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Nematicidal activity of silver nanoparticles of botanical products against root-knot nematode, Meloidogyne incognita

Moustafa A. Abbassy, Mona A. Abdel-Rasoul, Atef M. K. Nassar and Belal S. M. Soliman

Faculty of Agriculture, Plant Protection Department, Damanhour University, Damanhour, Egypt

ABSTRACT

Current study investigated the nematicidal activity of leaf extracts of *Conyza dioscoridis*, *Melia azedarach*, and *Moringa oleifera* that were prepared as silver nanoparticles (Ag-NP). The characterisation and size confirmation of the Ag-NP were done by UV–vis spectrophotometry and the scanning electron microscopy (SEM). The phytochemical contents of crude extracts and the nano formulations were analysed using gas chromatography-mass spectroscopy (GC-MS). Results revealed that silver nanoparticles of *C. dioscoridis* extractives had great nematicidal activity against the 2nd stage juvenile (J2) and eggs of *Meloidogyne incognita*. Also, the Ag-NP showed similar nematicidal effect to the reference nematicide; rugby. The GC-MS analysis revealed the increase of certain metabolites due to the formulation of the Ag-NPs. Aromadendrene, 1-hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecdiene, 6-epi-shyobunol, 4-hexylacetophenone, β-isocomene, caryophyllene, β- and α-selinene, α-cadinol, berkheyaradulen, and bis-(2-ethylhexyl)phthalate were increased more than 2.5-folds in the Ag-NP compared the extract. Therefore, the green synthesis of metal nanoparticles might be a safe, effective and affordable nematicide alternatives.

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KEYWORDS
Nematicide alternatives; metal nanoparticles; *Conyza dioscoridis*; *Melia azedarach*

Key message

The extractives of *Conyza dioscoridis*, *Melia azedarach*, and *Moringa oleifera* were synthesized in the silver nanoparticle (AgNPs) form.

The AgNPs showed nematicidal activity against root-knot nematode *M. incognita*.

The AgNPs of *C. dioscoridis* exerted great potential in inhibiting both the eggs and the 2nd juvenile (J2) stages of *M. incognita*.

CONTACT Atef M. K. Nassar atef.nassar@dmu.edu.eg

The increased activity of the nano-formulations might be a result of the elevated content of certain metabolites, for example, epi-shyobunol, aromdendrene, α- and t-cadinol, caryophyllene, α-humulone, β-isocomene, and α- and β-selinene.

Introduction

There are more than 4100 species of plant pathogenic nematodes that critically damage almost all crops (Nicol et al. 2011). Annually, the estimated crop loss due to nematodes is more than $100 billion worldwide (Khan et al. 2008). Nematode species infect all parts of the plant; roots, bulbs, rhizomes, stems, leaves, buds, flowers, and seeds. Specifically, the root-knot nematode (*Meloidogyne* spp.) is a serious pathogen to most crops and could cause up to 64% yield reduction in almost countries (Khan et al. 1996; Roberts et al. 2005; Sikora et al. 2007; Balbaa 2010).

Management tactics of nematodes in the field include enhanced cultural practices, cultivation of resistant cultivars, predators and parasites, allelochemicals of cover crops, organic amendments, sanitation, solarization, and the widely used synthetic nematicides (McSorley 2002; Browning et al. 2006; Williamson and Kumar 2006; Khan and Kim 2007; Okada and Harada 2007; Wang et al. 2007). The extensive use of nematicides might lead to environmental and health problems as well as the development of nematode resistance. Therefore, it's important to use alternative control strategies, which are effective, cheap, and safe to farmers, consumers, and the environment (Fernandez et al. 2001).

One of the possible alternatives is the utilisation of plant extractives since it is a very rich source for the development of new compounds. For example, the crude extractives of neem (*Azadirachta indica L.*) (Salawu 1992), African basil (*Ocimum gratissimum*) (Aralepo 1989), bitter leaf (*Vernonia amygdalina L.*) (Jahn 1989) and moringa (*Moringa oleifera*, Lam) (Ajayi 1990; Youssef et al. 2014) have been reported to contain nematicidal properties that inhibit egg hatchability and the development of *Meloidogyne* spp. The saponins of *Asparagus adscendens*, *Albizzia chinensis* and *Acacia concinna* were active against *Meloidogyne incognita* (Meher et al. 1988). Thiarubrine A of *Aspilia mossambicensis* was very effective to control *Meloidogyne javanica* (Rodriguez 1988). Thymol and benzaldehyde mixture exhibited broad-spectrum nematicidal effect against root-knot and cyst nematode (Soler-Serratosa et al. 1996). The alkaloid 1,2-dehydropyrrolizidine had effective nematicidal activity against *Meloidogyne hapla* (Thoden et al. 2009).

Moreover, the enhancement of the efficiency of plants' natural products against pathogens could be achieved through their formulation as nanoparticles. Nano-formulations of secondary metabolites were reported to have increased effectiveness against plant diseases with lessened side effects for human, animals, and the environment (Abdul Hameed 2012). Silver nanoparticle formulations of extractives (ethanol, ethyl acetate, petroleum ether, and water extracts) of *Urtica urens* showed up to 11-folds increase in the nematicidal activity compared to

their corresponding raw extractives against root-knot nematode (Nassar 2016). Therefore, the present study was conducted to test the effectiveness of silver-botanical nanoparticles formulations as nematicide alternatives against the root-knot nematode (*M. incognita*: Kofoid and White). The nano-formulations were synthesised via the atomization of silver and extractives (ethanol, ethyl acetate, petroleum ether, water, and essential oil) of leaves of *Conyza dioscoridis*, *Melia azedarach*, and *Moringa oleifera*.

Materials and methods

Chemicals

A commercial formulation of Rugby 20% CS (Cadusafos: organophosphorodithioate; an insecticide and nematicide) was used as a reference nematicide and was purchased from Food Machinery and Chemicals (FMC) Corporation, USA. Silver nitrate ($AgNO_3$; 99.99%) was purchased from Sigma Aldrich chemicals. All other reagents and solvents were HPLC-grade and were purchased from reputed local chemical suppliers.

Plant materials

Herbal and medicinal plants: *C. dioscoridis*, *M. azedarach*, and *M. oleifera* were used. The shoots were collected from Al-Bostan Region, El-Beheira Governorate. Plants were identified and confirmed with the help of Plant Pathology Department, Faculty of Agriculture, Damanhour University, Egypt.

Preparation of plant extractives and essential oils

Leaves were washed and air-dried at room temperature, then in an oven at 50° C until complete dryness. Samples were ground to a fine powder and a 100 g of each plant powder was extracted successively with 500 ml of each of petroleum ether, ethyl acetate, and absolute ethanol till exhaustion in a Soxhlet apparatus. Solvents in crude extracts were evaporated under reduced pressure in a rotary evaporator (Unipan vacuum rotary evaporator type 350p, Poland) at 35° C and stored in the refrigerator (−20° C) till been used in bioassay tests.

The preparation of aqueous extracts was done following the method of Claudius-Cole et al. (2010). Approximately 100 g of each powder was soaked in 1000 ml distilled water for 72 h at room temperature. Slurries were heated for 1 h over a boiling water bath. The extract was allowed to cool and filtered through Whatman filter paper No.1 and the filtrate was used as a crude extract.

