

Effectiveness of nanoparticles of some plant extracts against root-knot nematode, *Meloidogyne incognita* on tomato plants

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Abstract

The present study was carried out in order to investigate the efficiency of various formulations of silver-botanical nanoparticles, i.e. cloves, *lantana camara* and *conyza dioscoridis* as nematicide substitutes against the tomato root-knot nematode (*M. incognita*), in both laboratory and under greenhouse conditions. Results clarified that the tested normal and NE-extracts, significantly and drastically, increased (J2) mortality % and reduced egg-hatching and the effect increased with increasing compound concentration and exposure time. However, nanoparticles forms of all tested compounds consistently showed higher effect than normal extracts forms. Meanwhile, NP-*L. camara* showed the highest J2 mortality % and egg-hatching inhibition at all exposure times. The nanoparticles, dramatically reduced root galls, egg masses, eggs and final population of *M. incognita* infecting tomato plants at the application rate of 100 µg/ml. Meanwhile, the NP-*L. camara* treatment was the most efficient in reducing root galls, egg masses, eggs and population of J2 in soil, followed by NP-Cloves and NP-*C. dioscoridis*. Moreover, the different extracts treatments improved and increased the mean values of tomato growth parameters in the inoculated treated plants. NP-*L. camara* showed the highest growth parameters. However, most mean values of growth parameters for NP-Cloves treatment not significantly different from the NP-*L. camara*. Consequently, these nanoparticles extracts could be effective potentially and environmentally safe to control tomato knot-root nematode.

Keywords: Knot-root nematode; Nanoparticles (NPs); *M. incognita*; plant extracts; tomato.

1. Introduction

Plant pathogenic nematodes, which are more than 4100 species, severely infect nearly all types of crops causing serious diseases (Nicol *et al.*, 2011). *Meloidogyne* spp., a root-knot nematode, was reported to reduce agricultural yields by up to 64% in practically all over the world (Roberts *et al.*, 2005; Sikora *et al.*, 2007; Balbaa, 2010). Moreover, nematode feeding causes injuries on root tissue which enable entry of several soil-borne pathogens as fungi and bacteria. Incidence and severity of such soil-borne pathogens are

increased in nematode presence (Javed *et al.*, 2007).

Nematode diseases were routinely managed by the use of nematicides, however, the excessive use of nematicides exhibited negative effects on the environment and human health as well as the development of nematode resistance. Consequently, it's critical to employ alternative controlling methods that are successful and cost-efficient while still being safe for farmers, consumers, and the environment (Fernandez *et al.*, 2001).

Several nematode management strategies which are eco-friendly were introduced. This included use of certain agronomic measures, cultivation of resistant cultivars, predators and parasites, allelochemicals of cover crops, organic


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amendments, sanitation, solarization, resistant cultivars, plant extracts, and nanotechnology and the commonly used synthetic nematodes (Browning *et al.*, 2006; Williamson and Kumar, 2006; Khan and Kim, 2007; Okada and Harada, 2007; Wang *et al.*, 2007).

Several reports indicated that certain plant extracts such clove, *Conyza dioscoridis* and *Lantana camara* exhibited potentiality to control soil born fungi as well as several plant-parasitic nematodes (Pandey and Dwivedi, 2000; Park *et al.*, 2005; Salgado and Campos, 2003a; Salgado and Campos, 2003b; Sangwan *et al.*, 1990, Begum *et al.*, 2000, Begum *et al.*, 2000). Meanwhile, *M. javanica* juveniles were shown to have a substantial mortality rate when exposed in vitro to aqueous, methanol, ethyl acetate, and hexane extracts of *L. camara* leaves (Begum *et al.*, 2000). Also, extracts of *Conyza dioscoridis* showed nematicidal performance against *M. incognita*, which is a root-knot nematode. Meanwhile, nanoformulations of *C. dioscoridis* exhibited a significant amount of potentiality in suppressing both stages of *M. incognita*, eggs and J2 (Abbassy *et al.*, 2017).

Formulation of plant natural compounds as nanoparticles may enhance its effectiveness against infections. Several reports indicated that nano-synthesized secondary metabolites were shown to be more effective against plant diseases while having less negative impacts on humans, animals and the environment as well (Abdul Hameed, 2012). Nanoparticle formulations of extractives of *Urtica urens* recorded up to 11-folds increase in the nematicidal activity against root-knot nematode (*M. incognita*) when compared to their corresponding raw extractives (Nassar 2016). Therefore, the present study was conducted in order to investigate the efficiency of cloves, lantana camara and *conyza dioscoridis* plant extracts formulations in both normal and nanotype against the root-knot nematode (*M. incognita*) affecting tomato in El-Behera Governorate, Egypt.

