



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 (AgNO₃; 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° 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 35° 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 pyrrolidone (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 40° 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 Ag⁰ 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 $25 \pm 2^\circ \text{C}$ 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 $\mu\text{g ml}^{-1}$ 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 $25 \pm 2^\circ \text{C}$. 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 quadrupole 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 60°C for 3 min then elevated to 280°C at a rate of $8^\circ \text{C min}^{-1}$ and 10 min hold at 280°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 $\lambda_{\max}$ peaks at about (470 ± 10) nm (Fig 1).

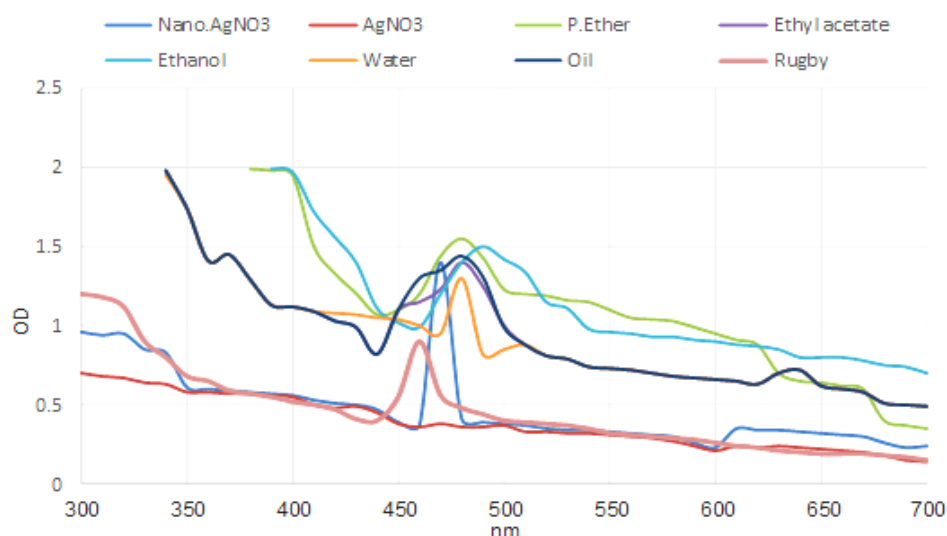


Fig 1: UV-spectrometry absorption peaks of Ag-nano of extracts of *A. judaica* (P. Ether, Ethyl Acetate, Ethanol, Water, and Oil), the synthesized silver nanoparticles (Nano AgNO₃), AgNO₃ solution and Rugby nanoparticles (Rugby).

Additionally, the scanning electron microscope (SEM) confirmed the shape and size of the silver nanoparticles of plant extracts. 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).

