

Nematicidal Potential of some Natural Botanical Extracts in Biocontrolling *Meloidogyne javanica* on Soybean under Laboratory and Greenhouse Conditions



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ABSTRACT

The nematicidal potential of certain plant part extracts against *Meloidogyne javanica* was determined under *in vitro* and *in vivo* experiments. Three different solvents were used in the preparation of the tested extracts including water, ethyl alcohol and acetone. The extracted plants included *Eugenia jambolana* (leaves), *Punica granatum* (Peels) and *Camellia sinensis* (Leaves). Results showed that ethyl alcohol peel extract of *E. jambolana*, and *P. granatum* completely inhibited the egg hatching of *M. javanica* while, ethyl alcohol leaf extract of *C. sinensis* inhibited the egg hatching by 60%. Nematode juveniles j_2 mortality gradually increased as the exposure time increased from 24 to 48 hours. Under greenhouse conditions, organic solvent extracts of all the tested plants were more effective in suppressing the nematode reproduction compared to the aqueous extracts. The ethyl alcohol leaf extract of *E. jambolana* completely suppressed the gall formation by *M. javanica* on soybean roots as well as number of egg-masses/plant, and the number of J_2 s in soil. Ethyl alcohol peel extract of *P. granatum* showed the greatest nematicidal effects against *M. javanica*, while its aqueous extract was the least effective. The plants treated with the ethyl alcohol leaf extract of *E. jambolana*, showed the highest increase in the plant growth parameters *viz.*, shoot weight, and length as well as root weight and length compared to the control treatment. Chemical analysis of leaf extract of *E. jambolana* by gas chromatography-mass spectrometry (GC-MS) showed that it contained thirty-three bioactive compounds, including, flavonoids, antioxidant, oxygenated hydrocarbons, unsaturated fatty acids, sterile, fatty acid ester, triterpene, and others. All plant extracts increased the activities of defense-related enzymes, including peroxidase, catalase, chitinase, β -1,3-glucanase enzymes as well as, the total phenol contents in soybean plants infected with *M. javanica* under greenhouse conditions.

Key words: *Glycine max*, *Meloidogyne javanica*, nematicidal effects, resistance enzymes, phenolic compounds, botanical extracts.

INTRODUCTION

Soybean, *Glycine max* (L.) Merr, is considered to be one of the most important legumes and oilseed crops cultivated in the world. Soybean is used for food, feed, and the processing of raw materials for the vegetable oil industry and other purposes as well. The cultivated areas account 121.53 million ha, worldwide with a total production of about 334.89 million metric tons in 2018 (FAO. 2018). Egypt has shown great interest in soybean cultivation due to the increasing lack of dietary

protein, as soybean protein provides all nine essential amino acids that considered the main protein source for human and animal consumption (Liebenberg, 2012).

Plant parasitic nematodes are major biotic factors that negatively affect the soybean production worldwide, causing an annual yield loss of about 10-15%. More than 100 nematode species belonging to 50 genera have been reported in association with soybeans. Of them, root-knot nematodes, *Meloidogyne* spp., and soybean cyst nematode, *Heterodera glycines*, are notably the most important nematodes attacking soybean plants worldwide. They, especially, cause deformation of the soybean root systems when galls are formed and consequently the nutrient and water uptake by these roots is adversely affected (Sikora et al., 2005 a, b). The most damaging species were found to be *M. javanica* ,and *M. incognita* which cause significant yield loss of susceptible soybean cultivars by as much as 90% (Fourie et al., 2001).

The continuous use of the classical nematicides to control plant-parasitic nematodes are likely to cause phytotoxicity, pollution of the environment and resistance to the nematode (Conway, 1995 &Yudelman et al., 1998). One of the effective control alternatives is the use of botanical extracts, which are safe, cheap and easy to use (Sultana et al., 2010, Khan et al., 2011). El- Nagdi and Youssef (2014) reported that aqueous extract of *P.granatum* as mashed fruit peels recorded 61.1% reduction in the number of the hatched juveniles of *M.incognia* and 87.7% in the number of juveniles in sugar beet soil. Some plants used for the nematode management to produce secondary compounds which do not only activate defense mechanisms of the treated plants, but also might have anti-pathogenic activity against plant nematodes (Stangarlin et al., 2011).

