University of Sadat City,
Environmental Studies and Research Institute
The4Th International Conference
Environmental for Studies and Research Smart Sustainable Environment

Journal of Environmental Studies and Researches (2017), 7(2-D):832 -844

EFFICACY OF SILVER NANOPARTICLES OF EXTRACTIVES OF *ARTEMISIA JUDAICA* AGAINST ROOT-KNOT NEMATODE

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ABSTRACT

Green synthesis of the nanoparticles is a novel technology that effectively uses the plants' crude extracts as safe and eco-friendly pesticide alternatives. The current study investigated the nematicidal activity of leave extracts of *Artemisia judaica* and their silver nanoparticles (Ag-NP). Characterization of the synthesized nanoparticles was done using the UV-Vis spectrophotometry and the scanning electron microscopy (SEM). Furthermore, the phytochemical contents of extracts and the Ag-NP formulations were characterized by the gas chromatography-mass spectroscopy (GC-MS). The results revealed that Ag-NP formulations were more toxic to the second juvenile (J2) of *Meloidogyne incognita* than the corresponding crude extracts. The inhibitory effects of all extractives on the egg and larval stages of the nematode were concentration dependent. The plant extracts synthesized in the form of silver nanoparticle showed comparable nematicidal activity against *M. incognita* to the recommended nematicide; rugby. The GC-MS analysis revealed the increase of certain secondary metabolites in the Ag-nano formulation, such as 4-(2',4',4'-trimethyl-yciclo[4.1.0]hept-2'-en-3'-yl)-3-buten-2-one, berkheyaradulene, caryophyllene, humulene, and allooaromadendrene were increased more than 20-30 folds. Silver nanoparticles of natural extracts might be considered as a suitable methodology to produce safe, effective and affordable nematicide alternatives.

Keywords: *Artemisia judaica*, SEM, GC-MS, nematicide alternatives, *Meloidogyne incognita*

INTRODUCTION:

Plant parasitic nematodes cause significant damage for almost all crops. They infect plant roots, bulbs, rhizomes, stems, leaves, buds, flowers, seeds etc. and cause losses to the plants directly or indirectly. Root-knot nematodes, *Meloidogyne* spp., are the most pathogenic specie of nematodes to most crops, which affecting the quantity and quality of yield in many annual and perennial crops and could cause up to 64% yield reduction (Khan *et al.*, 1996; Agrios, 2005). The population of plant-parasitic nematodes in the field can be decreased through several procedures such as enhancing the cultural practices (Okada and Harada, 2007), utilizing natural enemies (Khan and Kim, 2007), cultivating resistant cultivars (Williamson and Kumar, 2006), pesticides (Browning *et al*., 2006), nano-elements with natural products (Oka, 2001; Li et al. 2007; Nassar, 2016).

Plant natural products are considered a rich source to find effective control components against pathogens. Artemisia (*Artemisia judaica* L., Asteraceae) grows in the wild in Sinai Peninsula, Egypt. Its Arabic name is "Shih" and has a good prominence among herb experts in Egypt as a medicinal herb (Tackholm, 1974). Natural extractives of *A. judaica* were reported to be antimalarial, anti-inflammatory, and antibacterial (Saban *et al*., 2005) and as plant growth regulator and antitumor (El-Massry *et al*., 2002). Moreover, the antifeedant and fungicidal properties of *A. judaica* was due to two major constituents; piperitone and *trans*-ethyl cinnamate (Abdelgaleil *et al*., 2008). These components were isolated from the essential oil (EO) and been effective against *Spodoptera littoralis* and several pathogenic fungi (Abdelgaleil *et al*., 2008). The EOs of *A. judaica* caused immobilization of the second juvenile stage of *Meloidogyne javanica* (Oka et al., 2001).