The volatile oil was obtained by steam distillation using Clevenger trap apparatus (Guenther 1952). A 100-g powder of the air-dried leaves was placed in 2 L capacity flasks with 1000 ml distilled water. The distillation continued until no

further increase in the oil was observed (about 8 h of distillation). The essential oil extracts were dried over anhydrous sodium sulphate, kept in tightly brown colour bottles, and stored in a refrigerator until the use in different bioassay tests.

Synthesis of silver- nanoparticles of plant products (Ag-NP)

Nanoparticles of silver and botanicals products (Ag-NPs) were prepared using a modified method of Prasad and Elumalai (2011). Exactly 10 ml of plant extracts or oil (5000 µg ml^{-1}) were mixed with 90 ml of silver nitrate (1 mM) and 10 ml of ascorbic acid (0.1 M) as a reducing reagent and polyvinyl pyrrolidine (PVP) as a protecting agent in a 250-ml conical flask. The mixture was warmed at 40° C on the steam bath for 10 min and then tubes were kept in dark place for 24 h at room temperature. The change in colour from dark brown to reddish-brown was observed and was an indication of the formulation of the Ag-nanoparticles. Similarly, the synthesis of Ag nanoparticles of Rugby® was done by incubation of 10 ml of Rugby® (20%) with 90 ml of silver nitrate (1 mM) and 10 ml ascorbic acid (0.1 M) at 40° C for 12 h. The development of reddish-brown colour was an indication that the Ag- Rugby® nano-formulation was synthesised.

Characterisation of silver nanoparticles

The produced nanoparticles were studied by UV–vis spectroscopy. Approximately 1 ml of samples was diluted with 2 ml of double distilled water and subsequently measured by UV–vis spectroscopy at a resolution of 1 nm. The UV–vis spectrometric readings were recorded from 300 to 700 nm in a Jenway spectrophotometer (Model 6305, Bibby Scientific Limited Staffordshire, UK) (Rajesh et al. 2009). Also, the morphology and size of the silver nanoparticles were confirmed by the scanning electron microscopy (JEOL, JSM-5300, USA). The SEM microscopy depends on the transmission of a beam of electrons through an ultra-thin specimen. As its passage through the sample, it reacts with it. Silver nanoparticles were mounted on specimen stubs with double-sided adhesive carbon tape, coated with Au alloy to make the surface conducting in a sputter coater (Fine Coat, Model JFC-1100E, JOEL, USA), and examined under a Philips XL-30 SEM at 12–16 kV with a tilt angle of 45 °.

Culture of root-knot nematode

Eggs of root-knot nematode (*M. incognita*) were isolated from a 60-day old culture of inoculated eggplants (*Solanum melongena* L.) in the greenhouse. Root-knot nematodes were identified using perineal patterns of adult females as well as the morphology of second stage juveniles (Hartman and Sasser 1985; Jepson 1987). The egg masses were obtained from the eggplant roots using sodium hypochlorite 10% in sterilised distilled water (Hussey and Barker 1973) and incubated for

48 h at room temperature at 25 ± 2° C for hatching. The hatched second stage juveniles (J2) were collected daily. Only freshly hatched J2 (collected within 48 h) were used for bioassay experiments. The J2 and eggs of *M. incognita* were used for toxicity evaluations.

Nematicidal efficacy of Ag-nanoparticle formulations against M. incognita

The nematicidal activity of extracts, volatile oil and their corresponding Ag-nanoparticles were evaluated against egg and J2 stages of *M. incognita* under laboratory conditions. Based on the results of bioassay experiments, 4 concentrations: 125, 250, 500 and 1000 µg/ml of each extract and its nano-formulation were tested. Each concentration had four replicates each of about 100 eggs or individuals of *M. incognita* juveniles in 5-ml screw-cap glass vials. The control treatments were distilled water. Rugby 20% was used as reference nematicide. The vials were incubated at 25 ± 2 °C. Juvenile mortality was recorded after 48 h and egg hatchability percentages were observed after 7 d. The laboratory assays were conducted 4 times and the mathematical average was used to calculate the LC_{50} values according to Finney (1971) (Ehab Soft, LdP Line®).

Gas chromatography-mass spectrometry (GC-MS) analysis

An Agilent 6890 gas chromatography system equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column PAS-5 MS (30 mm × 0.25 µm film thickness) was used. About 1 µl of each sample was injected using helium as carrier gas (36 cm/sec) and flow 1 ml/min. The solvent delay time was 3 min. The mass spectrophotometric detector was operated in an electron impact ionisation mode with the energy of 70 eV and the scanning mode from 50 to 500 mass/charge (m/z). The ion source temperature was 230 °C and the quadruple temperature was 150 °C. The electron multiplier voltage (EM voltage) was maintained 1250v above the auto tune. The instrument was manually tuned using perfluorotributylamine (PFTBA). The GC temperature programme was started at 60 °C for 3 min then elevated to 280 °C at a rate of 8 °C/min and 10 min hold at 280 °C. The m/z ratio obtained was calibrated from the mass spectrum graph, which is the fingerprint of each molecule. Moreover, identification of the separated peaks was done using the Wiley-9 and NIST05 mass spectral database. Identified components in natural extractives and Ag nanoparticles were compared and the folds of change were calculated.

Results

Synthesis and characterisation of silver-botanical nanoparticles

Preparation, characterisation, and applications as nematicides of Ag-nanoparticles of natural products were studied in the laboratory. Results showed that the

synthesis of silver nanoparticles was rapidly achieved by reacting the petroleum ether, ethyl acetate, ethanol, water, and essential oil extractives of *C. dioscoridis, M. azedarach*, and *M. oleifera* with the silver mineral in the presence of reducing and stabilising agents. The formation of the nanoparticles was confirmed using the UV–vis spectroscopy. The UV–vis spectra of the dilute solution of Ag nano-formulations showed λ_{max} peaks at about 410 ± 10 nm. Additionally, the scanning electron microscope (SEM) was employed to confirm the shape and size of the silver nanoparticles of plant extractives. As shown in Figure 1, the SEM analysis verified that the three plants have great capability to synthesise silver nanoparticles, which were roughly spherical in shapes. The example pictures (Figure 1) from the SEM illustrated that the size of nanoparticles of 30–100 nm for all tested extracts and the reference nematicide (Ag-rugby).