A large number of enzymes are associated with induced resistance in plants against plant pathogens (Ojaghian et al., 2014). These enzymes include; peroxidases, β -1,3-glucanases, and chitinases. β -1,3-glucans and chitinase can degrade the cell walls of the pathogens, releasing molecules that act as elicitors in the initial stages of the resistance induction process of phytoalexins and phenolic compounds (Silva et al., 2004). In general, peroxidase plays a fundamental role in the growth and development of plants and is strongly related to defense mechanisms against pathogens (Kurabachew and Wydra, 2014). Generally, chitinase may play a secondary function as signal molecules, which elicit the induction of other pathogenesis-related (PR) proteins or metabolites, involved in plant-defense reactions (Rahimi et al., 1998). The objectives of this study: 1- evaluation of the effect of certain plant extracts on controlling the root-knot nematode, *M. javanica* on soybean plants under greenhouse conditions.2- determination of the bio-active, and chemical constituents of leaves extract by using GC/MS of *Eugenia jambolana*.3-determination of the biochemical changes in soybean plants treated with natural plant extracts.

MATERIALS AND METHODS

1.Preparation of plant extracts

A: Aqueous plant extracts:

Plants of three species belonging to three genera Table (1) were collected from different localities in El-Kharga, New Valley Governorate, Egypt. Leaves and/or peels of the collected plants were cut into small pieces, washed several times with running tap water, then with sterile water and air-dried at room temperature (≈ 25 °C)

for 15 days. Plant materials were ground to fine powders in a grinder, then a 100 g of each one was blended in 1 L of distilled water for 6 hours. The ground materials were squeezed through double cheesecloth sheets, then filtered through a Whatman No. 1 filter paper. The efficacy of the fresh extracts was evaluated against *M. javanica* J₂s at the concentration of 20% from the original ones (Abdel-Monaim et al., 2011).

Table 1: A list of the plant species and their parts that were tested for their nematocidal activity against *Meloidogyne javanica*.

Common name	Family name	Scientific name	Used parts
Stopper	Myrtaceae	<i>Eugenia jambolana</i> (Syn. <i>Syzygium cumini</i> or <i>Syzygium jambolana</i> or <i>Eugenia cumini</i>)	Leaves
Pomegranate	Lythraceae	<i>Punica granatum</i>	Fruit peels
Green tea	Theaceae	<i>Camellia sinensis</i>	Leaves

B: Organic solvents of plant extracts:

In this experiment, two organic solvents, i.e. ethanol 95% and acetone were used for preparing the plant extracts. The method was the same applied to the aqueous plant extracts except using organic solvents instead of distilled water. The extracted materials with each of the solvents were concentrated by a rotary vacuum evaporator at 50°C for 6 hours. The final concentration was prepared as those of water extracts (20%) by adding water for each organic solvent extract (Abdel-Monaim et al., 2011).

Nematode inocula: A pure culture of *M. javanica* was obtained from tomato plants grown in clay pots in the greenhouse. Nematode eggs were extracted from the *M. javanica*-infected tomato roots by the NaOCl 0.5% (Hussey and Barker, 1973). Eggs were then used immediately for hatching tests or incubated in water for juvenile hatching at room temperature. The freshly hatched J₂s were used for the evaluations of aqueous or solvent extracts.

2- Laboratory assay

A- Hatchability test:

One-ml of *M. javanica* egg suspension (about 100 eggs/ml) and nine-ml of each plant extract (whether aqueous or solvent plant extract) were transferred to glass cavity blocks and kept at room temperature (≈ 25 °C). An equal number of glass cavities containing 1- ml of the egg suspension and nine - ml distilled water served as controls. Each treatment was replicated thrice. After four days of exposure, the number of hatching juveniles was counted under a stereoscopic microscope. The toxicity of plant extracts was assessed as the mean percentages egg hatching inhibition.