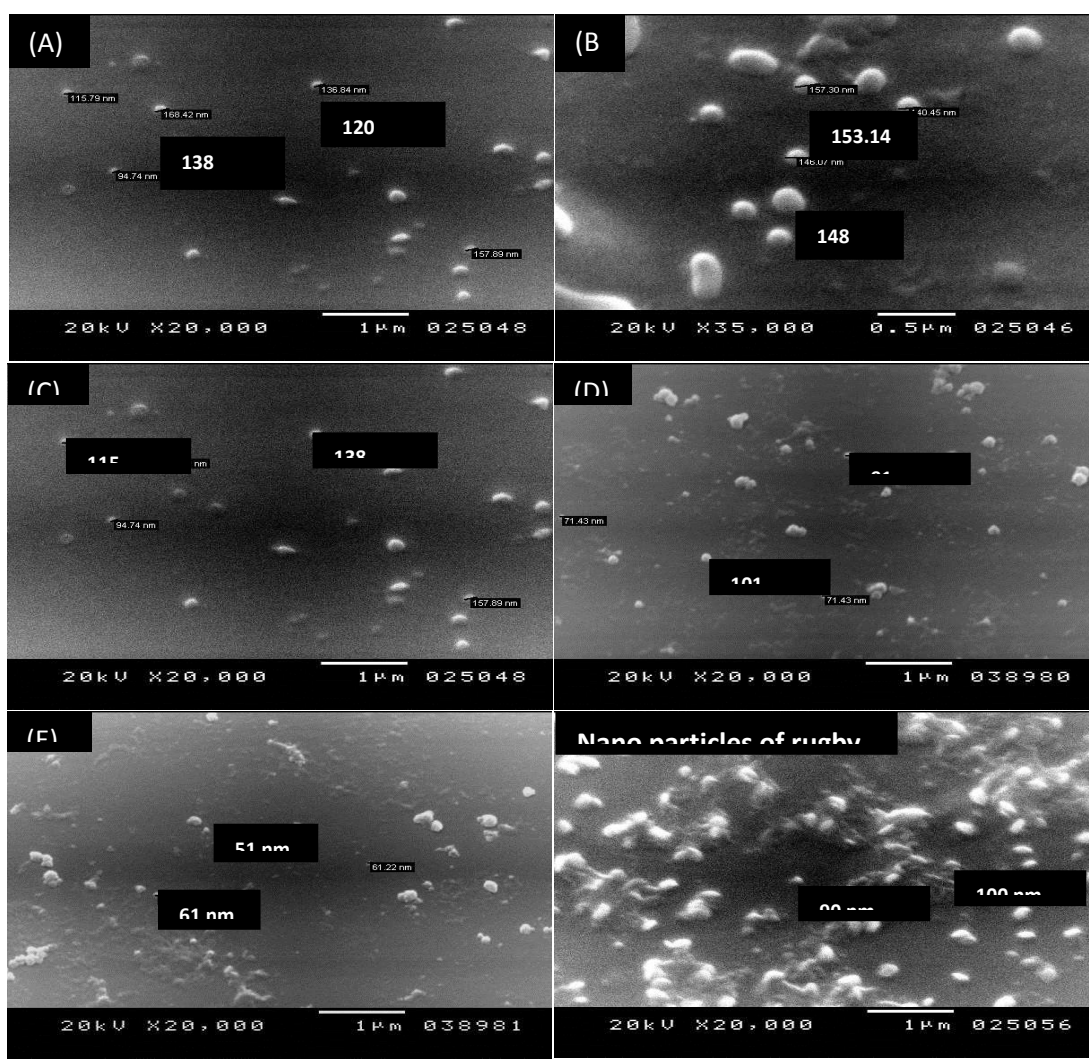


Fig (2): Nanoparticles of petroleum ether (A), ethyl acetate (B), ethanol (C), water extract (D), and essential oil (E) of *A. judaica* and the nano nematicide, Rugby.

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 LC₅₀ value of 14.39 µg ml⁻¹ compared to 25.97 µg ml⁻¹ for the rugby®. The Ag-petroleum ether showed the inhibitory action against the J2 compared to other extracts but was less than Ag-rugby and rugby. The LC₅₀ 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

^aThe median concentration that kill 50% of J2 stage, ^bSlope ± 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

LC₅₀ 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 LC₅₀ 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

^aThe median concentration that inhibit 50% of egg hatchability, ^bSlope ± 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	Alloaromadendrene	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.β.-hydroxy-.α.-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-thiazolidinone-1-[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-quinoline	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-azabicyclo[6.2.0]	43.88	0.16
65	Nonacosane	44.44	4.42
66	Triacotane	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-1-en-3'-yl)-3-buten-2-one	16.7	31.86
4	Berkheyaradulene	16.86	22.47
5	Junipene	17.36	0.16
6	<i>trans</i> -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-carboxaldehyde, 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 *et 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-cyclo[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.

➤ References Cited .

1. **Abdelgaleil, S.A.M., Abbassy, A.M., Belal, A.H. and Abdel Rasoul, M.A.A.** (2008). Bioactivity of two major constituents isolated from the essential oil of *Artemisia judaica* L. Bioresource Technology, 99: 5947 - 5950.
2. **Abd-Elhady, H.** (2012). Insecticidal activity and chemical composition of essential oil from *Artemisia judaica* L. against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)." Journal of Plant Protection Research 52(3):347–52.
3. **Abdul-Hameed, M.A.** (2012). Nanoparticles as Alternative to Pesticides in Management Plant Diseases - A Review. International Journal of Scientific and Research Publications. 2.