Synthesis of nanoparticles (size range of 1-100 nm) of minerals with natural products would increase the biological activity (Sharma et al., 2009). The nanoparticles display unique physical, chemical, catalytic, thermal, and biological properties due to their tiny sizes that would cover a higher surface area. Therefore, they exert high activity against devastating microbes (Nie and Emory, 1997; Ye *et al*., 1999). The frugally of the synthesis of metal nanoparticles of plant extracts made them an enormous source to identify alternatives to synthetic pesticides. For example, nanoparticles of silver with the plants' secondary metabolites have proven to have increased efficacy against the plant disease vectors (Gardea-Torresdey *et al.*, 2003; Ganesan *et al.*, 2013). The nanoformulation would be synthesized by physical, chemical, and biological methods. Specifically, the biogenic technique is eco-friendly, clean, non-toxic and economically applicable (Sastry *et al.*, 2004; Ganesan *et al.*, 2013). The silver nanoparticles of natural extracts of leaves of *Urtica urens* were reported to suppress egg hatchability and immobilization of the 2nd juvenile stage of *M. incognita* (Nassar, 2016).

Therefore, the effectiveness of plant extracts as nematicides could be increased through their biotransformation into metal nanoparticles. Thus, the present study was designed to evaluate the effectiveness of silver-nano-formulations of natural products as nematicide alternatives against root-knot nematode (*M. incognita*; Kofoid and White). The nano-formulations were synthesized via the atomization of the silver element and extractives (petroleum ether, ethyl acetate, ethanol, water, and essential oil) of leaves of *Artemisia judaica*.

MATERIALS AND METHODS

CHEMICALS

The pesticide Rugby 20% SC (Cadusafos: an insecticide and nematicide) was used as a reference nematicide and was purchased from FMC Corporation, Agriculture Chemical Group. 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

Artemisia. judaica was collected from Marsa Matrouh Governorate. The plant was identified and confirmed with the help of Plant Pathology Department, Faculty of Agriculture (El-Shatby), Alexandria University.

Preparation of Plant Extractives

The aerial parts of *A. judaica* plants were washed and air-dried at room temperature, then in an oven at 50^{o} C until complete dryness. Leaves were ground to a fine powder and a 100 g of the 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 35º C. Extractives were stored at -20 C till been used in the bioassay tests.

Preparation of aqueous extract was done following the method of Claudius-Cole *et al.*, (2010). About 100 g powder of dried leaves 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 at room temperature and filtered through Whitman filter paper No.1. The filtrate was used as the crude extract. The essential oil of *A. judaica* was obtained by steam distillation using a Clevenger trap apparatus (Gunther, 1952).

SYNTHESIS OF SILVER-NATURAL PRODUCTS (AG-NP) NANOPARTICLES.

Silver-natural products nanoparticles (Ag-NP) were synthesized using a modified method of Prasad and Elumalai (2011). Exactly 10 ml of plant extracts (5000 µg ml^{-1}) were mixed with 90 ml of silver nitrate (1mM), 10 ml of ascorbic acid (0.1 M) as a reducing reagent, and polyvinyl pyrrolidine (PVP) as protecting agent in 250 ml conical flask. The mixture was warmed at 40° C on the water bath for 10 min and then tubes were kept in dark place for 24 hours at room temperature. The change in color to reddish-brown was observed and was an indication of the formulation of the Ag-nanoparticles.

Similarly, the synthesis of Ag nanoparticles of Rugby® were prepared by the incubation of 10 ml of Rugby® (20%) with 90 ml of silver nitrate (1mM) and 10 ml ascorbic acid (0.1 M) at 40º C for 12 h. The development of reddish-brown color was an indication that the Ag-Rugby® nano-formulation was synthesized. The tubes were incubated in a dark chamber to minimize photo-degradation. Reduction of Ag^{+} to Ag^{0} was confirmed by the color change of solution from colorless to reddish-brown. Its formation was also confirmed by using UV-Visible spectroscopy and Scanning Electron Microscope (SEM).