2. Materials and methods

2.1. Culture of root-knot nematode

Culture of *M. incognita* (Kofoid and White) Chitwood was isolated from tomato plant (cv. Elisa) grown in a greenhouse at El-Bostan Faculty Agricultural farm, El-Behera Governorate. Perianal patterns of adult females and the morphology of J2 were used to identify root-knot nematodes according to Hartman and Sasser (1985), and Jepson (1987). Root knot nematode egg masses were incubated for 48 hours at room temperature ($25\pm 2^{\circ}\text{C}$) in sodium hypochloride (NaOCl) solution of sterilized distilled water (Hussey and Barker, 1973). Newly born second stage juveniles (J2) were collected every day. Toxicity experiments were conducted on *M. incognita* second-stage juveniles (J2) and eggs.

2.2. Preparation of plant extracts

Leaves of Clove and *L. camara* were obtained from Ornamental Plant Nursery and Leaves of *C. dioscoridis* were obtained from Faculty of Agriculture farm in El-Bostan. The leaves were washed several times in tap water then, rinsed with distilled water and air-dried at room temperature before being placed in an oven at a temperature of 50°C until they were completely dry. Then, samples were ground in a mortar to form a powder, and the synthesis of the plant extracts in accordance with the methodology described by Claudius-Cole *et al.* (2010) as follows:

A 100 g of each of *L. camara* and *C. dioscoridis* powder was allowed to sit at room temperature for three full days soaking in a 1000 ml of distilled water. The slurries were cooked for one hour in a bath containing boiling water. After allowing the extracts to cool down at room temperature, plant water extracts were filtered using Whatman filter paper No. 1, and the resulting filtrates were kept in a refrigerator until use as a crude extract.

Also, through the process of steam distillation with Clevenger trap equipment, the volatile oil was recovered (Guenther, 1952). Flasks with a

capacity of 2 L were filled with 1000 mL of distilled water and 100 g of powder made from air-dried leaves. The distillation continued until there was no longer any discernible was observed in the oil (about 8 h of distillation). After being dried over anhydrous sodium sulphate, the essential oil extracts were placed in well tight brown bottles, placed in a refrigerator and used in a variety of different bioassay tests as soon as they were ready.

2.3. Preparation of the nanoformulations of plant extracts and oil

Using a modified method of Prasad and Elumalai's (2011) approach, we were able to synthesize nanoparticles of silver and botanical compounds to use as Ag-NPs. In a conical flask with a capacity of 250 mL, exactly 10 mL of plant extracts or oil with a concentration of 5000 $\mu\text{g mL}^{-1}$ were mixed with 90 mL of silver nitrate with an average concentration of 1mM and 10 mL of ascorbic acid at a concentration of 0.1 M, and polyvinyl pyrrolidone (PVP) as a protecting agent. Following the heating of the mixture to 40 °C on the water bath for ten m., the tubes were stored in a dark area for 24 h at room temperature. The color shifted from a dark brown to a reddish-brown hue, which served as an indicator of the formation of the silver nanoparticles. Similarly, Ag nanoparticles of Oxamyl were produced by incubating 10 mL of Oxamyl (20 %), with 90 mL of silver nitrate (1mM). Thereafter, 10 mL of ascorbic acid (0.1 mM) were added, at a temperature of 40 °C for 12 h. The formation of a color that was reddish-brown in hue was an indicator that the Ag-Oxamyl nano-formulation was successfully synthesized.

2.4. The *in vitro* experiment

2.4.1. The nematocidal efficacy of normal and nano-formulations plant extracts and oil against *M. incognita*.

Under laboratory conditions, the nematocidal activity of extracts and their associated nano-formulations was tested *in vitro* against egg and J2 stages of *M. incognita*. Three concentrations were tested they were 250, 500 and 1000 $\mu\text{g mL}^{-1}$ for the normal-formulations and 25, 50 and 100

$\mu\text{g mL}^{-1}$ for the nano-formulations, in 5-ml screw cap glass vials. Each concentration comprised five replicates each of around 100 eggs or individuals of *M. incognita* juveniles and distilled water was used as a control treatment. The reference nematicide was Oxamyl 20 %. The vials were incubated at a temperature of 25 ± 2 °C. After 12, 24 and 48 h intervals, juvenile mortality% was assessed, while egg hatchability percentages were determined after 1, 3 and 7 days.