Nematicidal activity of botanical extracts and their Ag-nanoparticles

The nematicidal efficacy of the isolated extractives of *C. dioscoridis*, *M. azedarach*, and *M. oleifera* was screened against the J2 of root-knot nematode (Table 1). The reference nematicide, rugby, was the most toxic against *M. incognita*. The

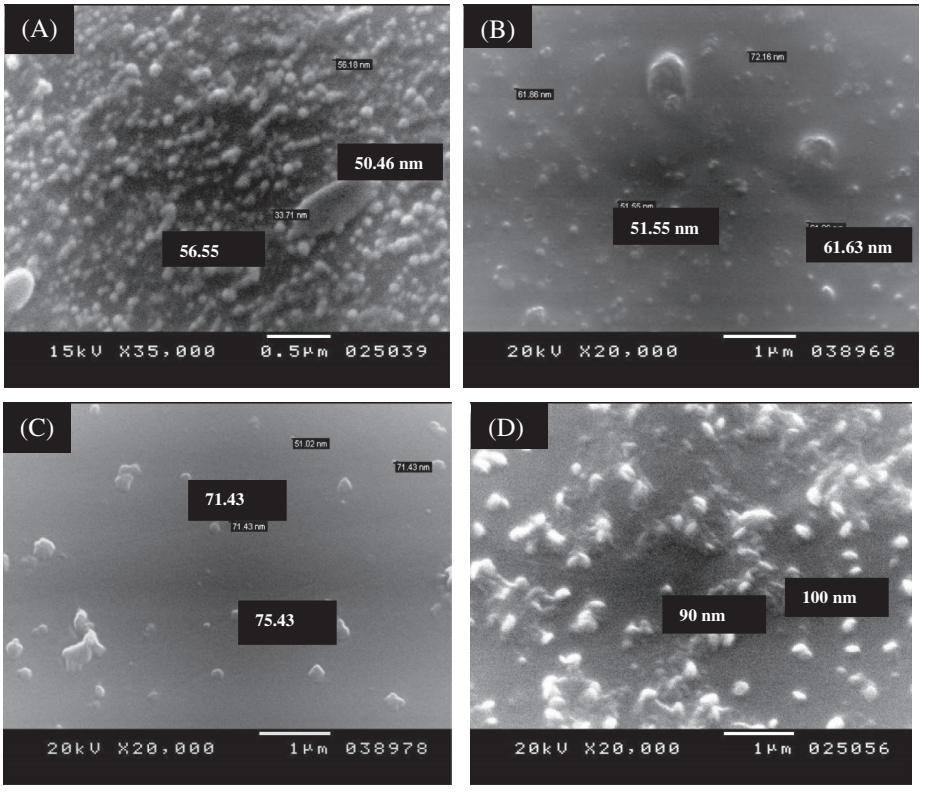

Figure 1. An example of nanoparticles of (A) water extract of *C. dioscoridis*, (B) *M. azedarach*, (C) *M. oleifera*, and (D) rugby.

Table 1. *In vitro* toxicity of extracts (PE; petroleum ether, EA; ethyl acetate, Et; ethanol, W; water, and oil; essential oil) of *C. dioscoridis*, *M. azedarach* and *M. oleifera* against the J2 of root-knot nematode *M. incognita*.

Treatment	Extract	LC_{50}[a] (µg ml^{-1})	95% Confidence limits (µg ml^{-1})		Slope ± SE[b]	X^{2c}	Toxicity index
			Lower	Upper			
Rugby	–	25.9	19.6	31.7	2.10±0.25	0.48	100.0
C. dioscoridis	PE	222.9	189.8	255.9	2.33±0.21	4.73	11.64
	EA	238.9	201.3	277.2	2.14±0.22	2.19	10.86
	Et	252.4	211.5	294.7	2.01±0.21	5.62	10.28
	W	274.6	232.6	319.2	2.05±0.21	4.57	9.45
	Oil	186.0	154.4	216.4	2.28±0.22	0.38	13.95
M. azedarach	PE	265.1	226.3	305.9	2.19±0.22	1.81	9.79
	EA	314.2	273.5	370.3	2.09±0.21	3.21	8.13
	Et	348.3	297.7	407.1	2.00±0.21	3.29	7.45
	W	370.1	316.7	433.9	1.99±0.21	3.81	7.01
	Oil	315.3	269.0	367.0	2.03±0.21	2.48	8.23
M. oleifera	PE	315.2	273.8	361.0	2.33±0.22	4.89	8.20
	EA	360.1	311.4	416.9	2.17±0.21	4.65	7.21
	Et	378.7	325.7	442.1	2.06±0.21	5.80	6.85
	W	408.7	352.1	478.4	2.07±0.21	5.91	6.35
	Oil	347.3	277.4	433.7	1.37±0.19	0.97	7.47

[a]The median concentration that kills 50% of larvae.
[b]Slope ± Standard Error.
[c]Chi Square.

inhibitory effect of all extractives on nematode activity or mortality was concentration dependent. The LC_{50} values of extracts ranged from 186 µg/ml for the essential oil extract of *C. dioscoridis* to 408.7 µg/ml for the water extract of *M. oleifera*. Petroleum ether (PE) and ethyl acetate (EA) extracts of all plants showed similar nematicidal effects.

The nematicidal activity of Ag-nanoparticles of extractives (Ag-NP) of *C. dioscoridis*, *M. azedarach*, and *M. oleifera* was screened against J2 of *M. incognita* (Table 2). The results revealed that the nanoformulations were more toxic than crude extracts. The 95% confidence limits (µg/ml) of rugby overlapped with those of the Ag nanoformulations of PE, EA, ethanol (EtOH), water (W), and essential oil extracts of *C. dioscoridis* and the EtOH extract of *M. azedarach*, which highlight similar nematicidal efficacy. Furthermore, the PE, EA, EtOH, and essential oil (EO) extracts of *C. dioscoridis* were more effective against *M. incognita* compared to *M. oleifera*, and *M. azedarach* but not different from the Et extract of *M. azedarach*.

The results presented in Tables 3 show the nematicidal activity of crude extracts of *C. dioscoridis*, *M. azedarach*, and *M. oleifera* against the eggs of *M. incognita*. It was obvious that the nematicide "rugby" was more effective in reducing the hatching of root-knot nematode compared to crude extracts by 5–10 folds (Table 3). The PE, EA, EtOH, W, and EO extracts of *C. dioscoridis* had significantly nematicidal effects compared to all other extracts except for the EA extract of all tested plants. The essential oil and PE extracts of *C. dioscoridis* were the most effective against eggs of *M. incognita* with LC_{50} values of 159.3 and 215.7 mg/ml, respectively.

Table 2. *In vitro* toxicity of silver Nanoformulations of extractives (PE; petroleum ether, EA; ethyl acetate, Et; ethanol, W; water, and oil; essential oil) of *C. dioscoridis*, *M. azedarach* and *M. oleifera* against the J2 of root-knot nematode *M. incognita*.

Treatment	Extract	LC_{50}[a] (µg ml^{-1})	95% Confidence limits (µg ml^{-1})		Slope ± SE[b]	X^{2c}	Toxicity index
			Lower	Upper			
Rugby	–	14.3	6.9	21.6	1.34 ± 0.21	1.41	100.0
C. dioscoridis	PE	53.7	20.1	87.3	1.36 ± 0.25	0.14	26.75
	EA	50.8	13.7	90.1	1.09 ± 0.23	0.23	28.30
	Et	52.9	15.5	92.3	1.10 ± 0.22	0.19	27.17
	W	58.0	19.4	97.1	1.15 ± 0.22	0.02	24.78
	Oil	52.2	19.8	84.4	1.44 ± 0.22	0.17	27.53
M. azedarach	PE	74.5	36.1	111.2	1.35 ± 0.22	0.66	19.29
	EA	80.7	38.1	120.4	1.26 ± 0.22	0.47	17.81
	Et	61.7	17.7	106.9	0.99 ± 0.21	1.72	23.28
	W	73.8	26.0	120.1	1.03 ± 0.21	1.06	19.49
	Oil	71. 2	24.6	116.7	1.04 ± 0.21	0.87	20.20
M. oleifera	PE	97.9	56.5	135.3	1.44 ± 0.22	2.51	14.69
	EA	119.4	74.7	159.5	1.42 ± 0.21	2.35	12.04
	Et	127.2	80.5	168.9	1.39 ± 0.21	1.41	11.31
	W	133.0	82.9	177.8	1.31 ± 0.21	1.49	10.81
	Oil	111.2	68.8	149.3	1.47 ± 0.22	0. 31	12.93

[a]The median concentration that kills 50% of larvae.
[b]Slope ± Standard Error.
[c]Chi Square.