CHARACTERIZATION OF SILVER NANOPARTICLES

The produced nanoparticles were studied by UV–Vis spectroscopy. About 1 ml of samples was diluted with 2 ml of double distilled water. The optical density of the solution was recorded from 300 to 700 nm at a 1 nm λ interval using a Jenway spectrophotometer (Model 6305, Bibby Scientific Limited Staffordshire, UK) (Rajesh *et al.,* 2009). The morphology and size of the Ag-NPs were confirmed by the scanning electron microscopy (JEOL, JSM-5300, USA).

Culture of Root-Knot Nematode

The *M. incognita* was isolated from inoculated plants of eggplant and identified using perineal patterns of adult females and the morphology of the 2nd stage juveniles (Hartman and Sasser, 1985; Jepson, 1987). Egg masses were obtained from the eggplant roots, which were incubated in a sodium hypochlorite (NaOCl) solution (Hussey and Barker, 1973) 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 that were collected within 48 h were used for experiments. The J2 and eggs of *M. incognita* were used for the bioassay evaluations.

Assessment of Nematicidal Efficacy of A. judaica Extractives and Ag-Nanoparticles of Extractives .

The nematicidal activity of extracts and their corresponding Ag nanoparticles were evaluated against eggs and the J2 of *M. incognita* under laboratory conditions. Four concentrations: 125, 250, 500 and 1000 µg ml^{-1} of each extract and its nano-formulation were prepared. Each concentration was tested in 4 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% and Ag-rugby nanoparticles were used as reference nematicides. The vials were incubated at 25 ± 2° C. Larval mortality was recorded after 48 h and egg hatchability percentages were observed after 7 d. The experiments of the biological activity of the nanoparticles and plant extractives were repeated four times. The median lethal concentration (LC_{50}) and toxicity index values were calculated using log-dose probit (Ldp) line software (Ehabsoft, Cairo, Egypt).

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS .

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 X 0.25 µm film thickness) was used. About 1 µl of each sample was injected using the helium as carrier gas (36 cm sec^{-1}) and flow 1 ml min^{-1}. The solvent delay time was 3 min. The mass spectrometric detector was operated in an electron impact ionization mode with energy of 70 eV and scanning mode from 50 to 500 m/z. The ion source temperature was 230˚C and the quadruple temperature was 150˚ C. The electron multiplier voltage (EM voltage) was maintained 1250 v above the auto tune. The instrument was manually tuned using perfluorotributylamine (PFTBA). The GC temperature program was started at 60º C for 3 min then elevated to 280º C at a rate of 8˚C min^{-1} and 10 min hold at 280º C. The m/z (mass/charge) ratio obtained was calibrated from the mass spectrum graph, which is the fingerprint of a molecule. Moreover, identification of the separated peaks was done using the Wiley2005 and NIST mass spectral database.

Results

Synthesis of Silver Nanoparticles

Results showed that the synthesis of silver nanoparticles of petroleum ether, ethyl acetate, ethanol, and water extractives of *A. judaica* was achieved and confirmed by the color change. The nanoparticles were further confirmed using the UV–Vis spectroscopy, which is one of the most widely used techniques for structural characterization of nanoparticles (Sun *et al.*, 2002). The UV-Vis spectra of the diluted solution of the nano-formulations showed λ_{max} peaks at about (470 ± 10) nm (Fig 1).