4. **Agrios, G.N.** (2005) Plant Diseases Caused by Nematodes. In: *Plant Pathology*, Elsevier Academic Press, Burlington, 838-842
5. **Badawy, M.E.I. and Samir A.M.A.** (2014). Composition and antimicrobial activity of essential oils isolated from Egyptian plants against plant pathogenic bacteria and fungi." *Industrial Crops and Products* 52:776-82.
6. **Balbaa, E.M.E.** (2010). Biological and environmental studies on root-knot nematode affecting Leguminosae. M.Sc. Thesis, Faculty of Agriculture, Alexandria University, Egypt.
7. **Browning, M., Wallace, D.B., Dawson, C., Alm, S.R., and Amador, J.** (2006). A. Potential of butyric acid for control of soil-borne fungal pathogens and nematodes affecting strawberries. *Soil Biol. Biochem*, 38(2), 401-404.
8. **Claudius-Cole, A. O., A. E. Aminu, and B. Fawole.** (2010). Evaluation of plant extracts in the management of root-knot nematode *Meloidogyne incognita* on cowpea [*Vigna unguiculata* (L) Walp]. *Mycopath.*, 2:53-60.
9. **El-Massry, K.F., El-Ghorab, A.H. and Farouk, A.** (2002). Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. *Food Chemistry*, 79:331 – 336.
10. **Elshamy, A., A. G. El gendy, A. H. Farrag, and M. Nassar.** (2015). Antidiabetic and antioxidant activities of phenolic extracts of *Conyza dioscoridis* L. Shoots. *International Journal of Pharmacy and Pharmaceutical Sciences*. 7.
11. **El-Sharabasy, Hamdy Mahmoud.** (2010). Acaricidal Activities of *Artemisia Judaica* L. Extracts against *Tetranychus urticae* Koch and Its Predator *Phytoseiulus persimilis* Athias Henriot (Tetranychidae: Phytoseiidae)." *Journal of Biopesticides* 3(2):514–19.
12. **Fernandez, C., R. Rodriguez-Kabana, P. Warrior, and J. W. Kloepper.** (2001). Induced soil suppressiveness to a root-knot nematode species by anematicide. *Biol. Control*. 22(2), 103-114.
13. **Ganesan, V., A. Astalakshmi, P. Nima, and C. Arunkumar.** (2013). Synthesis and haracterization of silver nanoparticles using *Merremia tridentata* (L.) Hall.f. *International Journal of Current Science*. 6:87-93.
14. **Gardea-Torresdey, J. L., E. Gomez, V. J. Peralta, J. G. Parsons, H. E. Troiani, and Y. Jose.** (2003). Synthesis of Gold nanotriangles and silver nanoparticles using Aloe vera plant extract. *Langmuir*. 13:1357.
15. **Gunther, E.** (1952). Essential oils volume 3 individual essential oils of the plant families Rutaceae and Labiatae. 2nd Ed. Van Nostrand company. Tornto, New York, London.
16. **Hartman, K.M. and Sasser, J.N.,** (1985). Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In: *An advanced treatise on Meloidogyne* (eds. K. R. Barker, C. C. Carter and J. N. Sasser). Vol. 2. Methodology. North Carolina State University Graphics, Raleigh, pp. 69-77.
17. **Hussey, R. S. and Barker, K. R.** (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Disease Report* 57:1925-1928.
18. **Jepson, S. B.** (1987). Identification of root-knot nematodes *Meloidogynespp.* Pp. 265, eds. CAB International, Wallingford, Oxon, UK.
19. **Khan, I. A., M. Sayed, S. S. Shaukat, and Z. A. Handoo.** (2008). Efficacy of four plant extracts on nematodes associated with papaya in Sindh, Pakistan. *Nematologia Mediterranea*. 36: 93 - 98.
20. **Khan, M.R; Khan, M.W and Khan, A.A** (1996). Effect of *Meloidogyne incognita* on dry weight, root gall and root nodulation of chickpea and cowpea cultivars. Test of agrochemicals and cultivars. 17: 70-71.
21. **Khan, Z.; Kim, Y. H.** (2007). A review on the role of predatory soil nematodes in the biological control of plant parasitic nematodes. *Appl.Soil Ecol.*, 35(2), 370-379.

