

Aqueous, Ethanolic and Nanoparticles of Two Plant Extracts Compared to Abamectin on Tomato Plant as Affected by *Meloidogyne incognita* and *Rotylenchulus reniformis* Infection



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ABSTRACT

The efficiency of the biopesticide, abamectin (Tervigo®) and plant extracts of lantana (*Lantana camara*) and mint (*Mentha longifolia*), as aqueous, ethanolic and nanoparticles was determined against the root knot nematode, *Meloidogyne incognita* and the reniform nematode, *Rotylenchulus reniformis* infecting tomato plant. All tested treatments caused a significant decrease in nematode infection parameters. Tervigo as a single application achieved the maximum percentage of reduction in nematode parameters followed by mint nanoparticles as a single application. Tervigo plus lantana nanoparticles showed the maximum reduction in nematode parameters. However, the triple application had a high synergistic effect on reducing nematode indices as well as achieving the maximum percentage increase in total plant height, total fresh plant weight and dried weight of leaves. It is also considered the most effective, as its values reached 36.3% (nitrogen), 30.3% (phosphorus), and 53.6% (total chlorophyll content) in tomato leaves, according to their susceptibility to *M. incognita* and *R. reniformis*. Oxamyl outperformed all treatments, with the percentage of reduction in total phenol reaching 26.2%. Simultaneous application showed a significant increase in salicylic acid compared to nematodes alone and single use. The highest percentage of increase (34.5%) in salicylic acid was recorded with the triple application. Oxamyl outperformed all treatments with an increase in salicylic acid (38.2%).

Keywords: Abamectin, *Lantana camara*, *Mentha longifolia*, root-knot nematode and reniform nematode

INTRODUCTION

Tomato, *Solanum lycopersicum* L. (Fam: Solanaceae) is a delicious tasting vegetable crop that is very essential in human nutrition and is used for fresh consumption to produce pastes, purees, ketchup and fruit drinks (Ogwulumba and Ogwulumba, 2018). Tomatoes are grown on various seasonal farms throughout a year in Egypt; as they are one of the most important and profitable vegetable crops that achieve higher incomes for both large and small farmers. Tomatoes are extremely susceptible to root-knot nematode, *Meloidogyne incognita*, which causes root galls in infected plants that disrupt the water relations and physiology leading to severe plant stunting (Pinkerton and Finn, 2005). Root-knot nematode interacts with other pathogenic microorganisms that cause dangerous diseases in tomatoes (Tian et al., 2015). This nematode was found in the most surveyed grown tomatoes fields (Bakr et al., 2011). *Rotylenchulus reniformis* is the second most important nematode species in Egypt and the world (Robinson et al., 1997). This species is an obligate semi-endoparasite on the roots of many plants. A significant decline in tomato growth parameters and production is assigned by nematodes (Nikman and Dbawan, 2003; Zhang et al., 2019). *R. reniformis* infection does not irritate root

tissue and leaf symptoms resembling those of nutrient deficiency and yield losses by these nematodes often go undetected (Koenning et al., 2004).

Chemical nematicides are used as effective measure to control these nematodes. The extreme toxic residual effect and toxicity to beneficial soil plants and animals led to search for alternative and cost-effective strategies for nematode control. One distinct alternative control measure against nematodes is the use of plant extracts and their nanoparticles (Khairy, 2020). Plant extracts contain bioactive compounds such as phenols, terpenes, organic acids, terpenoids and coumarin-like compounds that showed nematicidal activities for controlling plant nematodes (Khairy, 2016). Although plants have environmentally safe, toxicologically, selectively, and effective nematicidal potentials (Aoudia et al. 2012), they are generally considered to be unstable under field conditions and are easily transformed by light, oxygen, or microorganisms into less toxic products; Therefore, it is expected that less waste will result from the use of these natural products (Caponi et al. 2012). Several reports have indicated that some plant extracts such as *Mentha longifolia* and *Lantana camara* have shown potential to control several plant-parasitic nematodes (Park et al., 2005). Meanwhile, *M. javanica* juveniles have been shown to have significant mortality, when exposed *in vitro* to aqueous and methanol leaf extracts of *L. camara* (Begum et al., 2000) *M. longifolia* extract also showed nematicidal performance against *M. incognita*. Formulating natural plant compounds as nanoparticles may enhance their effectiveness against infections. Several reports have indicated that nano-synthesized secondary metabolites have proven to be more effective against plant diseases (Ali et al., 2020; Khairy, 2020; El-Qurashi et al., 2023; Ram et al., 2023), while having fewer negative effects on humans, animals and the environment as well.