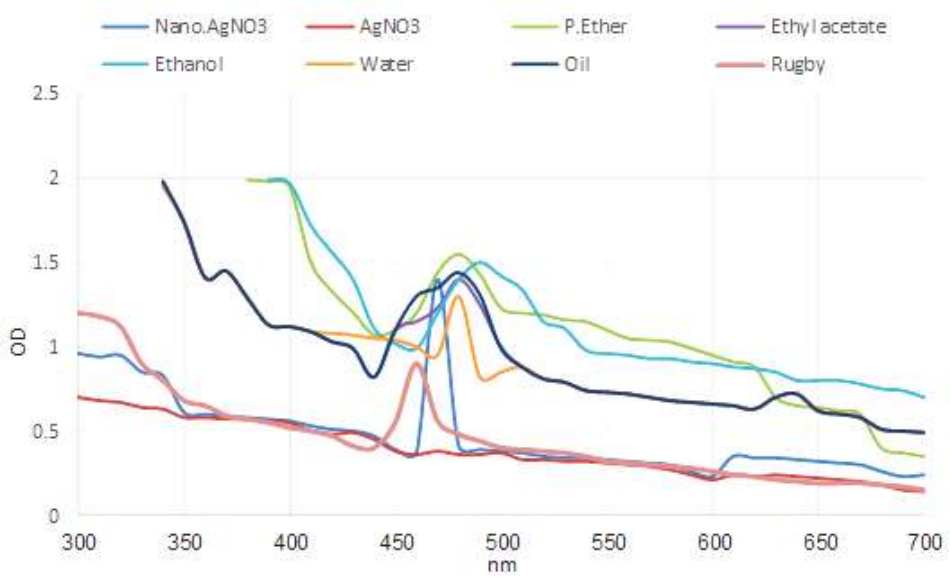

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Fig 1: UV-spectrometry absorption peaks of Ag-nano of extractives of *A. judaica* (P. Ether, Ethyl Acetate, Ethanol, Water, and Oil), the synthesized silver nanoparticles (Nano $AgNO_3$), $AgNO_3$ solution and Rugby nanoparticles (Rugby).

Additionally, the scanning electron microscope (SEM) confirmed the shape and size of the silver nanoparticles of plant extractives. As shown in Fig 2, the pictures of the SEM illustrated that the size of nanoparticles were of 50-150 nm for all tested extracts and the reference nematicide (Ag-rugby).