22. **Krishna, S., Bustamante, L., Haynes, R. K., & Staines, H. M.** (2008). Artemisinins: mechanisms of action and potential for resistance. *Trends in Pharmacological Science*, 29, 520–527.
23. **Li, S., Y. Shen, A. Xie, X. Yu, L. Qiu, L. Zhang, and Q. Zhang.** (2007). Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract. *Green. Chem.* 9:852–858.
24. **Li, Y., Huang, H., Wu, Y-L.** (2000). Qinghaosu (Artemisinin)-A fantastic antimalarial drug from a traditional Chinese medicine. In Liang & Fang (Eds.), *Medicinal chemistry of bioactive natural products*. Wiley, pp. 183–256.
25. **Mangala, R. and S. Mauria.** (2006). *Handbook of Agriculture Fact and figure for teacher, students and all interested farmers*. Indian Council of Agricultural Research, New Delhi 1346 pp.
26. **McSorley, R.,** 2002. Losses to Nematodes. In: *Encyclopedia of Pest Management*, Pimentel, D. (Ed.). CRC Press, Boca Raton, ISBN: 9781439870587.
27. **Meher, H.C., S. Walia and C.L. Sethi,** 1988. Effect of steroidal and triterpenic saponins on the mobility of juveniles of *Meloidogyne incognita*. *Indian J. Nematol.*, 18: 244-247.
28. **Naheed, A., Seema, S., Singh, V. N., Shamsi, S. F., Anjum, F. and Mehta, B. R.** (2011). Biosynthesis of Silver Nanoparticles from *Desmodium triflorum*: A Novel Approach Towards Weed. *Utilization Biotechnology Research International* 8 pages.
29. **Nassar, A.M.K.** (2016) Effectiveness of Silver Nano-particles of Extracts of *Urtica urens* (Urticaceae) Against Root-knot Nematode *Meloidogyne incognita*, *Asian Journal of Nematology*. 5: 14-19
30. **Nicol, J.M., S.J. Turner, D.L. Coyne, L. den Nijs, S. Hockland and Z.T. Maafi,** 2011. Current Nematode Threats to World Agriculture. In: *Genomics and Molecular Genetics of Plant-Nematode Interactions*, Jones, J., G. Gheysen and C. Fenoll (Eds.). Chapter 2, Springer, Berlin, Germany, ISBN: 978-94-007-0433-6, pp: 21-43.
31. **Nie, S. and S. R. Emory,** (1997). “Probing Single Molecules and Single Nanoparticles by Surface-enhanced Raman Scattering,” *Science*, 275, 1102.
32. **Oka, Y.** (2001). Nematicidal activity of essential oil components against the root-knot nematode *Meloidogyne javanica*. *Nematology* 3:159–164
33. **Okada, H.; Harada, H.** 2007. Effects of tillage and fertilizer on nematode communities in a Japanese soybean field. *Appl. Soil Ecol.*, 35(3), 582-598.
34. **Olowe, T. O.** (1992). Economic Importance of nematode on legume and Cereal crops in Nigeria. In: *proceedings of the first regional symposium on the biology and control of nematode pests of food crop in Africa*, held in Ibadan, Nigeria July 26-29. Fawole B, Egunjobi OA, Adesiyun SO, Babalola JO, Idowu AA, eds. His will Info Resources Management Limited, Ibadan.
35. **Prasad, T. N. V. K. V. and E. K. Elumalai.** (2011). Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity. *Asian Pacific Journal of Tropical Biomedicine*. 1(6):439-442.
36. **Rajesh, W. R., R. L. Jaya, S. K. Niranjana, D. M. Vijay, and B. K. Sahelebrao.** (2009). Phyto synthesis of silver nanoparticles using *Gliricidia sepium* (Jaeq). *Curr. Nano. sci.*, 5:117–122.
37. **Ramteke, C., Chakrabarti, T., Sarangi, B.K. and Pandey, R.M.** (2013). Synthesis of silver nanoparticles from the aqueous extract of leaves of *Ocimum sanctum* for enhanced antibacterial activity. *Journal of Chemistry*, - ID 278925
38. **Roberts, P.A., J. R. Matthews and J. D. Ehler,** (2005). Root-knot nematode resistant cowpea cover crops in tomato production systems. *Agron. J.*, 97:1626-1635.
39. **Rodriguez, E.,** (1988). Dithiopolyacetylenes as Potential Pesticides. In: *Biologically Active Natural Products: Potential Use in Agriculture*, Cutler, H.G. (Ed.). 2nd Edn., The Society, London, UK., ISBN: 9780841215566, pp: 432-437.