Abamectin produced by *Streptomyces avermitilis* fermentation (Putter et al., 1981), is one of the new bio-nematicides that has been registered over the past few years in Egypt. It has been widely used as soil applications for nematode control in vegetable, fruit and crop fields due to its nematicidal properties (Jansson and Rabatin, 1998; Korayem et al., 2008; Khairy, 2020; El-Marzouki et al., 2022). Abamectin affects the aminobutyric acid (GABA) nervous system of the nematode. It showed a high ability to bind among soil particles, insoluble in water, and is not hydrophilic (Wislocki et al., 1989).

Therefore, in the present study, the efficiency of the biopesticide, abamectin and plant leaf extracts of *L. camara* and *M. longifolia*, as aqueous, ethanolic and nanoparticles was determined against *M. incognita* and *R. reniformis* on tomato plant under greenhouse conditions.

MATERIALS AND METHODS

Plant leaf extracts

A. Aqueous extracts of plant leaves

Twenty five grams of *L. camara* (Fam: Lantaneae) and *M. longifolia* (Fam: Lamiaceae) fresh leaves were separately grinded with an electric mixer in 100 ml of distilled water, then centrifuged at 5000 rpm for five minutes and filtered through Whatman No. 1 filter paper and considered as standard solutions (Ranjitsingh and Sucheta, 2009).

B. Ethanolic leaf extracts

Leaf powder of *L. camara* and *M. longifolia* (0.4 g) leaf powders were soaked separately in 100 ml of the chosen solvent (ethanol 95 %) for two hours, then centrifuged at 2000 rpm for

ten minutes and filtered through Whatman filter paper No. 1 and was considered as standard solutions (Oka et al., 2012).

C. Nano formulations of leaf extracts

Using method of Abbassy et al. (2017), nanoparticles of *L. camara* and *M. longifolia* leaf extracts were prepared out in the Department of Genetic Engineering, Faculty of Science, Mansoura University, Mansoura, Egypt.

Commercial bio-products

Tervigo® 2% SC (Abamectin). was applied at the recommended rate 2.5 L / feddan.

Greenhouse design

The tested soil used was collected from experimental field, Faculty of Agriculture, Mansoura University, Dakahlia Governorate, Egypt, naturally infested with *M. incognita* (450 J2s/250g soil) and *R. reniformis* (150/250g soil). Pot experiment was done within pots (14 cm-diam) filled with 850 g clay loamy soil (Rough sand 2.2; Soft sand 20.41; Silt 25.09; Clay 48.62) based on soil analysis. Each pot was planted with a 35-day-old tomato seedling cv. Nova. After 10 days, aqueous, ethanolic and nanoparticles extracts of lantana and mint as well as Tervigo (Abamectin) were applied as experimental design. Treatments were divided to three groups (A. single treatments) as follows: oxamyl (0.3g/pot); Tervigo (T) 2% (0.3ml/pot); Aqueous leaf extract of *L. camara* (AL) (5ml/pot); Ethanolic leaf extract of *L. camara* (EL) (5ml/pot); Nanoparticles of *L. camara* (Ag-Nps-L) (5ml/pot); Aqueous leaf extracts of mint (AM) (5ml/pot); Ethanolic leaf extract of mint (EM) (5ml/pot); Nanoparticles of mint (Ag-Nps-M) (5ml/pot); (B. double treatments at half rate), ½ (T+ Ag-NPs -L); ½ (T+ Ag-NPs -M); (C. triple treatment), ⅓ (T+ Ag-NPs -L + Ag-NPs -M); and left four pots with nematode only (N) without any treatment as check. Each treatment was replicated four times and irrigated as needed. All pots were randomly arranged on a block design under greenhouse conditions (26±2°C).

Vegetation criteria

All plants of each treatment were harvested and uprooted, 48 days from nematode inoculation, and both vegetative and root systems were used as fresh and dried tissues for the evaluation analyses. All plants morphology parameters including, plant length, fresh weight as well as dry shoot weight were measured and recorded.

Nematode reproduction

Nematode juveniles in 250g soil were extracted using sieving and modified Baermann technique (Goodey 1957). The nematode suspensions were examined in a Hawksley counting slide with a dissecting microscope to quantify the numbers of juveniles. A scale of 0-5 (Taylor and Sasser, 1978) for root gall index (RGI) or egg mass index (EI) was calculated.