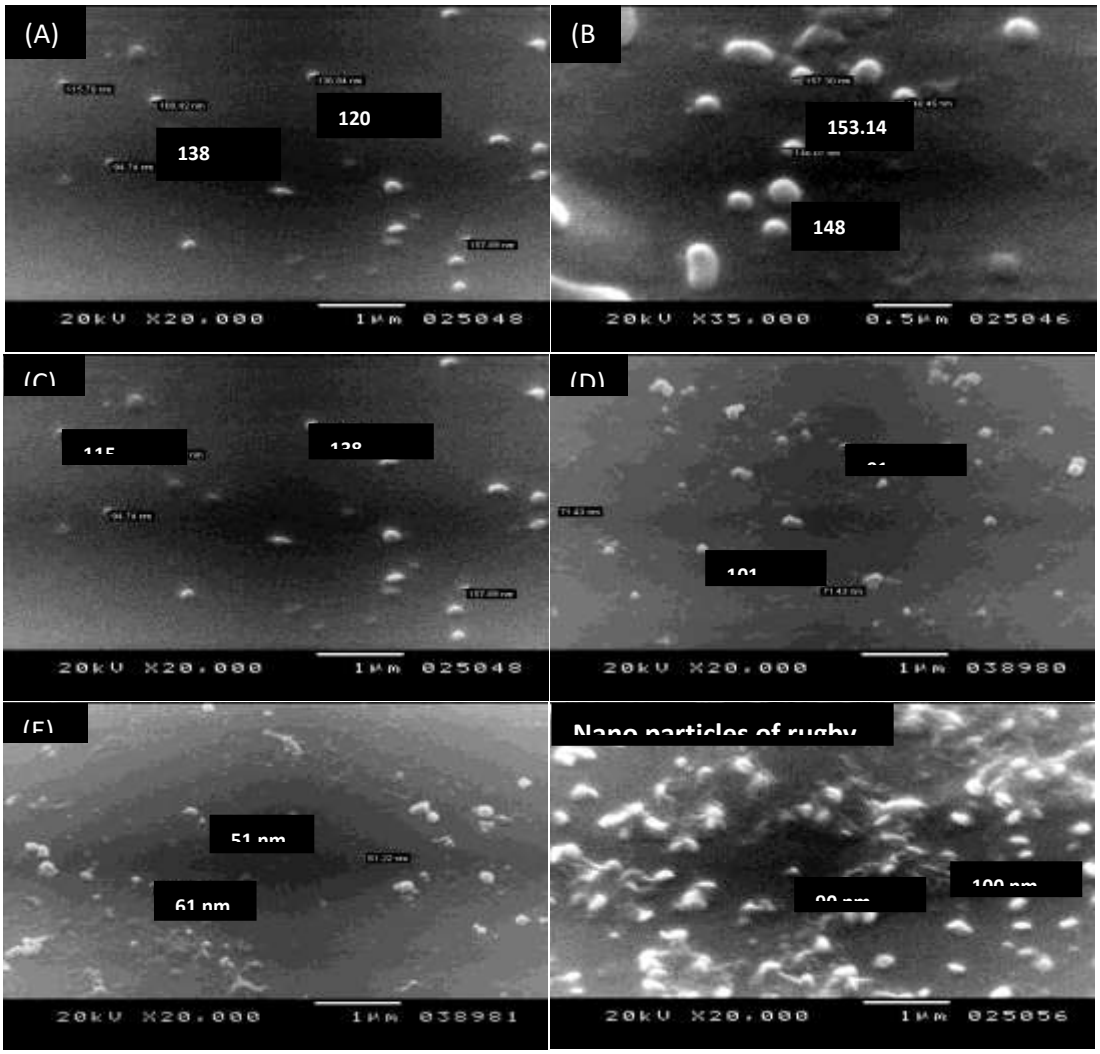

Fig (2): Nanoparticles of petroleum ether (A), ethyl acetate (B), ethanol (C), water extract (D), and essential oil (E) of *A. judaica* and the nano nematicide, Rugby.

Nematicidal Activity of Botanical Extracts and their Ag-Nanoparticles

The results presented in Table 1 show the nematicidal bioactivity of natural extracts of *A. judaica* and their Ag-nano-formulations against root-knot nematode compared to rugby® . The nanoformulations and rugby® were more toxic to the J2 of *M. incognita* than crude extracts. The inhibitory effects of all extractives on nematode activity were concentration dependent. The Ag-rugby® nanoparticles suppressed the nematode activity with an LC_{50} value of 14.39 µg ml^{-1} compared to 25.97 µg ml^{-1} for the rugby®. The Ag-petroleum ether showed the inhibitoriest action against the J2 compared to other extracts but was less than Ag-rugby and rugby. The LC_{50} values of extracts ranged from 93.87 µg ml^{-1} for Ag-petroleum ether to 370.5 µg ml^{-1} for water extract. The preparation of *A. judaica* extractives into Ag-nanoparticles increased their biological activity up to 3-fold.

Table (1) *In vivo* toxicity of normal and silver nano-formulations of extractives of *A. judaica* against the J2 of root-knot nematode *M. incognita*

Treatment	LC_{50}[a]	95% Confidence Limits (µg ml^{-1})		Slope ± SE[b]	Toxicity Index
		Lower	Upper		
Ag-Rugby	14.39	6.99	21.62	1.34±0.21	100
Rugby	25.97	19.56	31.72	2.10±0.25	55.40
Ag-Petroleum ether	93.87	51.52	132.32	1.38±0.22	15.32
Ag-Ethyl acetate	113.42	70.01	152.35	1.44±0.22	12.68
Ag-Essential oil	119.41	70.72	162.99	1.30±0.22	12.05
Ag-Ethanol	119.6	75.32	159.40	1.44±0.21	12.02
Ag-Water	142.4	97.77	182.82	1.51±0.22	10.10
Petroleum ether	312.6	270.85	358.78	2.28±0.22	4.60
Essential oil	334.5	275.46	404.08	1.60±0.22	4.30
Ethanol	357.1	312.31	408.58	2.39±0.22	4.02
Ethyl acetate	359.3	313.30	412.53	2.32±0.22	4.00
Water	370.5	324.27	424.16	2.40±0.21	3.88

[a]The median concentration that kill 50% of J2 stage, [b]Slope ± Standard Error.