2.5. Greenhouse experiment

2.5.1. Efficacy of normal and nano-formulations plant extracts and oil against *M. incognita* on tomato.

The most effective concentrations of plant extracts and oil, in both the normal and nano-formulations, revealed in the previous *in vitro* test, were further tested for their efficacy to control *M. incognita* at the concentration of 100 $\mu\text{g/ml}$ under greenhouse conditions. Tomato 30-day-old uniform seedlings (cv. Elisa) were transplanted singly in a plastic pots (20 cm - diameter) filled with 3 Kg mixture of sand and peat moss (3: 1, v: v). Ten days later, 5000 eggs of root-knot nematode were injected in holes (5-7 cm deep & 2-cm radius) surrounding the seedling within a two-centimeter radius. Each treatment had four duplicates,

Treatments with the tested plant extracts or oil were conducted by extract application to pot at the same time of nematode eggs pot infection. Positive control pots were inoculated but treated with nematicide oxamyl at the recommended rate (3L per feddan, i.e. 100 ml/pot), while negative control pots were infected but not treated with any of the plant extracts or nematicide. Four replicate pots were prepared for each treatment or control. Greenhouse temperature ranged between 25-30°C, and potted seedlings were treated according to the normal agricultural practices for tomato.

Sixty days after transplantation, potted tomato plants were carefully uprooted. Roots were dyed for 15 minutes in an aqueous solution of Phloxine B stain (0.15 g/l water) before being gently rinsed

in tap water (Holbrook *et al.*, 1983). Total number of egg masses per root system and galls, as well as the final J2 population were recorded. Henderson and Tilton's (1955) equation was used to calculate the percent reduction in galls number, egg masses, and nematode population density.

2.5.2. Effect of normal and nano-formulations plant extracts and oil on plant growth of tomato plants inoculated with *M. incognita*.

At the end of the experiment, 60 days after transplantation, plant growth characteristics, i.e. shoot fresh weight, shoot dry weight, as well as root fresh weight, were determined and percentage of increase was calculated.