Biochemical analyses

Fresh tomato leaves from each replicate/treatment were taken to evaluate the amount of chlorophyll according to Goodwin methodology (Goodwin, 1965). Dried leaves of tomato plants were ground and wet digested to determine their nitrogen, potassium, and phosphorus contents (Jones et al., 1991). Total phenol contents were extracted and calculated at 520 nm via a spectrophotometer by Chachiol according to standards (Slinkard and Singleton, 1977). Salicylic acid was extracted from feed with 0.1% hydrochloric acid in methanol. Separation

was achieved in 8 min in a gradient elution using 0.1% formic acid and acetonitrile. The analyte was detected using negative electrospray tandem mass spectrometry (Protasiuk and Olejnik, 2018).

Statistical analysis

Data were statistically performed using Costat (2005) computer software version 6.303. Statistically significant differences among means were compared using analysis of variance (ANOVA) with the least significant difference (LSD) with a probability of 0.05.

RESULTS

The efficacy of aqueous, ethanolic and nanoparticles of lantana and mint extracts in comparison with commercial product, abamectin as singly or integrated applications in controlling *M. incognita* and *R. reniformis* infecting common tomato was carried-out under greenhouse conditions. Data in Table (1) showed that all the tested treatments significantly reduced the numbers of nematode juveniles in soil, gall and egg mass numbers for *M. incognita* infection. Tervigo (T) as a single application achieved the maximum percentage of reduction in nematode parameters with the values of 71.0, 58.3 and 83.3% for nematodes in soil, galls and egg masses numbers, respectively (Table 1).

Table 1: Reproduction of *Meloidogyne incognita* infecting tomato plants cv. Nova as affected by aqueous, ethanolic and nanoparticles of two plant extracts comparing to commercial product abamectin under greenhouse conditions.

Treatments	No. juveniles in soil	Red.%	No Galls	Red. %	RGI	No. egg masses	Red. %	EI
T	700.0 ^{ef}	71.0	5.0 ^{b-d}	58.3	1.7	1.0 ^{b-d}	83.3	0.7
AL	1116.7 ^b	53.8	8.7 ^{ab}	27.5	2.0	2.3 ^b	61.7	1.3
EL	983.3 ^{b-d}	59.3	6.7 ^{bc}	44.2	2.0	1.7 ^{b-d}	71.7	1.3
Ag-NPs –L	816.7 ^{de}	66.2	7.3 ^b	39.2	2.0	2.0 ^{bc}	66.7	1.3
AM	1216.7 ^b	49.7	7.7 ^b	35.8	2.0	2.0 ^{bc}	66.7	1.3
EM	1000.0 ^{b-d}	58.6	8.0 ^b	33.3	2.3	1.7 ^{b-d}	71.7	1.0
Ag-NPs –M	850.0 ^{c-e}	64.8	6.0 ^{bc}	50.0	2.0	1.0 ^{b-d}	83.3	0.7
T+ Ag-NPs –L	450.0 ^{fg}	81.4	5.0 ^{b-d}	58.3	2.0	0.3 ^d	95.0	0.3
T+ Ag-NPs –M	483.3 ^{fg}	80.0	6.0 ^{bc}	50.0	2.0	0.6 ^{cd}	90.0	0.7
T+ Ag-NPs -L+ Ag-NPs –M	333.3 ^g	86.2	3.0 ^{cd}	75.0	2.0	0.3 ^d	95.0	0.3
Oxamyl	316.7 ^g	86.9	2.0 ^d	83.3	1.3	0.3 ^d	95.0	0.3
Nematode only (N)	2416.7 ^a	----	12.0 ^a	----	3.0	6.0 ^a	----	2.0
LSD	273.7	---	3.8	---	---	3.8	--	--

Means of four replicates in each column followed by the same letter (s) did not differ at $P < 0.05$ according to Duncan multiple-range test. N= 450 J₂s of *M. incognita*/250g soil

T= Tervigo (Abamectin); AL =Aqueous leaf extract of *L. camara*; EL =Ethanolic leaf extract of *L. camara*; Ag-Nps-L=Silver Nanoparticles of *L.camara*; AM=Aqueous leaf extracts of *M. longifolia*; EM=Ethanolic leaf extract of *M. longifolia*; Ag-Nps-M=ilver Nanoparticles of *M. longifolia*;