Table 3. *In vitro* toxicity of extractives (PE; petroleum ether, EA; ethyl acetate, Et; ethanol, W; water, and oil; essential oil) of *C. dioscoridis*, *M. azedarach* and *M. oleifera* against eggs of root-knot nematode *M. incognita*.

Treatment	Extract	LC_{50}[a] (µg ml^{-1})	95% Confidence limits (µg ml^{-1})		Slope ± SE[b]	X^{2c}	Toxicity index
			Lower	Upper			
Rugby	–	41.4	36.5	46.3	3.18 ± 0.29	4.66	100.0
C. dioscoridis	PE	215.7	177.0	253.0	2.03 ± 0.23	2.90	19.20
	EA	263.2	225.0	302.0	2.27 ± 0.22	3.34	15.73
	Et	280.0	241.0	323.4	2.22 ± 0.22	4.34	14.75
	W	296.3	254.0	341.4	2.21 ± 0.22	4.98	13.97
	Oil	159.3	124.9	191.3	2.01 ± 0.22	0.61	25.99
M. azedarach	PE	302.0	260.9	347.0	2.26 ± 0.22	1.51	13.71
	EA	362.2	315.4	416.4	2.3 ± 0.22	1.59	11.43
	Et	400.8	351.0	460.1	2.39 ± 0.22	1.99	10.33
	W	403.5	351.7	465.7	2.29 ± 0.22	3.93	10.26
	Oil	342.4	295.9	395.6	2.1 ± 0.21	3.35	12.09
M. oleifera	PE	354.3	309.7	405.4	2.38 ± 0.22	5.81	11.69
	EA	385.8	335.8	444.9	2.27 ± 0.22	5.35	10.73
	Et	417.6	363.3	484.0	2.25 ± 0.22	5.37	10.03
	W	412.9	354.4	485.9	2.01 ± 0.21	5.94	9.91
	Oil	377.3	291.8	494.5	1.17 ± 0.19	0.27	10.97

[a]The median concentration that inhibits 50% of egg hatchability.
[b]Slope ± Standard Error.
[c]Chi Square.

The nematicidal activity of the Ag-NP against the eggs of root-knot nematode was reported in Table 4. Results revealed that preparation of Ag- botanical

Table 4. *In vitro* toxicity of silver nano-formulations of extractives (PE; petroleum ether, EA; ethyl acetate, Et; ethanol, W; water, and oil; essential oil) of *C. dioscoridis*, *M. azedarach* and *M. oleifera* against eggs of root-knot nematode *M. incognita*.

Treatment	Extract	LC_{50}[a] (µg ml^{-1})	95% Confidence limits (µg ml^{-1})		Slope ± SE[b]	X^{2c}	Toxicity index
			Lower	Upper			
Rugby	–	19.4	13.8	24.2	2.49 ± 0.34	5.19	100.0
C. dioscoridis	PE	95.4	60.1	126.8	1.75 ± 0.25	0.20	20.42
	EA	105.3	61.1	144.4	1.40 ± 0.23	1.02	18.51
	Et	114.6	73.0	151.8	1.52 ± 0.22	0.11	17.00
	W	134.0	92.3	171.5	1.59 ± 0.22	0.04	14.54
	Oil	87.5	54.7	116.3	1.90 ± 0.22	0.59	22.27
M. azedarach	PE	113.7	71.6	151.4	1.50 ± 0.22	0.11	17.14
	EA	155.5	111.4	195.8	1.30 ± 0.21	1.16	18.28
	Et	165.0	119.7	206.7	1.55 ± 0.21	0.18	11.80
	W	169.9	122.6	213.4	1.50 ± 0.21	0.11	11.47
	Oil	123.8	83.9	159.6	1.63 ± 0.23	3.51	15.73
M. oleifera	PE	127.1	86.1	164.0	1.59 ± 0.22	0.43	15.32
	EA	155.5	111.4	195.8	1.56 ± 0.21	0.26	12.52
	Et	176.8	126.1	223.7	1.41 ± 0.20	0.06	11.02
	W	200.5	152.9	245.9	1.57 ± 0.20	0.21	9.72
	Oil	165.7	114.7	212.3	1.38 ± 0.21	0.73	11.76

[a]The median concentration that inhibits 50% of egg hatchability.
[b]Slope ± Standard Error.
[c]Chi Square.

nanoparticles significantly increased their nematicidal activity compared crude extracts. The nano-formulations of PE of *C. dioscoridis*, *M. azedarach*, and *M. oleifera* were highly efficient in inhibiting the egg hatchability of root-knot nematode. The LC_{50} values were 95.4, 113.7, and 127.1 mg/ml, respectively. Also, the Ag-essential oil nanoparticles of *C. dioscoridis*, *M. azedarach*, and *M. oleifera* had more nematicidal activity compared to that of EA, EtOH, and W Ag-nanoparticles. The results revealed that Ag-rugby and rugby were the most efficient compounds in reducing the hatching of eggs of *M. incognita* with LC_{50} values of 19.4 and 41.4 µg/ml, respectively. It was obvious that plant extracts and their corresponding Ag-NPs of *C. dioscoridis* were more effective as nematicides compared to other extracts.

GC-MS analysis of extracts of C. dioscoridis and their Ag-nano formulations

The phytochemical contents of PE, EA, and EO extracts of *C. dioscoridis* were identified using the GC-MS analysis because of their high potential potency against eggs and J2 of *M. incognita*. Moreover, their nematicidal activities were comparable to or equal to that of rugby, the reference nematicide. The chemical compounds from the GC-MS analysis were identified with NIST and Willey database libraries and re-confirmed by the online search on the PubChem, ChemSpider, and the Chinese chemical online databases. Data presented in Figure 2 and Tables 5–7 clarified the compounds that were identified in the Ag-NP nano-formulations, in the crude extract (NP), and folds of difference of compounds in Ag-NPs to the NP.