40. **Saban, K., Recep, K., Ahmet, M., Ahmet, C. A. A., Ali, Y.,** (2005). Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *Artemisia dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. *Journal of Agricultural Food Chemistry*, 53: 9452 - 9458.
41. **Salawu, E. O.** (1992). Effect of neem leaf extract and ethoprop singly and in combination on *Meloidogyne incognita* and growth of sugarcane. *Pak. J. Nematol.*, 10(1): 51-56.
42. **Sastry, M., A. Ahmad, M. I. Khan, and R. Kumar.** (2004). Microbial nanoparticles production in Nanobiotechnology ed. Niemeyer CM and Mirkin CA. Wiley-VCH, Weinheim. 126.
43. **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. *N. J. Phys. Chem. C.* 111:3901–3907.
44. **Sharma, K Virender., Yngard, A Ria., Lin, Yekaterina** (2009). Silver Nanoparticles: Green Synthesis and their Antimicrobial Activities. *Advances in Colloid and Interface Science*, 145, 83 -9.
45. **Sikora, R.A., K. Schafer and A.A. Dababat** (2007). Modes of action associated with microbially induced in planta suppression of plant-parasitic nematodes. *Australasian Plant Pathol.*, 36: 124-134.
46. **Soler-Serratos, A., N. Kokalis-Burelle, R. Rodriguez-Kabana, C.F. Weaver and P.S. King** (1996). Allelochemicals for control of plant-parasitic nematodes. 1. In vivo nematocidal efficacy of thymol and thymol/benzaldehyde combinations. *Nematropica*, 26: 57-71.
47. **Song, J. Y., H. K. Jang, and B. S. Kim.** (2009). Biological synthesis of gold nanoparticles using *Magnolia kobus* and *Diopyros kaki* leaf extracts. *Process Biochemistry*. 44: 1133-1138.
48. **Sun, Y., Y. Yin, B. T. Mayers, T. Herricks, Y. Xia, and Chem. Mater.** (2002). 14:4736.
49. **Tackholm, V.** (1974). Student Flora of Egypt, 2nd ed. Cairo University Press, Cooperative printing Co., Beirrut, Lebanon, 581.
50. **Thoden, T.C., J. Hallmann and M. Boppre** (2009). Effects of plants containing pyrrolizidine alkaloids on the Northern root-knot nematode *Meloidogyne hapla*. *Eur. J. Plant Pathol.*, 123: 27-36.
51. **Wang, K.H., C.R. Hooks and A. Ploeg** (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 Disease, July 2007, University of Hawaii, pp: 1-6.
52. **White, R.** (2009). Luque, V.L. Budarin, J.H. Clark, D.J. Macquarrie, *Chem. Soc. Rev.* 38 481.
53. **Williamson, V. M. and A. Kumar** (2006). Nematode resistance in plants: The battle underground. *Trends Genet.* 22 (7), 396-403.
54. **Ye, L., Z. Lai, J. Liu, and A. Tholen** (1999). "Effect of Ag Particle Size on Electrical Conductivity of Isotropically Conductive Adhesives," *IEEE Trans. Electron. Packag. Manuf.*, 22 (4), 299.