Moreover, the plant received mint nanoparticles (Ag-NPs –M) as a single application was ranked the second in reducing nematode parameter values reaching 64.8, 50.0 and 83.3% for

the same parameters. As for dual applications, Tervigo plus *lantana* nanoparticles at their half rates showed the highest reduction values for the number of developmental nematodes stages in the soil (81.4%), the number of galls (58.3%), and egg masses (95.0%). Moreover, when abamectin was added to nanoparticles of *L. camara* and *M. longifolia* as a triple application of [$\frac{1}{3}$ (T+ Ag-NPs -L+ Ag-NPs -M)], an obviously high synergistic effect was recorded in decreasing nematode parameters with values of 86.2, 75.0 and 95.0%, respectively, for the same parameters. It is worth noting that oxamyl recorded the highest percentages of reduction in the number of nematode juveniles in the soil (86.9%), number of galls (83.3%), and number of egg masses (95.0%), as it was rated the first in this regard. Likewise, significant results were observed between gall and egg mass indices for the entire treatment tested and nematodes only, ranging from 1.3 to 2.3 for gall index and 0.3 to 1.3 for egg mass index versus 3.0 and 2.0 for nematodes alone (Table 1).

Data presented in Table (2) showed that all tested treatments resulted in a significant reduction in the numbers of soil nematode juveniles per pot and egg masses per root for *R. reniformis* infection. Among individual applications, Tervigo (T) achieved the maximum percentage of reduction in nematode parameters with the values of 68.1 and 69.7% for numbers of soil nematodes and numbers of egg masses, respectively (Table 2).

Table 2: Nematode parameters of *Rotylenchulus reniformis* infecting tomato plants cv. Nova as affected by aqueous, ethanolic and nanoparticles of two plant extracts comparing to commercial product. abamectin under greenhouse conditions.

Treatments	No. nematodes in	Red.%	No. egg	Red.%	EI
	soil		masses		
T	250.0 ^{de}	68.1	1.0 ^{b-d}	69.7	1.0
AL	383.3 ^{bc}	51.1	1.7 ^b	48.5	1.0
EL	366.7 ^{bc}	53.2	1.3 ^{bc}	60.6	1.0
Ag-NPs -L	326.7 ^{cd}	58.3	1.3 ^{bc}	60.6	1.0
AM	416.7 ^b	46.8	1.7 ^b	48.5	1.0
EM	366.7 ^{bc}	53.2	1.3 ^{bc}	60.6	1.0
Ag-NPs -M	310.0 ^{cd}	60.4	1.0 ^{b-d}	69.7	1.0
T+ Ag-NPs -L	226.7 ^{ef}	71.1	0.7 ^{cd}	78.8	0.7
T+ Ag-NPs -M	223.3 ^{ef}	71.5	0.7 ^{cd}	78.8	0.7
T+ Ag-NPs -L+ Ag-NPs -M	170.0 ^f	78.3	0.3 ^d	90.9	0.3
Oxamyl	180.0 ^{ef}	77.0	0.3 ^d	90.9	0.3
Nematode only (N)	783.3 ^a	----	3.3 ^a	----	2.0
LSD	76.7	----	0.9	----	----

Means of four replicates in each column followed by the same letter (s) did not differ at $P < 0.05$ according to Duncan multiple range test. N=150 immature females of *R. reniformis*/250g soil.

T= Tervigo (Abamectin); AL =Aqueous leaf extract of *L. camara*; EL =Ethanolic leaf extract of *L. camara*; Ag-Nps-L= Silver Nanoparticles of *L.camara*; AM=Aqueous leaf extracts of *M. longifolia*: EM=Ethanolic leaf extract of *M. longifolia*; Ag-Nps-M= Silver Nanoparticles of *M. longifolia*;

Moreover, plants receiving mint nanoparticles (Ag-NPs -M) as a single treatment were ranked the second in reducing nematode parameter values of 60.4 and 69.7% for the same parameters. For dual applications, Tervigo plus Mint nanoparticles at half rates achieved the highest reductions in numbers of soil nematodes (71.5%) and egg masses (78.8%). A similar trend was

observed, when Tervigo was added to nanoparticles of lantana and mint as a triple application, where a high synergistic effect in decreasing nematode parameters was recorded with values of 78.3 and 90.9% for nematode in soil and egg masses per root, respectively. It is worth noting that oxamyl recorded a degree equal to the previous triple treatment in terms of reduction percentages in the number of young nematodes in the soil (77.0%) and the number of egg masses (90.9%). Likewise, significant results were observed among egg mass indices for the entire treatment tested and nematodes only, ranging from 0.3 to 1.0 for the applications tested versus 2.0 for nematodes alone (Table 2).