B- Effect of plant extracts on juvenile mortality:

Nine ml of each plant extract was separately poured into each Petri dish was added to 1 one- ml of the nematode suspension containing 100 freshly hatched juveniles. The Petri dishes with distilled water taken as control. All treatments were replicated three times. The Petri dishes were incubated at room temperature (≈ 25 °C).

Percentages juvenile mortality were calculated after 24 and 48 hours using Abbott's formula (Abbott, 1925) as follows:

Mortality (%) = [(mortality percentage in treatment – mortality percentage in control) / (100 – mortality percentage in control)] × 100.

The greenhouse experiments were conducted during the summer season, 2018 at the New Valley Agriculture Research Station.

3- Greenhouse experiment

Fifty clay pots, 30 -cm diameter (five kg capacity) were, filled with a steam-sterilized mixture of sandy clay (4:1) soil. Three seeds of soybean cv. "Giza 35" were planted in each pot, and the seedlings were thinned to one seedling/pot, 7 days after emergence. Each seedling was inoculated with 2000 eggs of *M. javanica*. Nine plant extracts plus a control (10 treatments), with five replicates each, were established as follows:

- 1- *E. jambolana* aqueous leaf extract + *M. javanica*.
- 2- *E. jambolana* ethyl alcohol leaf extract + *M. javanica*.
- 3- *E. jambolana* acetone leaf extract + *M. javanica*.
- 4- *P. granatum* aqueous leaf extract + *M. javanica*.
- 5- *P. granatum* ethyl alcohol peel extract + *M. javanica*.
- 6- *P. granatum* acetone peel extract + *M. javanica*.
- 7- *C. sinensis* aqueous leaf extract + *M. javanica*.
- 8- *C. sinensis* ethyl alcohol leaf extract + *M. javanica*.
- 9- *C. sinensis* acetone leaf extract + *M. javanica*.
- 10- Control (*M. javanica* only).

The plant extracts were added three times as follows: A- two days before nematode inoculation, B- Simultaneously with nematode inoculation and, C- two days after nematode inoculation.

The extracts were added @ 40 ml/ pot by pouring the solution into holes made around the seedlings in each pot. Plants inoculated with *M. javanica* only served as control. Pots were arranged in a completely randomized design with five replicates for each treatment.

Sixty days after inoculation, the experiment was terminated. Root systems were gently washed with tap water and stained with Phloxine B (0.15 g/l liter water) for 20-min. to emphasize the presence of egg masses (Daykin and Hussey, 1985). Numbers of root galls, and egg masses/root system were counted and indexed on a 0-5 scale (Taylor and Sasser 1978), where 0: = no galls (or egg masses), 1= 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5= more than 100 galls (or egg masses) /root system. Second-stage juveniles (J₂s) were extracted from an aliquot of 250 g soil from each pot using sieving and Baermann pan technique (Barker et al., 1985). The extracted juveniles were counted using 1-ml counting slide under a compound microscope. Finally, shoot and root lengths (cm), and shoot and root fresh and dry weights (g) were measured.

4-Gas chromatography-mass spectrometry (GC/MS) analysis

The protocol for GC-MS analysis was performed according to (Weckwerth et al., 2004). The analysis extract of *E. jambolana* was carried-out using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary

column (30 m × 0.25 mm i. d. and 0.25 µm film thickness). The carrier gas was helium with the linear velocity of 1ml/min. The injector and detector temperatures were 200 and 250 °C, respectively. The volume of injection of each sample was 1µl. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 250 °C, and acquired mass range 50–800, the identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature. This was carried out by the Regional Center for Food & Feed, Agricultural Research Center, Giza.