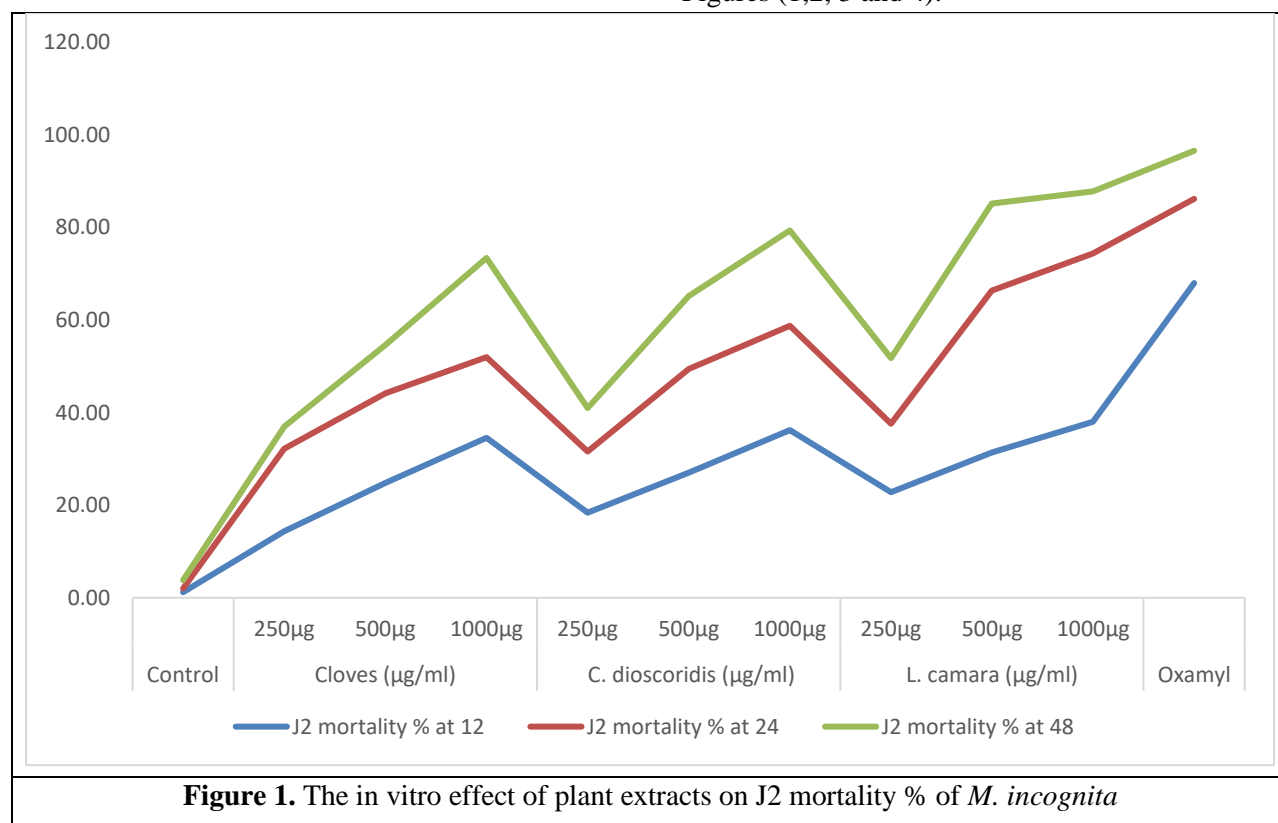
2.6. Analytical statistics

Using SPSS 20.0 software they obtained data were statistically analyzed using one-way analysis of variance (ANOVA), and means were compared by least significant difference test (LSD) at 0.05 of probability.

3. Results

3.1. Toxicity of normal and NP- extracts against J2 juveniles and egg hatching of *M. incognita*

In the bioassay test, the effects of normal and NP-extracts of *Conyza dioscoridis*, *Lantana camara*, and Cloves on J2 of *M. javanica* at different concentrations of (250, 500 and 1000 µg/ml, for normal, and 25, 50 and 100 µg/ml, for nanoparticles) were evaluated and shown in Figures (1,2, 3 and 4).



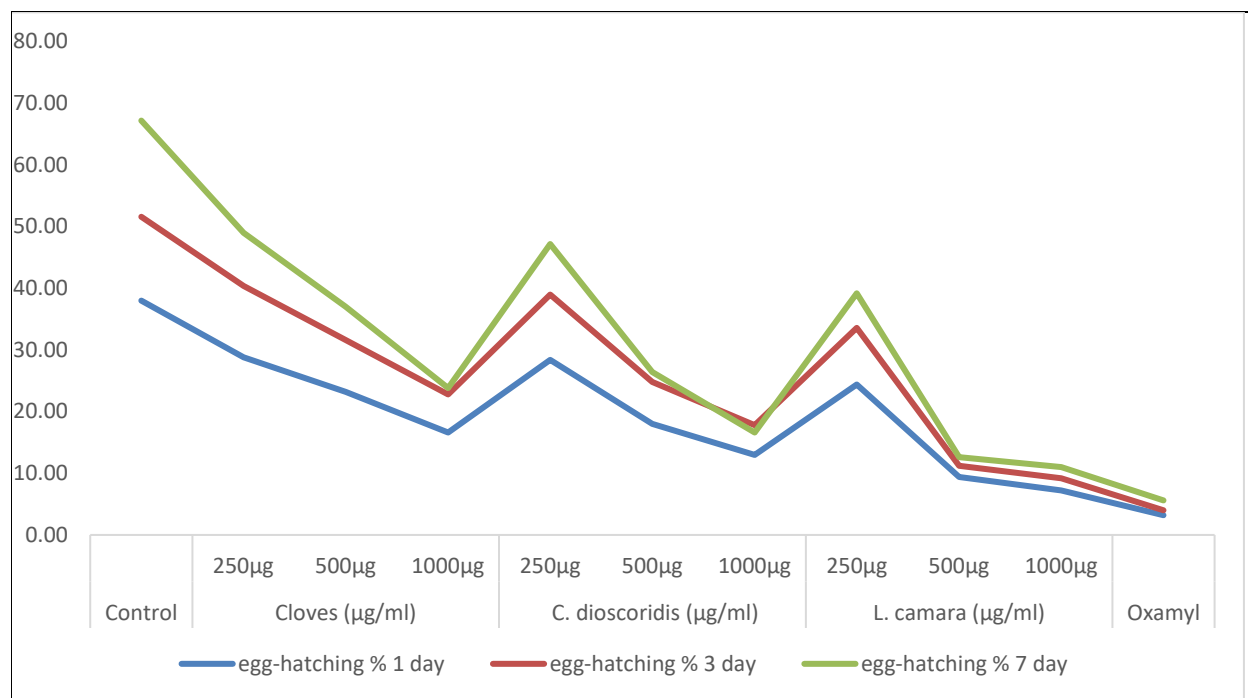


Figure 2. The in vitro effect of plant extracts on egg-hatching % of *M. incognita*