Data in Table (3) presented that the tested materials significantly enhanced the plant testing parameters. Among the individual applications, abamectin-treated plants outperformed other single treatments tested in growing shoots for total plant height (50.5%), total plant fresh weight (71.9%) and dried weight of shoots (188.9%), followed by lantana nanoparticles in this respect. Moreover, among the dual treatments tested, abamectin plus lantana nanoparticles with its half rates, the other double treatments outperformed the percentage of increase in the values of vegetative growth characteristics of tomato plants, such as total plant length (63.1%), total fresh plants, and weight (85.9%). and dry weight of shoots (227.8%). Likewise, a similar trend was evident, where application of lantana nanoparticles plus mint nanoparticles mixed with abamectin at triple rates each achieved the maximum percentage of increase values of 78.1, 100.0 and 261.1% for the same parameters. Oxamyl recorded a moderate significant increase in the values of plant growth criteria, reaching 94.4, 115.6, and 277.8% for plant height and dried weights of the shoots, respectively, compared to nematodes alone (Table 3).

Data in Table (4) illustrated the leverage of plant extracts (aqueous – ethanolic – nanoparticles) and Tervigo, the commercial product of abamectin only or integrated applications on nitrogen; phosphorus; potassium and chlorophyll total contents in leaves of tomato as affected by *M. incognita* and *R. reniformis* infection under greenhouse conditions. All screened treatments showed a significant improvement in N, P and K contents with various degrees. The triple application, T+ Ag-NPs -L+ Ag-NPs -M were considered the best effective in improving N, P and K with values reaching 36.3, 30.3 and 53.6%, respectively compared to control plants. The impact of evaluated treatments on chlorophyll a, b and total chlorophyll in tomato revealed a significant induction in total chlorophyll compared to control plants. The increase in total chlorophyll A+B was recorded with treatments using three components viz. oxamyl and concomitant triple application T+ Ag-NPs-L +Ag-NPs-M at percentages of increases of 16.6 and 15.4%, respectively.

Data in Table (5) showed the efficacy of two plant extracts (aqueous, ethanolic and nanoparticles) as well as Tervigo (abamectin) only or integrated applications on two resistance related compounds, salicylic acid, and total phenol in leaves of tomato as affected by *M. incognita* and *R. reniformis* infection under greenhouse conditions. All screened treatments showed a significant reduction in salicylic acid, and total phenol in tomato with various degrees. Herein, triple application T+ Ag-NPs-L +Ag-NPs showed the highest reduction in total phenol in dried leaves of tomato (23.7%) compared to untreated plants. Oxamyl surpassed all treatments with percentage of reduction in total phenol reached (26.2%). Concomitant application showed the highest reduction (34.5%) in salicylic acid compared to nematode alone and single application. Oxamyl surpassed all treatments with percentage of decrease in salicylic acid reaching 38.2%.

Table 3. Effect of aqueous, ethanolic and nanoparticles of two plant extracts compared to the commercial product, abamectin on tomato plants as affected by *Meloidogyne incognita* and *Rotylenchulus reniformis* infection under greenhouse conditions.