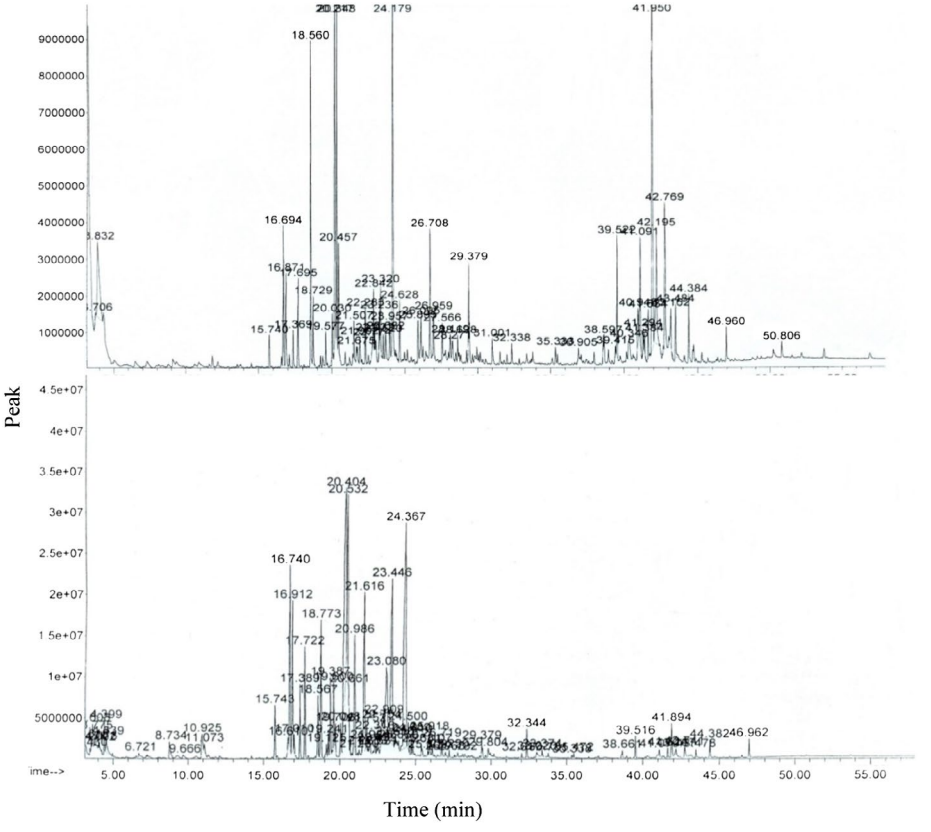

Figure 2. An example chromatogram: gas chromatography – mass spectrometry analysis of (A) petroleum ether extract of *C. dioscoridis* and (B) silver nano formulation of petroleum ether extract.

About 83.82% of the detected compounds in the PE-Ag-NPs were reported in Table 5. The 8S-driman-11-al (15.62%), naphthalene-hexahydro-4,7-dimethyl-1-(1-methylethyl) (14.09%), 2',2'-dimethyl-6'-methylene-1'-cyclohexylcarbaldehyde (5.90%), 4-hexylacetophenone (5.26%), and elemol (3.88%) were the major compounds detected in the Ag-NPs and not found in the NP. Also, the major compounds reported in both Ag-NPs and NP with folds of increase in favour to Ag-NPs were 1-hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene (9.60 folds), *t*-cadinol (5.29 folds), alloaromadendrene (3.37 folds), β-isocomene (2.56 folds), α-selinene (2.53 folds), berkheyaradulene (1.87 folds), bicyclo[4.4.0] dec-1-ene-2-isopropyl-5-methyl-9-methylene (1.71 folds), caryophyllene (1.70 folds), and phytol (1.65 folds).

Results in Table 6 showed the GC-MS analysis of the ethyl acetate (EA) extract and its Ag-nanoparticle of *C. dioscoridis*. The major metabolites were 2-(3-butenyl)cyclopentanone (11.74%), 4-hexylacetophenone (10.43%), *t*-cadinol (6.73%), endo-1-bourbonanol (5.68%), spiro[1,2-dihydro-1-oxoacenaphthylene-2,7',8'-oxabicyclo-[4.2.0]-octane (5.23%), elemol (3.03%), selina-4,11-diene (2.52%), and

Table 5. Gas chromatographic-mass spectrometry (GC-MS) analysis of petroleum ether extracts of leaves and their corresponding silver nanoparticle formulations of *C. dioscoridis*.

Chemical name	Area (%)		Ratio (Ag-NP:NP)
	Ag-NP*	NP	
t-Cadinol	8.52	1.61	5.29
1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecdiene	6.16	0.64	9.62
Alloaromadendrene	4.05	1.20	3.37
Berkheyaradulene	3.70	1.98	1.87
Bicyclo[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene	3.48	2.04	1.71
Caryophyllene	2.86	1.68	1.70
α-Selinene	1.62	0.64	2.53
β-Isocomene	1.79	0.70	2.56
α-Humulene	1.66	6.06	0.27
Bis(2-ethylhexyl)phthalate	0.51	2.18	0.23
Squalene	0.15	1.09	0.14
4-(2′-Ethyl-5′-phenyl-pyrrol-3′-yl) pyridine	0.26	0.96	0.27
Heptacosane	0.24	0.91	0.26
Phytol	0.66	0.40	1.65
Tetracosane	0.44	0.63	0.69
Docosane	0.19	1.38	0.14
m-Xylene	0.59	5.30	0.11
8S-Driman-11-al	15.62	–	–
Naphthalene-hexahydro-4,7-dimethyl-1-(1-methylethyl)	14.09	–	–
2′,2′–Dimethyl-6′-methylene-1′-cyclohexylcarbaldehyde	5.90	–	–
4-Hexylacetophenone	5.26	–	–
Elemol	3.88	–	–
Naphthalene-decahydro-4a-methyl-1-methelene-7-(1-methylethenyl)	2.07	–	–
Naphthalene-1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)	1.47	–	–
8,9-dihydrocycloheptaphenalene-6-one	–	11.66	–
Hexenyl cyclopentanone	–	10.35	–
Cyclohexanol-3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)	–	9.13	–
6-Epi-shyobunol	–	5.89	–
N-(6-Aminosulfonyl-2-benzothiazolyl-3-chlorobenzamide	–	4.22	–
Alcyopterosin B	–	3.98	–
Longifolenaldehyde	–	2.86	–
4-(2′,4′,4′-trimethyl-yciclo[4.1.0]hept-2′-en-3′-yl)-3-buten-2-one	–	2.66	–
3-Methyl-5-methylene-2-thiazolidinone-1-[2-pyridylethylidene] hydrazone	–	2.32	–
3,4-Epoxy-6-methyl-6-(3′-isopropenyl-1′-cyclopropen-1′-yl)-2-heptanone	–	1.30	–
2,4-Bisphenol	–	1.10	–
12-Oxabicyclo[9.1.0]dodeca-3,7-diene,1,5,5,8-tetramethyl	–	1.08	–
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,8,8a-octahydro-naphthalen-2-ol	–	1.03	–
Geranyl-α-terpinene	–	1.01	–

*Ag-NP; nano-silver with natural products formulation, NP; natural products extract.

β-selinene (2.33%). The compounds that were increased in the Ag-nano formulation were alloaromadendrene (6.08 folds), β-isocomene (3.27 folds), cyclohexanol-3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethenyl) (2.93 folds), α-cadinol (2.78 folds), berkheyaradulene (2.64 folds), α-selinene (2.60 folds), caryophyllene (2.58 folds), and 6-epi-shyobunol (2.44 folds).