5-Biochemical changes in soybean plants treated with some natural plant extracts in soil infested with M. javanica under greenhouse conditions

Clay pots, 30 -cm diam. (4-kg soil capacity) were filled with a sterilized mixture of clay and sand (4:1), and planted with soybean seeds cv. "Giza 35". Seven days after emergence, seedlings were thinned to one seedling/pot and inoculated with 500 eggs of *M. javanica*/seedling. Plant extracts were added as mentioned previously. Each treatment was replicated 3 times, and pots were arranged in a complete randomized design. Three days after the third extract addition, one g of root tissues from each treatment was used for enzyme extraction according to McCord and Fridovich (1969).

Peroxidase activity: The activity of peroxidase enzyme was determined directly using a spectrophotometrical method according to Hammerschmidt et al. (1982). Activity was expressed as units min⁻¹ (mg protein)⁻¹.

Catalase activity: The activity of catalase was assayed according to Verma and Dubey (2003). Activity was expressed as units min⁻¹ (mg protein)⁻¹.

Chitinase activity: The Chitinase activity was determined using the method described by (Wirth and Wolf, 1992). Enzyme activity was expressed as an enzyme min⁻¹ (mg protein)⁻¹.

β-1,3-glucanase: β-1,3-glucanase activity was assayed by the laminarin-dinitrosalicylic acid method (Pan et al., 1991). The enzyme activity was expressed as µg glucose released min⁻¹ (mg protein)⁻¹. Increasing percentages (%) of enzyme activity were counted in comparison with a control treatment.

Determination of phenolic compounds

For assessing the total phenolic contents, 1g fresh root of each treatment was homogenized in 10 ml of 80% methanol and agitated for 15- min. at 70 °C. One ml of the extract was added to 5-ml distilled water plus 250 µl of 1 N Folin-Ciocalteu reagent, and the solution was kept at 25 °C. The absorbance was measured with a spectrophotometer at 725- nm. Catechol was used as a standard. The amount of phenolic content was expressed as phenol equivalents in mg /g fresh tissue (Saikia et al., 2006). Increasing percentages (%) of total phenolic contents were counted in comparison with a control treatment.

Statistical analysis

All experiments were performed twice. Analyses of variance were carried-out using MSTAT-C program version 2.10 (Anonymous,1991). Means were separated using the least significant differences (LSD) method at $P \leq 0.05$ (Gomez and Gomez, 1984).

RESULTS

Egg hatching and juvenile mortality:

All extracts affected egg hatching and juvenile mortality of *M. javanica* Table (2). Ethyl alcohol extracts of *E. jambolana* (Leaves), and *P. granatum* (Peels) completely inhibited egg hatching of *M. javanica* after 4 days. However, the ethyl alcohol leaf extract of *C. sinensis* inhibited the egg hatching by 60%. Nematode juvenile mortality gradually increased as the exposure time increased Table (2).

It was noticed that ethyl alcohol extracts of *E. jambolana* (Leaves), and *P. granatum* (Peels) caused 100% mortality after 48 hours. But, ethyl alcohol leaf extract of *C. sinensis* caused 65% mortality after the same time. The least effective treatment was a acetone leaf extract of *C. sinensis*, as it recorded 32% only after 48hours.

Table 2: Effects of some plant extracts on egg hatching of *Meloidogyne javanica* in vitro.

Plant extracts	Extraction medium (Solvents)	% of egg hatching inhibition after 4 days	Juvenile mortality (%)	
			After 24 hours	After 48 hours
<i>Eugenia jambolana</i>	Distilled water	23	26	42
	Ethyl alcohol	100	62	100
	Acetone	44	38	64
<i>Punica granatum</i>	Distilled water	38	21	53
	Ethyl alcohol	100	54	100
	Acetone	50	30	48
<i>Camellia sinensis</i>	Distilled water	56	23	50
	Ethyl alcohol	60	38	65
	Acetone	42	17	32
Control (distilled water)		0.0	0.0	0.0
LSD _{0.05}				
Plant extracts (A) =	-	4.41	6.1	5.9
Solvents (B) =	-	4.41	6.1	5.9
Interactions (A×B) =	-	7.63	10.48	10.21

Data are averages of 3 replicates.