Inhibition of Egg Hatchability by Extractives and Ag-nanoparticles of *A. judaica*

The results presented in Table 2 indicated that Ag-nano and crude extracts of *A. judaica* were effective against eggs of *M. incognita*. It was obvious that the nematicide rugby was more effective in reducing the hatchability of the root-knot nematode compared to crude extracts by 4 to 10 folds. The results revealed that Ag-rugby and rugby were the most efficient in reducing the hatching of eggs of *M. incognita* with LC_{50} values of 19.4 and 41.4 µg ml^{-1}, respectively. Ag-nano for petroleum ether, ethyl acetate, volatile oils, ethanol, and the aqueous extracts of *A. judaica* reduced the hatching eggs of *M. incognita* with LC_{50} values of 141.12, 167.73, 215.7, 178.72, 194.33 and 205.33 µg ml^{-1}, respectively compared to the control.

Table (2): *In vivo* toxicity of normal and silver nano-formulations of extractives of *A. judaica* against eggs of root-knot nematode *M. incognita*.

Treatment	LC_{50}[a]	95% Confidence Limits (µg ml^{-1})		Slope ± SE[b]	Toxicity Index
		Lower	Upper		
Ag-Rugby	19.49	13.86	24.24	2.49±0.34	100
Rugby	41.42	36.51	46.37	3.18±0.28	47.05
Ag-Petroleum ether	141.12	101.62	176.9	1.71±0.22	13.81
Ag-Ethyl acetate	167.73	126.16	206.22	1.70±0.21	11.62
Ag-Volatile oil	178.72	132.45	221.82	1.56±0.21	10.90
Ag-Ethanol	194.33	150.79	235.81	1.69±0.21	10.031
Ag-Water	205.33	162.77	246.53	1.76±0.21	9.49

Petroleum ether	357.04	311.93	408.83	2.37±0.21	5.46
Ethanol	369.23	319.34	427.9	2.16±0.21	5.28
Volatile oil	382.75	329.49	446.69	2.07±0.21	5.09
Ethyl acetate	383.72	336.87	438.46	2.46±0.21	5.08
Water	411.79	358.34	476.7	2.25±0.21	4.73

[a]The median concentration that inhibit 50% of egg hatchability, [b]Slope ± Standard Error.

GC-MS Analysis of Extracts of *A. judaica* and their Ag-nano Formulations

GC-MS analysis the petroleum ether extract of *A. judaica* revealed the presence of sixty-seven (67) peaks (Table 3)**.** The spectra of the compounds were verified by National Institute of Standards and Technology (NIST) and Willey libraries. The compounds were identified by the percentage similarity values. They confirmed via the study of classical fragmentation pattern, base peak, and molecular ion peaks of the compounds. The major compounds were found to be 6-octadecanoic acid (peak area = 13.19%), n-hexadecanoic acid (10.94%), 1,3-dimethylbenzene (6.27%), bis(2-ethylhexyl)phthalate (5.35%), and octacosane (5.19%).

The GC-MS analysis of the Ag-petroleum ether nanoparticles of *A. judaica* revealed the presence of twelve (12) peaks (Table 4). The major compounds were found to be 4-trimethyl-yciclo-hept-'-en-3'-yl]-3-buten-2-one (peak area=31.8%), berkheyaradulene (22.47%), aromadendrene (13.29%) and trans-caryophyllene (10.51%).