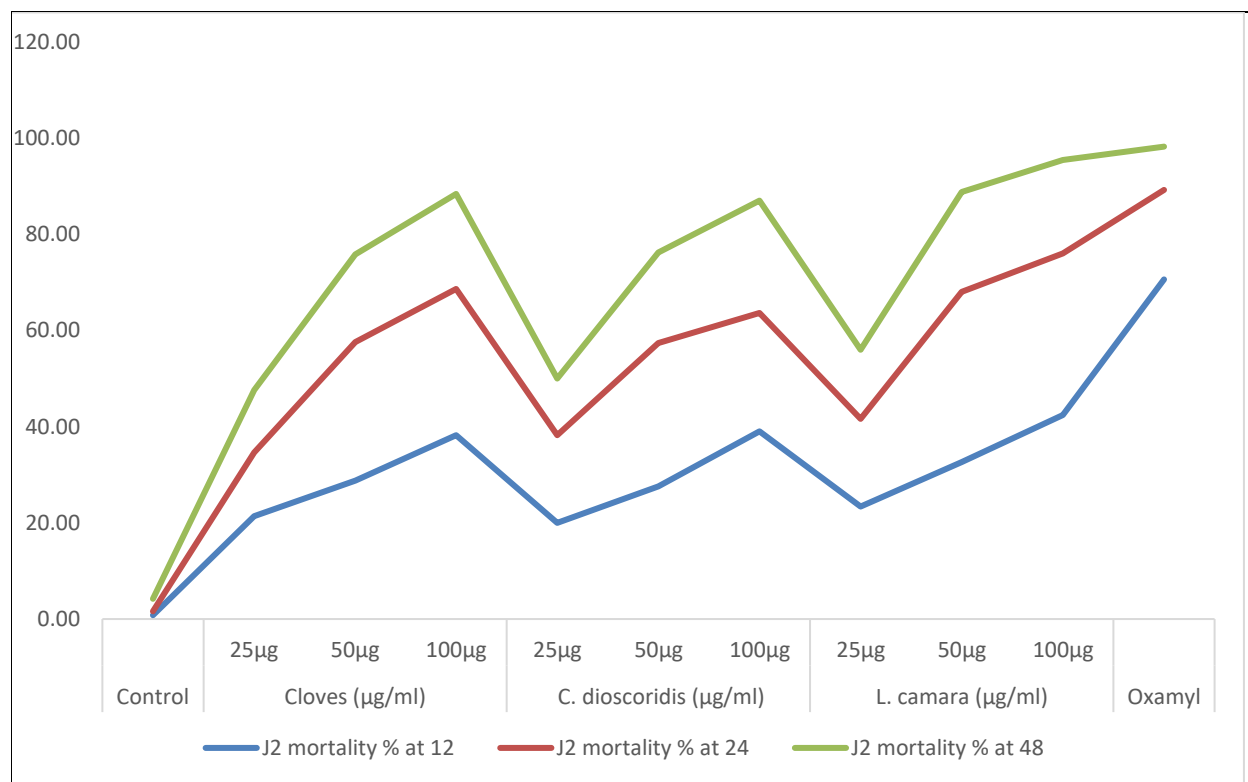
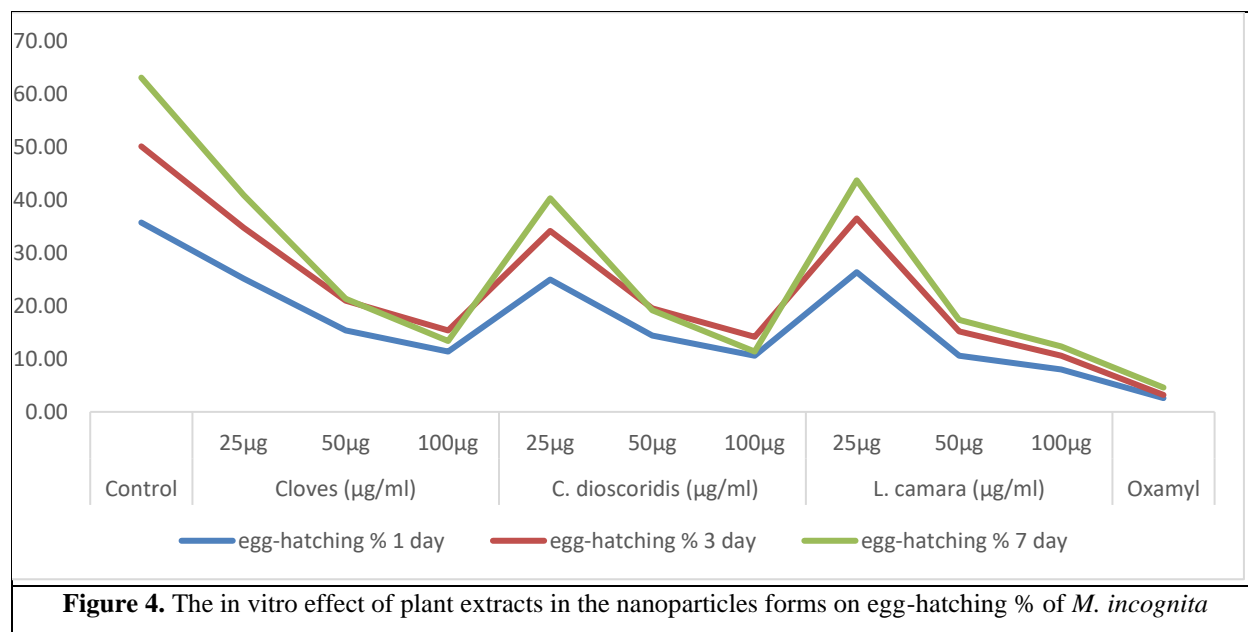