Treatments	Plant Growth Response									
	Length(cm)		Plant length (cm)	Inc. %	Fresh weight (g)		Plant F.wt (g)	Inc.%	Shoot Dry wt. (g)	Inc.%
	Shoot	Root			Shoot	Root				
T	34.3 ^{cd}	22.0 ^{cd}	56.3	50.5	9.3 ^{ab}	1.7 ^{cd}	11.0	71.9	5.2 ^{c-e}	188.9
AL	28.3 ^{fg}	16.7 ^{ef}	45.0	20.3	6.4 ^{d-f}	1.3 ^{ef}	7.7	20.3	2.8 ^g	55.6
EL	30.0 ^{ef}	19.0 ^{de}	49.0	31.0	6.8 ^{de}	1.5 ^{de}	8.3	29.7	4.0 ^f	122.2
Ag-NPs -L	32.0 ^{de}	20.3 ^{de}	52.3	39.8	8.6 ^{bc}	1.6 ^{de}	10.2	59.4	4.8 ^{d-f}	166.7
AM	26.3 ^g	15.0 ^f	41.3	10.4	6.0 ^{ef}	1.2 ^f	7.2	12.5	2.6 ^{gh}	44.4
EM	28.3 ^{fg}	19.3 ^{de}	47.6	27.3	6.1 ^{ef}	1.4 ^{d-f}	7.5	17.2	4.2 ^f	133.3
Ag-NPs -M	31.0 ^{ef}	19.7 ^{de}	50.7	35.6	7.7 ^{cd}	1.5 ^{de}	9.2	43.8	4.6 ^{ef}	155.6
T+ Ag-NPs -L	37.0 ^c	24.0 ^{bc}	61.0	63.1	9.7 ^{ab}	2.2 ^b	11.9	85.9	5.9 ^{a-c}	227.8
T+ Ag-NPs -M	35.7 ^c	24.3 ^{bc}	60.0	60.4	9.7 ^{ab}	2.1 ^{bc}	11.8	84.4	5.6 ^{b-d}	211.1
T+ Ag-NPs -L+ Ag-NPs -M	40.3 ^b	26.3 ^{ab}	66.6	78.1	10.3 ^a	2.5 ^b	12.8	100.0	6.5 ^{ab}	261.1
Oxamyl	43.7 ^a	29.0 ^a	72.7	94.4	10.5 ^a	3.3 ^a	13.8	115.6	6.8 ^a	277.8
Nematode only (N)	22.7 ^h	14.7 ^f	37.4	----	5.3 ^f	1.1 ^f	6.4	----	1.8 ^h	----
LSD	3.3	3.1	----	----	1.4	0.4	----	----	1.0	----

Means of four replicates in each column followed by the same letter (s) did not differ at P<0.05 according to Duncan multiple-range test. N= 450 juveniles (J₂) of *M. incognita*/250g soil. N=150 immature females of *R. reniformis* 250g soil.

T= Tervigo (Abamectin); AL =Aqueous leaf extract of *L. camara*; EL =Ethanolic leaf extract of *L. camara*; Ag-Nps-L= Silver Nanoparticiles of *L.camara*; AM=Aqueous leaf extracts of *M. longifolia*; EM=Ethanolic leaf extract of *M. longifolia*; Ag-Nps-M= Silver Nanoparticles of *M. longifolia*;

Table 4: Chemical constituents of tomato infected with *Meloidogyne incognita* and *Rotylenchulus reniformis* as affected by aqueous, ethanolic and nanoparticles of two plant extracts comparing to commercial product abamectin under greenhouse conditions.

Treatments	Chemical components									
	Leaves									
	N	Inc. %	P	Inc. %	K	Inc. %	Chlorophyll content m/g			
Chlo. A							Chlo. B	Total	Inc. %	
T	2.97 ^f	23.8	0.27 ^e	19.9	2.43 ^h	26.6	0.63 ⁱ	0.44 ⁱ	1.07 ⁱ	5.7
AL	2.90 ⁱ	20.8	0.26 ^f	18.6	2.34 ⁱ	21.9	0.62 ^j	0.44 ^j	1.06 ^j	3.9
EL	2.95 ^g	22.9	0.24 ^h	10.0	2.50 ^g	30.2	0.65 ^h	0.45 ^h	1.10 ^h	7.7
Ag-NPs –L	3.03 ^e	26.3	0.27 ^e	19.9	2.67 ^e	39.1	0.66 ^e	0.46 ^g	1.12 ^e	10.7
AM	2.65 ^j	10.4	0.24 ^h	9.5	2.16 ^j	12.5	0.61 ^k	0.43 ^k	1.04 ^k	2.8
EM	2.94 ^h	22.5	0.25 ^g	14.5	2.59 ^f	34.9	0.66 ^g	0.45 ^f	1.11 ^g	9.3
Ag-NPs –M	2.95 ^g	22.9	0.25 ^g	14.5	2.59 ^f	34.9	0.66 ^f	0.46 ^e	1.12 ^f	9.9
T+ Ag-NPs –L	3.20 ^c	33.3	0.27 ^d	22.6	2.80 ^d	45.8	0.67 ^d	0.47 ^d	1.14 ^d	12.1
T+ Ag-NPs –M	3.18 ^d	32.5	0.28 ^c	26.7	2.88 ^c	50.0	0.68 ^c	0.47 ^c	1.15 ^c	13.9
T+ Ag-NPs –L+ Ag-NPs –M	3.27 ^b	36.3	0.29 ^b	30.3	2.95 ^b	53.6	0.69 ^b	0.48 ^b	1.17 ^b	15.4
Oxamyl	3.34 ^a	39.2	0.30 ^a	35.3	3.04 ^a	58.3	0.70 ^a	0.49 ^a	1.19 ^a	16.6
Nematode alone	2.40 ^k	0.0	0.22 ⁱ	0.0	1.92 ^k	0.0	0.60 ^l	0.41 ^l	1.01 ^l	0.0
LSD	0.02	---	0.003	---	0.02	--	0.002	0.002	0.004	---