Table (3): Chemical composition of the petroleum ether extract of *A. judaica* analyzed by GC-MS

Peak No.	Component name	Rt (min)	Area (%)
1	1,3-dimethylbenzene (m-Xylene)	3.81	**6.27**
2	1,4-dimethylbenzene (p-Xylene)	4.25	0.20
3	Undecane	9.09	0.41
4	Dodecane	11.83	0.49
5	alpha-Guaiene	15.73	0.30
6	4-(2',4',4'-trimethyl-yciclo[4.1.0] hept-2'-en-3'-yl)-3-buten-2-one	16.69	1.22
7	Berkheyaradulene	16.88	1.23
8	Tetradecane	17.10	0.38
9	β-isocomene	17.36	0.47
10	β –Caryophyllene	17.69	0.78
11	α – Humulene	18.56	1.52
12	Allooaromadendrene	18.72	0.35
13	Pentadecane	19.57	0.60
14	α-Amorphene	20.03	0.32
15	Cyclohexanol,3-ethenyl-3-methyl-2(1-methylethenyl)-6(1-methylethyl)	20.22	**4.19**
16	β-Elemene	20.36	2.03
17	Germacrene-4-ol	21.50	0.36
18	1-hexadecene	21.74	0.74
19	Phenol,2,5-bis(1,1-dimethylethyl)	21.90	0.82
20	1,4-dimethylcyclohexene-4-carboxaldehyde	22.28	0.33

21	4-methyl-endo, exo-tetracyclo-dodecan-11-syn-ol	22.85	0.58
22	α-cadinol	23.32	0.55
23	5,8-decadien-2-allylcyclohexanone	23.96	0.32
24	Cyclohexanol-3-ethenyl-3-methyl-2-(1-methylethenyl) -6-(1-methylethyl)	24.19	**4.03**
25	2-iso propylidene-3-methylhexa-3,5-dienal	24.64	0.36
26	3-cyclohexene-1-carboxylic acid, 4-methyl-, methyl ester	25.89	0.55
27	α-octadecylene	26.10	0.82
28	Octadecane	26.25	0.41
29	(2R)-3,4-dihydro-3,4-dihydroxy-2-3'-methyl-2'-butenyl)-1(2H) -naphthalenone	26.43	0.28
30	3-(propenyl)bicycle-heptan-2-one	26.73	1.02
31	2,3dichlorobenzyl alcohol-isopropyl ether	26.98	0.72
32	2-pentadecanone, 6,10,14-trimethyl	27.23	1.45
33	2-cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)	27.59	0.27
34	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	27.79	1.33
35	Culmorin		
36	Nonadecane	28.26	0.55
37	Benzene(1-methyldodecyl)	28.50	0.27
38	Dibutyl phthalic acid	28.71	0.54
39	Methyl-3-(3,5-ditertbutyl-4-hydroxyphenyl)propionate	29.24	0.50
40	Geranyl-alpha-terpinene	29.38	1.08
41	Dibutylphthalate	29.64	0.68
42	n-hexadecanoic acid	30.09	**10.94**
43	Heneicosane	32.03	0.27
44	Phytol isomer	32.36	0.36
45	6-octadecanoic acid	33.45	**13.19**
46	9-octadecenoic acid	33.69	0.74
47	Cycloeicosane	35.38	0.16
48	Tricosane	35.49	0.25
49	4,8,12,16-tetramethyl heptadecane-4-olide	36.49	0.64
50	Tetracosane	37.11	0.73
51	1,2-dihydro-11.beta.-hydroxy-.alpha.-santonin	37.44	0.83
52	5-Eicosene	38.65	1.25
53	Phenol, 2,4-bis(1-methyl-1-phenylethyl)	38.96	0.74
54	Bis (2-ethylhexyl)phthalate	39.58	**5.35**
55	Eicosane	40.18	0.24
56	Phenol, 2,4-bis(1-methyl-1-phenylethyl)	40.32	0.41
57	3-methyl-5-methylene-2-thiazolidinone1-[2- pyridylethylidene]hydrazine	41.14	2.00
58	Cyclohexane carboxylic acid, 2,2-dimethylpropyl ester	41.33	0.26
59	Heptacosane	41.67	1.27
60	9,11-dimethyl-6H-indolo-quinline	42	**4.90**
61	O-ethyl O-N-octyl ethylphosphonat diethyl ethylidenemalonate	42.25	2.81
62	Cyclohexane, bis 1,1' –(1,4-butanediyl)	42.87	1.07
63	Squalene	43.50	0.37
64	(+)-6-acetyl-7-hydroxy-6-[2-(4-methylphenyl)ethyl]-9-phenoxy-1-	43.88	0.16

	azabicyclo[6.2.0]		
65	Nonacosane	44.44	**4.42**
66	Triacontane	46.51	1.52
67	Octacosane	47.03	**5.19**