Also, the GC-MS analysis of essential oil of *C. dioscoridis* was presented in Table 7. Several compounds were found in great abundance in the Ag-nanoparticles of the essential oil; dihydro-1H,5H-dipyrrolo[1,2a:1,2d]

Table 6. Gas chromatographic-mass spectrometry (GC-MS) analysis of ethyl acetate extracts of leaves and their corresponding silver nanoparticle formulations of *C. dioscoridis*.

	Area (%)		
Chemical name	Ag-NP*	NP	Ratio (Ag-NP/NP)
Cyclohexanol-3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)	17.42	5.94	2.93
6-Epi-shyobunol	8.41	3.45	2.44
Berkheyaradulene	6.66	2.52	2.64
Alloaromadendrene	6.02	0.99	6.08
Caryophyllene	4.49	1.74	2.58
β-Isocomene	2.85	0.87	3.27
α-Humulene	2.27	4.20	0.54
α-Cadinol	2.25	0.81	2.78
α-Selinene	1.95	0.75	2.60
2-(3- Butenyl) cyclopentanone	11.74	–	–
4-Hexylacetophenone	10.43	–	–
t-Cadinol	6.73		
Endo-1-bourbonanol	5.68	–	–
Spiro[1,2-Dihydro-1-oxoacenaphthylene-2,7′-8′-oxabicyclo-[4,2.0]-octane]	5.23	–	–
Elemol	3.03	–	–
Selina-4,11-diene	2.52	–	–
β-Selinene	2.33	–	–
Buta-1,3-diyne-1,4-bis-(2-methoxycarbonyl cyclopropyl)	–	8.85	–
Hexenyl cyclopentanone		6.56	
1-Butyl-1-tosyl-2-methylene cyclopropane	–	4.08	–
Hexadecanoic acid	–	4.08	–
1,2-Dihydro-11-β-hydroxy-α-santonin	–	3.90	–
Squalene	–	2.83	–
Benzene-1,3-dimethyl	–	2.78	–
Benzene, 1,3-bis (1,1-dimethylethyl)-5-methyl	–	2.94	–
(+-) – Alloaromadendrene2–4. α- α diol	–	2.22	–
Bis-(2-ethylhexyl)-phthalate	–	2.10	–
9-methoxy-11H-benzofluorene	–	1.77	–
Nanocosane	–	1.77	–
9,12-octadecadienoic acid	–	1.73	–
1-Octadecene	–	1.24	–
Phytol		1.23	
Heptacosane	–	1.19	–
Docosane	–	1.15	–
6-Hydroxyethylbicyclo-[3.2.0]-heptan-2-one	–	1.09	–

*Ag-NP; nano-silver with natural products formulation, NP; natural products extract.

pyrazine-3,5,8,10-(2H,5ah,10ah)-tetraone (15.47%), 2,2-dimethyl-2H-1-benzopyran-6-carboxylic acid (7.65%), and ledene (5.11%). The major compounds that were increased in the Ag-essential oil compared to the essential oil were 6-epi-shyobunol (3.53 folds), cyclohexanol-3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethenyl) (1.78 folds), and bicyclo-[4.4.0]-dec-1-ene-2-isopropyl-5-methyl-9-methylene (1.68 folds).

Discussions

Confirmation and characterisation studies of the Ag nanoparticles were done in previous studies using UV-spectrometry and SEM (Naheed et al. 2011; Ramteke et al. 2013; Nassar 2016). The UV–vis spectra showed λ_{max} peaks at about

Table 7. Gas chromatographic-mass spectrometry (GC-MS) analysis of essential oil extract of leaves and their corresponding silver nanoparticle formulations of *C. dioscoridis*.

Chemical name	Area (%)		Ratio (Ag-NP/NP)
	Ag-NP*	NP	
Cyclohexanol-3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl); syn.: Elema-1,3-dien-6 α-ol; Shyobunol	21.90	12.28	1.78
6-Epi-shyobunol	13.62	3.86	3.53
α-Cadinol	7.65	8.24	0.92
Berkheyaradulene	5.23	5.47	0.96
1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene; syn.: Germacren D-4-ol	4.57	6.68	0.68
Alloaromadendrene	3.18	5.65	0.56
t-Caryophyllene	2.92	3.82	0.76
Bicyclo-[4.4.0]-dec-1-ene-2-isopropyl-5-methyl-9-methylene	2.44	1.45	1.68
β-Isocomene	2.04	2.37	0.86
Naphthalene-decahydro-4a-methyl-1-methylene-7-(1-methylethylidene); syn.: γ-Selinene	1.89	2.57	0.74
α-Humulene	1.67	2.07	0.81
α-Selinene	0.64	3.75	0.17
γ-Muurolene	0.32	0.41	0.78
Elemol	0.26	5.04	0.05
Dihydro 1H,5H-dipyrrolo-[1,2-a:1,2-d]-pyrazine-3,5,8,10- tetraone	15.47	–	–
2,2-dimethyl-2H-1-benzopyran-6-carboxylic acid	7.65	–	–
Ledene	5.11	–	–
2-Cyclohexen-1ol, 2-methyl-5-(1-methylethenyl)-; syn.: cis-Carveol	–	12.28	–
2-(1-Hydroxy-2-methylpropyl)-5- butylphenol	–	7.76	–
Santolina triene; syn.: 3-ethenyl-2,5-dimethylhexa-1,4-diene	–	3.64	–
(3S,5R)-3-(hydroxymethyl)-1,4,4,5-tetramethylcyclopentene	–	1.63	–
1,5-heptadien-4-ol,3,3,6-trimethyl-2-pyrrolidinone; Isoartemisia ketone	–	1.22	

*Ag-NP; nano-silver with natural products formulation, NP; natural products extract.

410 ± 10 nm, the occurrence of this peak is due to the phenomenon of surface Plasmon resonance, which occurs due to the excitation of the surface Plasmon's on the outer surface of the silver nanoparticles that get excited due to applied electromagnetic field (Naheed et al. 2011). In the literature, it was reported that the UV absorption peaks of silver nanoparticles were ranged from 410 to 450 nm (Ramteke et al. 2013). The SEM analysis illustrated that the size of nanoparticles is 30–100 nm for all tested extracts and the reference nematicide (Ag-rugby). Similar results of the size and shape of silver nanoparticles were reported for ethanol, ethyl acetate, and petroleum ether extracts of *Urtica urens* (Nassar 2016).

The green synthesis of natural products into the Ag-nanoparticles increased the activity up to 5 times against the J2 and 2 times against the eggs of *M. incognita*. Such findings highlighted the importance of the use of natural products to be incorporated into the nano-technology to find safe nematicide alternatives. Results reported herein emphasised the potential application of Ag-NPs as alternatives or synergic to synthetic nematicides. The preparation of Ag-NP of plant secondary metabolites was reported in current study and others to be rapid, applicable in large-scale, size and shape-controlled, and effective process and produced formulations with no phytotoxic effects (Gardea-Torresdey et al. 2003; Sastry et al. 2004; Ganesan et al. 2013). Moreover, silver nanoparticles have distinct properties such

as being chemically stable, catalytic activity, and antimicrobial activity (Chen et al. 2007; Li et al. 2007; Setua et al. 2007).