Means of four replicates in each column followed by the same letter (s) did not differ at $P < 0.05$ according to Duncan multiple-range test.

T= Tervigo (Abamectin); AL =Aqueous leaf extract of *L. camara*; EL =Ethanolic leaf extract of *L. camara*; Ag-Nps-L= Silver Nanoparticiles of *L.camara*; AM=Aqueous leaf extracts of *M. longifolia*; EM=Ethanolic leaf extract of *M. longifolia*; Ag-Nps-M= Silver Nanoparticles of *M. longifolia*;

DISCUSSION

Integrated management is a sustainable approach for the management of plant parasitic nematodes. In the present investigation the effectiveness of certain biotic inducers i.e. aqueous, ethanolic and nanoparticles of lantana and mint extracts as singly or integrated applications in combination with biopesticide against *M. incognita* and *R. reniformis* infecting tomato plants was assessed under greenhouse. The enhancement of the efficiency of plants' natural products against pathogens could be achieved through their formulation as nanoparticles (Nassar, 2016; Abbassy et al., 2017). Therefore, the effectiveness of leaf plant extracts nanoparticles was evaluated as nematicidal alternatives on growth plant parameters of tomato grown in soil naturally infested with *M. incognita* and *R. reniformis* under greenhouse and field conditions.

Silver nanoparticles (AgNPs) possess nematicidal effects against root-knot nematodes (Cromwell et al., 2014; Nour El-Deen and El-Deeb, 2018). In the present study, silver nanoparticles of leaf plant extracts of *L.camara* and *M. longifolia*; were used in improving tomato plant fresh weights infected with *M. incognita* and *R. reniformis*. Nematode population, root galling, number of females and egg masses were significantly suppressed by all treatments. Previous study has shown a potential nematicidal effect of AgNPs on root-knot nematodes (Khalil and Badawy, 2012; Bernard et al. 2019; Khairy, 2020) and reniform nematode as well (Khairy, 2020).

Table 5. Resistance related compounds of tomato infected with *Meloidogyne incognita* and *Rotylenchulus reniformis* as affected by aqueous, ethanolic and nanoparticles of two plant extracts comparing to commercial product, Abamectin under greenhouse conditions.

Treatments	T. phenol mg/100g	Dec. %	SA ppm	Dec. %
T	516.6e	10.9	6.57f	16.9
AL	528.6b	8.8	6.98b	11.8
EL	522.9d	9.8	6.88c	13.0
Ag-NPs -L	498.8h	13.9	6.37g	19.5
AM	526.3c	9.2	6.98b	11.8
EM	513.7g	11.4	6.85d	13.4
Ag-NPs -M	515.4f	11.1	6.77e	14.4
T+ Ag-NPs -L	456.5j	21.2	5.51i	30.3
T+ Ag-NPs -M	472.6i	18.4	5.79h	26.8
T+ Ag-NPs -L+ Ag-NPs -M	442.3k	23.7	5.18j	34.5
Oxamyl	427.4l	26.2	4.89k	38.2
Nematode alone	579.5a	0.0	7.91a	0.0
LSD	310.0	---	2.08	---

Means of four replicates in each column followed by the same letter (s) did not differ at $P < 0.05$ according to Duncan multiple-range test.

T= Tervigo (Abamectin); AL =Aqueous leaf extract of *L. camara*; EL =Ethanolic leaf extract of *L. camara*; Ag-Nps-L= Silver Nanoparticiles of *L.camara*; AM=Aqueous leaf extracts of *M. longifolia*; EM=Ethanolic leaf extract of *M. longifolia*; Ag-Nps-M= Silver Nanoparticles of *M. longifolia*;

On the other hand, a synergistic effect on root-knot nematode population was more evidenced with all combinations being the most effective by two plant extracts integrated with Abamectin. In general, combining different nematode management practices are a good option to prevent deleterious effect of plant parasitic nematodes and secure yield. The present study demonstrated the potential of such treatments to control *M. incognita* and *R. reniformis* in tomato under greenhouse conditions.