Figure 3. The in vitro effect of plant extracts in the nanoparticles forms on J2 mortality% of *M. incognita*



Results clarified that the tested normal and NP-extracts, significantly and drastically, increased J2 mortality % and reduced egg-hatching and the effect increased with increasing compound concentration and exposure time. However, nanoparticles forms of all tested compounds consistently showed a higher effect even at tenth of the concentration applied by the normal extracts forms. Meanwhile, NP- *L. camara* at 100µg/ml showed the highest J2 mortality % and egg-hatching inhibition at all exposure times which were not significantly different from the oxamyl nematicide effect for both parameters. This was followed by NP- *C. dioscoridis* and NP-Cloves at 100µg/ml with J2 mortality % and egg-hatching inhibition.

3.2. Greenhouse experiments

3.2.1. Effects of extracts in both the normal and NP- formulations on *M. incognita* disease parameters on tomato under greenhouse conditions.

Table (1) indicate that the highest numbers of egg masses per root system, galls and eggs and the final population, were recorded in the untreated inoculated control (UI control). The nanoparticles, dramatically reduced root galls, egg masses, eggs and final population of *M. incognita* infecting tomato plants at the application rate of 100 µg/ml. Meanwhile, the

Oxamyl and NP- *L. camara* were the most efficient treatments in reducing root galls, which reduced the number of galls by 98.78 and 94.16%, respectively. Also, the most effective treatments for lowering the number of egg masses were Oxamyl and NP- *L. camara*, which reduced the number of egg masses by 99.00 and 94.09 %, respectively, followed by NP-Cloves and NP-*C. dioscoridis*, which reduced the number of egg masses by 86.42 and 84.71%, respectively. Moreover, all tested compound, dramatically, decreased the number of eggs where the most effective treatments for lowering the number of eggs were Oxamyl and NP-*L. camara*, which reduced the by 99.60 and 96.05%, respectively followed by NP-Cloves and NP-*C. dioscoridis*, which reduced the number of eggs by 91.66 and 90.31%, respectively. On the other hand, application of the tested compound, dramatically, decreased the population of J2 in soil where Oxamyl and NP- *L. camara* was the most effective and suppressed the final population of *M. incognita* by 99.30 and 95.96%, respectively, followed by NP-Cloves and NP-*C. dioscoridis*, which reduced the population of J2 in soil by 91.17 and 90.90%, respectively.

Table 1. Effects of extracts in both the normal and NP- formulations on *M. incognita* disease parameters on tomato under greenhouse conditions.

