Ag-nanoparticles, oxamyl: Ag-nanoparticles, PVP, fenamiphos and fenamiphos: Ag-nanoparticles as they were 57.52, 47.44, 43.82, 40.17, 39.03 and 38.55 cm, respectively compared with both control and control infested with nematode, as they were (36.1 and 26.89 cm) respectively. While, the order of different treatments of dry weight measures were fenamiphos, fenamiphos: Ag-nanoparticles, Ag-nanoparticles, oxamyl: Ag-nanoparticles, PVP and oxamyl depend on the biggest dry weight results.

Both results observed under laboratory condition and greenhouse were in agreement with these of Sharon *et al.*[1] and Batchelor-McAuley *et al.*[14] who mentioned that silver nano-particles have more toxic effect as compared to the bulk silver and this return to the high surface area and high fraction of surface atoms. Also, Cromwell *et al.*[10] mentioned that the laboratory assays attested to the nematicidal effect of AgNP and the field evaluation demonstrated its benefits for mitigating damage caused by root-knot nematode in bermudagrass. Beside, Kim *et al.*[2] studied the antifungal effectiveness of colloidal silver nanoparticles (1.5 nm average diameter) solution, against rose powdery mildew caused by *Sphaerotheca pannosa* var rosae. After two days using silver nano-particles as spray more than 95% of rose powdery mildew faded out. Silver nanoparticles colloid are both well dispersed and stabilized. Silver nanoparticles solution either is more adhesive on bacteria or fungus, hence, are better than fungicide. This popularity of nanoparticles has caused concern about regulating and classifying the silver nano-particles as pesticide[3].

The results also agree with the demonstrated observers, Ag/chitosan nano formulation which had significant antifungal activity against the fungi tested, *Aspergellus flavus*, *Alternaria alterneta* and *Rhizoctonia solani* and this was much higher than silver or chitosan nanoparticles used independently[15].

Used nematicides reduced the reproduction of the *Meloidogyne* on tomato proved their potentiality in controlling this serious pest by inhibiting cholinesterase in nematodes e.g., carbofuran, oxamyl and fenamiphos in *M. incognita* and *M. javanica*[16], this observation agreement with the results in both of laboratory and greenhouse for investigate juvenile's under stereoscopic microscope and the percentage of juvenile's mortality in Fig. 2 and Table 2. Carbamates have influenced on juvenile development and growth in the root. This direct effect may be the result of toxic action on nematode physiology or disturbance in nematode nutrition through indirect effects on the activity of the syncytium[17].

It can be effectively use Ag/chitosan nano formulation against plant phytopathogenic fungi to protect the various crop plants and their products instead of commercial available synthetic fungicides, which show higher toxicity to humans. The area of mode of action of silver-chitosan composites on the phytopathogenic fungi needs more investigation. More research needs to evaluate the influence impact of this new technology on all environmental components[15].

CONCLUSION

The results clarified the positive effect of silver nanoparticles alone and in combination with the nematicides on *M. incognita* and the enhancement on growth parameters of tomato seedlings. More research needs to determine the long run effect.

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