Table (4): GC-MS analysis of Ag-petroleum ether extract of *A. judaica*

Peak No.	**Compound**	**Rt (min)**	**Area (%)**
1	2-Thiazoline, 5-methyl-2-(2-pyridylmethyl)amino	4.52	1.06
2	β-chamigrene	15.73	0.56
3	4-(2',4',4'-trimethyl-cyclo[4.1.0]hept-'-en-3'-yl]-3-buten-2-one	16.7	**31.86**
4	Berkheyaradulene	16.86	**22.47**
5	Junipene	17.36	0.16
6	*trans*-caryophyllene	17.69	**10.51**
7	α-Humulene	18.54	3.74
8	Allo-aromadendrene	18.73	**13.29**
9	4,8-Methanoazulen-9-ol, decahydro-2,2,4,8-tetramethyl.	20.19	5.73
10	3-cyclohexene-1-carbxaldehyde, 1,3,4-trimethyl.	20.32	5.83
11	1,1-cyclohexane diacetic acid	21.52	4.78
12	Pyrrolidine, 1-(1-cyclopentene-1-yl)	24.14	1.44

Discussions:

The secondary metabolites of several plants have been used in the formulation of nanoparticles to increase their effectiveness against plant pathogens. This technology is known as green synthesis (for example the silver nanoparticles) and is a very cost-effective, safe, non-toxic, eco-friendly, and could be mass-produced (Gardea-Torresdey *et al.*, 2003; Sastry *et al.*, 2004; Ganesan *et al.*, 2013). Moreover, silver nanoparticles have special properties such as being chemically stable and catalytic, antimicrobial, and anti-nematicidal activity (Li *et al.*, 2007; Setua *et al.*, 2007; Nassar, 2016). The preparation of natural products into the Ag-nanoparticle form increased their activity against J2 and eggs of *M. incognita* at least 3 times.

Such findings highlighted the importance of the incorporation of natural products into the nano-technology to find nematicide alternatives. The results reported in current study showed that the extracts of *A. judaica* were successfully synthesized in AgNPs and confirmed for the size and type by UV-Vis absorption patterns and the SEM microscope. The occurrence of the peak around 470 ± 10 nm is due to the phenomenon of surface Plasmon resonance, which occurs due to the excitation of the surface Plasmon's present on the outer surface of the silver nanoparticles that gets excited due to applied electromagnetic field (Naheed *et al.*, 2011). Moreover, it was reported that the UV absorption peak of silver nanoparticles were ranged from 450 – 500 nm (Ramteke *el al.*, 2013). Furthermore, the GC-MS analysis revealed the increase of certain secondary metabolites in the Ag-nano formulation, such as 4-(2',4',4'-trimethyl-yciclo[4.1.0]hept-2'-en-3'-yl)-3-buten-2-one, caryophyllene, berkheyaradulene, and allo-aromadendrene that were increased from 20 to 30-folds than in the extract. The AgNPs showed nematicidal activity against root-knot nematode *M. incognita*. So, the herbal plants might present good sources for finding phytochemical bionematicides. Moreover, the biological activity might be increased through the preparation of secondary metabolites into the nano-sized formulations.

In conclusion, results reported in current experiments offered a good potential of extracts of *A. judaica* and its nanoparticles formulation as new nematicide of activators of nematicides. The benefits of the synthesis of metal nanoparticles of plant extractives are versatile; energy efficient, cost effective, non-toxic to human and environment, use of safer products. Therefore, the use of silver nanotechnology in evolving and developed countries will bring dramatic changes in in pest management programs. This would truly be a journey to green nanobiorevolution.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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