Treatment	Number of galls	Reduction %	Eggs	Reduction %	Egg-masses	Reduction %	Number of J2 / 250 g Soil	Reduction %
UI control	479.2±19.51a	-	116587±5089a	-	467.0±10.69a	-	529.8±19.34a	-
N-Cloves	210.4±13.33b	56.09	59848±1431b	48.67	185.60±17.33b	60.26	115.6±2.86b	78.18
NP-Cloves	64.2±1.50d	86.60	9721±284ef	91.66	63.4±3.79d	86.42	46.8±0.74d	91.17
N- <i>C. dioscoridis</i>	196.0±18.78b	59.10	51032±640c	56.23	182.2±14.03b	60.99	105.6±2.86b	80.07
NP- <i>C. dioscoridis</i>	66.8±1.20d	86.06	11295±373e	90.31	71.4±3.63d	84.71	48.2±1.11d	90.90
N- <i>L. camara</i>	109.4±11.86c	77.17	22656±405d	80.57	108.6±20.59c	76.75	72.2±4.63c	86.37
NP- <i>L. camara</i>	28.0±1.45e	94.16	4602±434fg	96.05	27.6±1.29e	94.09	21.4±0.51e	95.96
Oxamyl nematicide	5.80±1.16e	98.78	466±148f	99.60	4.20±0.97e	99.00	3.60±1.63e	99.30
Sig.	0.003	-	0.001	-	0.009	-	0.002	-

3.2.2. Effects of extracts in both the normal and NP- formulations on *M. incognita* growth parameters on tomato under greenhouse conditions

In pot experiments under greenhouse conditions, the results presented in Table (2) showed that *M. incognita* infection decreased all tested plant growth parameters in the untreated inoculated plants. However, oxamyl and the different extracts treatments improved and increased tomato plant growth parameters in the inoculated

treated plants. Oxamyl and NP-*L. camara* showed the highest shoot growth values being (53.10 and 51.12g for fresh weight, and 13.34 and 12.74g for dry weight), respectively, and 18.64 and 17.14g for fresh weight of root system, respectively. However, NP-Cloves treatment showed growth values for most parameters not significantly different from the NP-*L. camara*. Meanwhile, most growth parameters with *C. dioscoridis* treatment were not significantly different from NP-Cloves treatment (Table 2).

Table 2. Effects of extracts in both the normal and NP- formulations on *M. incognita* growth parameters on tomato under greenhouse conditions.

Treatment	Shoot system				Root system	
	Fresh weight (g)	I (%)	Dry weight (g)	I (%)	Fresh weight (g)	I (%)
UI control	21.960±0.69g	-	4.740±0.30f	-	8.020±1.63c	-
N-Cloves	32.220±2.29f	46.72	6.820±0.51e	43.88	8.960±0.60c	11.72
NP-Cloves	47.140±2.24bc	114.66	11.160±0.31bc	135.44	13.700±0.67b	70.82
N- <i>C. dioscoridis</i>	37.900±2.97e	72.59	7.840±0.54de	65.40	11.800±0.57b	47.13
NP- <i>C. dioscoridis</i>	43.780±0.62cd	99.36	9.560±0.81cd	101.69	13.300±0.53b	65.84
N- <i>L. camara</i>	41.240±1.24de	87.80	8.040±0.53de	69.62	13.540±0.51b	68.83
NP- <i>L. camara</i> 2	51.120±1.82ab	132.79	12.740±0.74ab	168.78	17.140±0.99a	113.72
Oxamyl nematicide	53.10±1.21a	141.8	13.34±0.65a	181.43	18.64±1.10a	132.41
Sig	0.002	-	0.001	-	0.007	-

UI control=Untreated inoculated control; N-= normal formulations; NP- = nanoparticles f

4. Discussions

Applications of Ag-NP enhanced the activity up to 5 and 2 times against the J2 the eggs of *M. incognita*, respectively (Abbassy *et al.*, 2017). Clearly, the role of natural green products was visible, emphasizing the importance of nanotechnology to serve as safe and effective nematicide options. Furthermore, Gardea-Torresdey *et al.* (2003), Sastry *et al.* (2004), Ganesan *et al.* (2013) and Abbassy *et al.* (2017) reported that the preparation of Ag-NP of plant secondary metabolites was fast, large-scale, size and shape-controlled and effective process that produced formulations with no phytotoxic effects. Therefore, silver nanoparticles offer unique characteristics, i.e., chemical stability, catalytic activity and antibacterial activity (Chen *et al.*, 2007; Li *et al.*, 2007; Setua *et al.*, 2007). Abbassy *et al.* (2017) showed that increased potency of the Ag-NP compared to both the

extractives and the reference nematicide highlights the presence of certain secondary metabolites. For example, the presence of the sesquiterpenoids: aromdendrene, cyclohexanol-3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethenyl), epi-shyobunol, β -isocomene, α - and t-cadinol, caryophyllene, 1-hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene, α -humulone, and α - and β -selinene at high concentrations in the Ag-NP nanoparticle.