Results reported herein about the increased potency of the Ag-NP nanoparticles 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 great concentrations in the Ag-NP nanoparticle. It was reported that the terpenoids and their nanoparticles had antimicrobial, anthelmintic, and anticancer activities (Cavar et al. 2012; Chizzola 2013; Elshamy et al. 2015). The sesquiterpenoids were found to have insecticidal effects (Ebadollahi 2013; Kreuzwieser et al. 2014; Piesik and Wenda-Piesik 2015).

Plant secondary metabolites that were identified in the current study included hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols, and phenol ethers) (Guenther 1972). Those component changes due to oxidation, isomerization, cyclization, or dehydrogenation reactions because of the presence of unsaturated chemical bonds and active groups (Jukic and Milos 2005; Scott 2005; Jha et al. 2009) and the antioxidant activity of the alkaloids, flavonoids, phenols, and tannins and they possess anionic radicals that are responsible for the reduction and stability of silver salts into metallic nanoparticles (Mukunthan and Balaji 2012; Awwad et al. 2013). Since almost all identified metabolites in the Ag-NP nanoparticle of extractives of *C. dioscoridis* were alcohols, aldehydes, ketones, phenols, unsaturated alkenes and alkynes, there was a great chance for most of the chemical reactions to occur. Based on that, the increased activity of Ag-nanoparticles might be due to chemical conversion, the presence of the silver mineral, and the increased cellular uptake (Rahimi et al. 2016).

Therefore, the herbal plants are good sources for finding phytochemical bionematicides that might have a great impact in the organic agriculture systems. Moreover, their biological activity could be increased through their preparation in the nano-sized formulations. More importantly, more research must be conducted to investigate (1) the chemical mechanism behind the increased activity of Ag-nanoparticles compared to the plant crude extractives, (2) the kinetics and chemical conversion of secondary metabolites in the nano-form, and (3) the methods that might increase the stability and nematicidal activity of the Ag-nano-formulations.

Conclusions

The plants' secondary metabolites have been used in the formulation of nanoparticles to increase their effectiveness against plant diseases. Green synthesis of silver nanoparticles (natural biopesticides) is cost-effective, safe, non-toxic, eco-friendly

route of synthesis, and could be manufactured at a large scale. Extractives of *Conyza dioscoridis*, *Melia azedarach*, and *Moringa oleifera* showed great capability to be synthesised in AgNP formulations. The AgNPs showed nematicidal activity against root-knot nematode *M. incognita*. Specifically, the AgNPs of *C. dioscoridis* exerted great potential in inhibiting both stages; eggs and J2 of *M. incognita*. The increased activity of the nano-formulations might be a result of the elevated content of certain metabolites, for example, epi-shyobunol, aromdendrene, α- and *t*-cadinol, caryophyllene, α-humulone, β-isocomene, and α- and β-selinene. However, more studies need to be performed to isolate specific secondary metabolites and prepare them in the nano-form, which are safe, potent, environmentally friendly and has low side effects to mammals.

Author contribution statement

MA and MA designed and revised the writing of the manuscript, AN designed and conducted the experiments and wrote the manuscript, and BS conducted the experiments. All authors read and approved the manuscript.

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Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Atef M. K. Nassar http://orcid.org/0000-0002-0394-1530

References

Abdul Hameed MA. 2012. Nanoparticles as an alternative to pesticides in management plant diseases - a Review. Int J Sci Res Publ. 2:1–4.

Ajayi VA. 1990. Comparison of Nematicidal potential of *Vernonia amygdalina* (Bitter leaf) and carbofuran (Furadan) on the growth and yield of root-knot infested soybean *Glycine max* [L. M.Sc. Dissertation]. Nigeria: Department of Agric. Biology, University of Ibadan; p. 283.

Aralepo OE. 1989. Nematicidal potentials of pirimiphos-methyl and leaf extracts of *Ocimum gratissimum* in the control of *Meloidogyne incognita* on cowpea [M.Sc. Thesis]. Nigeria: Department of Agric. Biology, University of Ibadan; p. 205.

Awwad AM, Salem NM, Abdeen AO. 2013. Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. Int J Ind Chem. 4:29–35.
Balbaa EME. 2010 Biological and environmental studies on root-knot nematode affecting Leguminosae [M.Sc. Thesis]. Egypt: Faculty of Agriculture, Alexandria University; p. 50.
Browning M, Wallace DB, Dawson C, Alm SR, Amador JA. 2006. Potential of butyric acid for control of soil-borne fungal pathogens and nematodes affecting strawberries. Soil Biol Biochem. 38:401–404.
Cavar S, Maksimovica M, Vidica D, Paric A. 2012. Chemical composition and antioxidant and antimicrobial activity of essential oil of *Artemisia annua* L. from Bosnia. Ind Crops Prod. 37:479–485.
Chen M, Feng Y-G, Wang X, Li T-C, Zhang J-Y, Qian D-J. 2007. Silver nanoparticles capped by oleylamine: formation, growth, and self-organization. Langmuir. 23:5296–5304.
Chizzola R. 2013. Regular Monoterpenes and Sesquiterpenes (Essential Oils). In: Ramawat KG, Merillon JM editors. Natural products: phytochemistry, botany and metabolism of alkaloids, phenolics and terpenes. Chapter 97. Springer-Verlag Berlin Heidelberg. doi:10.1007/978-3-642-22144-6_183; p. 2992–2998
Claudius-Cole AO, Aminu AE, Fawole B. 2010. Evaluation of plant extracts in the management of root-knot nematode *Meloidogyne incognita* on cowpea (*Vigna unguiculata* (L) Walp). Mycopathology. 2:53–60.
Ebadollahi A. 2013. Plant essential oils from Apiaceae family as alternatives to conventional insecticides. Ecologia Balkanica. 5:149–172.
Elshamy A, El Gendy AG, Farrag AH, Nassar M. 2015. Antidiabetic and antioxidant activities of phenolic extracts of *Conyza dioscoridis* L. shoots. Int J Pharm Pharm Sci. 7:65–72.
Fernandez C, Rodriguez-Kabana R, Warrior P, Kloepper JW. 2001. Induced soil suppressiveness to a root-knot nematode species by a nematicide. Biol Control. 22:103–114.
Finney DJ. 1971. Probit analysis. Cambridge: Cambridge Univ. Press; p. 333.
Ganesan V, Astalakshmi A, Nima P, Arunkumar C. 2013. Synthesis and characterization of silver nanoparticles using *Merremia tridentata* (L.). Int J Curr Sci. 6:87–93.
Gardea-Torresdey JL, Gomez E, Peralta-Videa JR, Parsons JG, Troiani H, Yacaman MJ. 2003. Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. Langmuir. 19:1357–1361.
Guenther E. 1952. Essential oils (volume 3) individual essential oils of the plant families Rutaceae and Labiatae. 2nd ed. New York (NY): Van Nostrand Company.
Guenther E. 1972. The essential oils. Malabar (FL): Krieger Publishing Company.
Hartman KM, Sasser JN. 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In: Barker KR, Carter CC, Sasser JN, editors. An advanced treatise on Meloidogyne. vol 2. Methodology, Raleigh, NC: North Carolina State University Graphics; p. 69–77.
Hussey RS, Barker KR. 1973. A comparison of methods of collecting inocula of Meloidogyne spp. including a new technique. Plant Dis Rep. 57:1925–1928.
Jahn SA. 1989. *Moringa oleifera* for food and water purification – selection of clones and growing of annual short stems. Entwicklung Landlicher Raum. 23:22–25.
Jepson SB. 1987. Identification of root-knot nematodes *Meloidogyne* spp. Wallingford: CAB International; p. 265.
Jha AK, Prasad K, Kumar V, Prasad K. 2009. Biosynthesis of silver nanoparticles using Eclipta leaf. Biotechnol Prog. 25:1475–1477.
Jukic A, Milos M. 2005. Catalytic oxidation and antioxidant properties of thyme essential oils (*Thymus vulgarae* L.). Croatica Chemica Acta. 78:105–110.
Khan Z, Kim YH. 2007. A review on the role of predatory soil nematodes in the biological control of plant-parasitic nematodes. Appl Soil Ecol. 35:370–379.