Furthermore, the impact of the tested materials on chemical constituents and resistant related compounds in dried leaves of tomato showed significantly increment in NPK, photosynthetic pigments i.e. Chlorophyll, A, B, A+B, and salicylic acid with different degrees compared to untreated plants. Our findings were in agreement with earlier observations made by many scientists (Gottstein and Ku, 1989; Radwan et al., 2012; Vafadar et al., 2013; Molinari and Loffredo, 2006). A positive correlation was noticed, since phenol content in treated plants decreased as the root galling formation decreased. This result contradicts with those reported by Nguyen et al. (2011). Phenolic compounds interfere with the relationship between plants and nematodes. Phenolics found in the feeding sites of root-knot nematodes are associated with the hypersensitivity response of plants to nematode infection (Oliveira et al., 2019). Salicylic acid (SA) has been found to inhibit CAT (catalase) activity in many plants, and thus, to be involved in plant systemic acquired resistance (SAR) (Chen et al., 1993).

CONCLUSION

This study showed that silver nanoparticles of both lantana and mint leaf extracts in integration with abamectin, provided early protection against root-knot nematode, *M. incognita* and reniform nematode, *R. reniformis* infection under greenhouse conditions. The utilization of botanical products formulated in nanoparticles formulations as nematicidal alternatives is viable to control such nematode species. This point needs to further studies with a focus on working under field conditions to study the impact of this technique on the survival of nematode and give adequate recommendations for use as an appropriate method in IPM programs.

DECLARATION

The authors declare that they do not have any actual or potential conflict of interest.

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الملخص العربي

فعالية المستخلص المائي والكحولي والنانو لكل من اللانتانا والنعناع مقارنة بالمنتج التجاري أبامكتين علي نبات الطماطم تحت تأثير الإصابة بنيماتودا تعقد الجذور والنيماتودا الكلوية.

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أجريت تجربة تحت ظروف الصوبة الزراعية لتحديد كفاءة المبيد الحيوي ابامكتين وثلاثة انواع (مائي - كحولي - نانو) من المستخلصات النباتية لنباتي *Lantana camara* و *Mentha longifolia* لمكافحة نيماتودا تعقد الجذور *Meloidogyne incognita* والنيماتودا الكلوية *Rotylenchulus reniformis* التي تصيب نباتات الطماطم ومع دراسة تأثير ذلك علي المقاييس النيماتودية والخضرية وكذا التركيب الكيماوي للنباتات تحت الدراسة. وقد اظهرت النتائج ما يلي:

- ١- ادت جميع المعاملات المختبرة إلى انخفاض معنوي واضح في الإصابة بكل نوعي النيماتودا تحت الدراسة بدرجات متفاوتة.
- ٢- سجل المبيد الحيوي Tervigo (ابامكتين) كمعاملة منفردة القيم الاعلي في خفض المقاييس النيماتودية تحت الدراسة تليها جزيئات مستخلص النعناع النانوية كمعاملة مفردة.
- ٣- أظهرت معاملة النانو من اللانتانا مع المبيد الحيوي ترفيجو أعلى معدلات الانخفاض في المقاييس النيماتودية المختبرة.
- ٤- كان من الواضح أن المعاملة الثلاثية كان له تأثير تعاوني عالي في خفض قيم المعاملات النيماتودية بالإضافة إلى تحقيق أقصى نسبة زيادة في إجمالي طول النبات وإجمالي الوزن الطازج للنبات والوزن الجاف للأوراق.
- ٥- سجلت المعاملة الثلاثية القيم الأفضل ٣٦,٣ و ٣٠,٣ و ٥٣,٦% لكلا من النيتروجين والفوسفور والبوتاسيوم على التوالي والمحتوى الكلي من الكلوروفيل في أوراق الطماطم، وكذلك انخفاض ملحوظ في حمض الساليسيليك مقارنة بالمعاملات الاخري (٣٤,٥%).
- ٦- تفوقت المعاملة بالأوكساميل على جميع المعاملات حيث بلغت نسبة الخفض في الفيول الكلي (٢٦,٢%) و انخفاض نسبة حامض الساليسيليك بقيم (٣٨,٢%).