Patel *et al.* (2004) went almost to similar findings, they have found that aqueous leaf extract of *L. camara* was highly efficient in complete inhibition of egg hatching. Also, Begum *et al.* (2008) extracted some effective compounds, i.e., pomolic acid, lantanolic acid, lantoic acid, camarin, lantacin, camarinin, and ursolic acid from the aerial part of *L. camara* and investigated their nematicidal activity against root-knot nematode *M. incognita*. Pomolic acid, lantanolic acid, and lantoic acid exhibited 100% mortality at

1 mgmL⁻¹ concentration after 24 h, while camarin, lantacin, camarinin, and ursolic acid produced similar effect after 48 h at same concentration. Ahmad *et al.* (2010) also found that an aqueous leaf extract from *L. camara* was effective against eggplant-borne *M. incognita* juveniles when tested *in vitro*. Nematodes were totally paralyzed after 12 h and 96% of juveniles were killed after 48 h of exposure to the optimum concentration 'S' of leaf extract. Clove oil was reported to diminish *M. incognita* egg hatch and J2 viability in microwell experiments by Meyer *et al.* (2008).

Similarly, extracts of *Conyza dioscoridis* showed nematicidal action against root-knot nematode *M. incognita* in a study performed by Abbassy *et al.* (2017). *C. dioscoridis* AgNPs were particularly effective in inhibiting *M. incognita* in its egg and J2 phases. For example metabolites such as aromdendrene, caryophyllene and caryophyllene, -humulone, isocomene and t-selinene may be responsible for the enhanced activity of nano-formulations.

We found that the application of *L. camara* root, a plant growth-promoting rhizobacterium, greatly decreased nematode population densities in roots and subsequent root-knot infection and enhanced plant growth in greenhouse experiments. In addition, Patel *et al.* (2004) found that *L. camara* aqueous leaf extract was highly efficient in preventing *M. incognita* larvae from entering the banana, indicating that it has ovicidal effects. To that end, Montasser *et al.* (2012) and Ahmad *et al.* (2013) found that the root-dip treatment with the standard concentration of aqueous extract from *L. camara* leaves effectively inhibited larval penetration in tomato roots, improved plant growth, and reduced root-knot development in roots. The saponin of *L. camara* was also shown to be effective against the migration of *Meloidogyne* sp. second stage larvae, with an EC50 value of 4906.8 ppm and a 100% suppression of root gall formation at 5000 ppm concentration, according to Ibrahim *et al.* (2014). Ghimire *et al.* (2015) also studied the *L.*

camara's nematicidal efficacy against *Meloidogyne* sp. juveniles was *in vitro* using an aqueous leaf extract. *Meloidogyne* sp. larvae were effectively immobilized by 50% leaf extract at 48 h of incubation time and above, and 57.66% of nematode juveniles were discovered dead within 48 h. A 100 % leaf extract was also extremely nematostatic, killing 99.66 % of the test worm juveniles within 48 h. This is further supported by the findings of Park *et al.* (2005) and Salgado and Campos (2003a and b) that the extracts of clove were most effective against plant-parasitic nematodes.

Abdelrasoul and El-Habashy (2021) discovered that the conversion of monoterpenes to nanoemulsions considerably increased their nematicidal activity against the main plant pathogenic nematode *M. javanica*, which is in agreement with the findings of this study. Nanoemulsion, on the other hand, proved to be a successful treatment for root-knot nematodes in tomato, with no harmful effects on plants and other non-parasitic nematodes.

5. conclusion

The findings of this study suggested that the conversion of extracts to nanoparticles greatly enhanced its nematicidal activity against the major plant pathogenic nematode *M. incognita*. The utilization of such technique in root-knot nematode control could gain a new trend, safe and effective nematode management programme. As a result, more research is required to be conducted in order to isolate particular secondary metabolites and preparing them in the nano-form. These secondary metabolites must be safe, effective, eco-friendly and have a low risk of adverse effects on mammals.

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Institutional Review Board Statement

All Institutional Review Board Statement are confirmed and approved.

Data Availability Statement

Data presented in this study are available on fair request from the respective author.

Ethics Approval and Consent to Participate

This work carried out at Department of Plant Pathology and followed all the department instructions.

Consent for Publication

Not applicable.

Conflicts of Interest

Declare no conflict of interest.

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