Khan MR, Khan MW, Khan AA. 1996. Effect of *Meloidogyne incognita* on dry weight, root gall and root nodulation of chickpea and cowpea cultivars. Test Agrochem Cult. 17:70–71.
Khan IA, Sayed M, Shaukat SS, Handoo ZA. 2008. Efficacy of four plant extracts on nematodes associated with papaya in Sindh, Pakistan. Nemato Medit. 36:93–98.
Kreuzwieser J, Scheerer U, Kruse J, Burzlaff T, Honsel A, Alfarraj S, Georgiev P, Schnitzler J-P, Ghirardo A, Kreuzer I, et al. 2014. The Venus flytrap attracts insects by the release of volatile organic compounds. J Exp Bot. 65:755–766. doi:10.1093/jxb/ert455.
Li S, Shen Y, Xie A, Yu X, Qiu L, Zhang L, Zhang Q. 2007. Green synthesis of silver nanoparticles using *Capsicum annum* L. extract. Green Chem. 9:852–858.
McSorley R. 2002. Losses to nematodes. In: Encyclopedia of pest management, Pimentel, D. editor. Boca Raton (FL): CRC Press, ISBN: 9781439870587; p. 392–394.
Meher HC, Walia S, Sethi CL. 1988. Effect of steroidal and triterpenic saponins on the mobility of juveniles of *Meloidogyne incognita*. Indian J Nematol. 18:244–247.
Mukunthan KS, Balaji S. 2012. Cashew apple juice (*Anacardium occidentale* L.) Speeds up the synthesis of silver nanoparticles. Int J Green Nanotechnol. 4:71–79. doi:10.1080/19430892.2012.676900.
Naheed A, Seema S, Singh VN, Shamsi SF, Fatma A, Mehta BR. 2011. Biosynthesis of silver nanoparticles from *Desmodium triflorum*: A novel approach towards weed. Biotech Res Int, ID. 454090:8.
Nassar AMK. 2016. Effectiveness of silver nano-particles of extracts of urtica urens (Urticaceae) against root-knot nematode Meloidogyne incognita. Asian J Nematol. 5:14–19.
Nicol JM, Turner SJ, Coyne DL. 2011. Current nematode threats to world agriculture. In: Jones J, Gheysen G, Fenoll C, editors. Genomics and molecular genetics of plant-nematode interactions. Berlin: Springer; p. 21–43 ISBN 978-94-007-0433-6 pp.
Okada H, Harada H. 2007. Effects of tillage and fertilizer on nematode communities in a Japanese soybean field. Appl Soil Ecol. 35:582–598.
Piesik W, Wenda-Piesik A. 2015. *Sitophilus granarius* responses to blends of five groups of cereal kernels and one group of plant volatiles. J Stored Prod Res. 63:63–66.
Prasad TNVKV, Elumalai EK. 2011. Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity. Asian Pac J Trop Biomed. 1:439–442.
Rahimi HR, Nedaeinia R, Shamloo AS, Nikdoust S, Oskuee RK. 2016. Novel delivery system for natural products: Nano-curcumin formulations. Avi J Phytomed. 6:383–398.
Rajesh WR, Jaya RL, Niranjan SK, Vijay DM, Sahelebrao BK. 2009. Phyto synthesis of silver nanoparticles using *Gliricidia sepium* (Jaeq). Curr Nano Sci. 5:117–122.
Ramteke C, Chakrabarti T, Sarangi BK, Pandey RM. 2013. Synthesis of silver nanoparticles from the aqueous extract of leaves of *Ocimum sanctum* for enhanced antibacterial activity. J Chem. 278925:7.
Roberts PA, Matthews JR, Ehler JD. 2005. Root-knot nematode resistant cowpea cover crops in tomato production systems. Agronomy J. 97:1626–1635.
Rodriguez E. 1988. Dithiopolyacetylenes as potential pesticides. In: Cutler HG editor. Biologically active natural products: potential use in agriculture, 2nd Ed., London: The Society; p. 432–437. ISBN: 9780841215566.
Salawu EO. 1992. Effect of neem leaf extract and ethoprop singly and in combination on *Meloidogyne incognita* and growth of sugarcane. Pak J Nematol. 10:51–56.
Sastry M, Ahmad A, Khan MI, Kumar R. 2004. Microbial nanoparticles production in nanobiotechnology. In: Niemeyer CM and Mirkin CA, editor. Weinheim: Wiley-VCH; p. 126.
Scott RPW. 2005. Essential oils. In: Worsfold P, Townshend A, Poole C, editors. Encyclopedia of analytical science. 2nd ed. London: Elsevier; p. 554–561.

Setua P, Chakraborty A, Seth D, Bhatta MU, Satyam,PV, Sarkar, N. 2007. Synthesis, optical properties, and surface enhanced raman scattering of silver nanoparticles in nonaqueous methanol reverse Micelles. J Phys Chem C. 111:3901–3907.

Sikora RA, Schafer K, Dababat AA. 2007. Modes of action associated with microbially induced in planta suppression of plant-parasitic nematodes. Australas Plant Pathol. 36:124–134.

Soler-Serratosa A, Kokalis-Burelle N, Rodriguez-Kabana R, Weaver CF, King PS. 1996. Allelochemicals for control of plant-parasitic nematodes. 1. *In vivo* nematicidal efficacy of thymol and thymol/benzaldehyde combinations. Nematropica. 26:57–71.

Thoden TC, Hallmann J, Boppre M. 2009. Effects of plants containing pyrrolizidine alkaloids on the northern root-knot nematode Meloidogyne hapla. Eur J Plant Pathol. 123:27–36.

Wang KH, Hooks CR, Ploeg A 2007 Protecting crops from nematode pests: using marigold as an alternative to chemical nematicides. Cooperative Extension Service, College of Tropical Agriculture and Human Resources [Plant Dis]. University of Hawaii at Manoa, p. 1–6.

Williamson VM, Kumar A. 2006. Nematode resistance in plants: the battle underground. Trends Genet. 22:396–403.

Youssef MMA, El-Nagdi WMA, Eissa MFM. 2014. Population density of root-knot nematode, *Meloidogyne incognita* infecting date palm under stress of aqueous extracts of some botanicals and a commercial bacterial byproduct. Middle East J Appl Sci. 4:802–805.