Greenhouse experiment

Effect of some natural plant extracts on soybean infected with *M. javanica* under greenhouse conditions

Results of the nematicidal effect of some aqueous and organic solvent extracts of *E. jambolana*, *P. granatum* and *C. sinensis* against *M. javanica* reproduction on soybean plant are shown in Table (3). All treatments significantly reduced the root

galling and *M. javanica* reproduction on soybean plants. Generally, organic solvent extracts of all the tested plants were more effective in reducing nematode reproduction than aqueous extracts.

Table 3: Effect of some natural plant extracts on root galling and nematode reproduction of *Meloidogyne javanica* infecting soybean under greenhouse conditions.

Plant Extracts	Extraction medium (Solvents)	No. galls/ root system	No. egg masses/ root system	No. J ₂ /250g soil
<i>Eugenia jambolana</i>	Distilled water	180.33	27.67	680.33
	Ethyl alcohol	0.00	0.00	0.00
	Acetone	110.67	7.33	300.0
	Mean	97.00	11.67	326.78
<i>Punica granatum</i>	Distilled water	280.33	45.33	560.33
	Ethyl alcohol	20.33	0.00	0.00
	Acetone	170.67	14.67	300.67
	Mean	157.11	20.00	287.00
<i>Camellia sinensis</i>	Distilled water	90.67	25.0	320.33
	Ethyl alcohol	70.33	8.33	160.00
	Acetone	170.33	31.00	400.67
	Mean	110.44	21.44	293.67
Control (nematode only)		320.00	62.67	1600.33
LSD _{0.05}				
Plant extracts (A)	=	7.155	1.74	47.69
Solvents (B)	=	4.020	1.10	11.63
Interactions (A×B)	=	8.040	2.18	23.25

Data are average of 3 replicates.

* Root gall index (RGI) or egg masses index (EI) was determined according to (Taylor and Sasser,1978) where G.I and E.I were determined as follows: 0: = no galls, 1= 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5= more than 100 galls or egg- masses per root system.

The ethyl alcohol leaf extract of *E. jambolana*, completely suppressed gall formation induced by *M. javanica* and egg masses / root system, and the second stage juveniles (J₂) in soil. The least effective treatment was the aqueous leaf extract of *E. jambolana* in reducing number of root galls (180.33) / root system, gall index (5.0), and the second stage juveniles in the soil (680.33 / 250 g soil). At the same time, ethyl alcohol peel extract of *P. granatum* showed that the greatest nematocidal effect against *M. javanica* as root galling was (20.33) galls/ root system, compared with control treatment (320) galls/ root system, in addition to its complete suppressing effect on egg masses per root system, and the second stage juveniles (J₂) in soil. The least effective treatment in reducing root galls (280.33) /, and egg masses (45.33)/ root system, as well as gall, and egg mass indices (5) occurred with the aqueous peel extract of *P. granatum*.

Effect of some natural plant extracts on the growth parameters of soybean plants infected with *Meloidogyne javanica*

The growth parameters (root and shoot lengths, fresh and dry weights of roots and shoots) of soybean plants infected with *M. javanica* were measured (Table 4). Ethyl alcohol leaf extract of *E. jambolana* gave the highest shoot fresh weight (17.25 g), shoot length (55.23 cm), root fresh weight (6.86g), and root length (60 cm) compared to 3.52g; 22.43cm; 1.93g and 22.5cm in control treatment, respectively.

However, plants treated with acetone leaf extract of *C. sinensis* showed the lowest fresh shoot weight (7.25 g), root fresh weight (3.25 g), shoot dry weight (1.69 g), and root dry weight (0.75g) compared to 3.52g; 1.93g; 0.75g and 0.41g in control treatment, respectively.

Table 4: Growth parameters of soybean plants infected with *Meloidogyne javanica* and treated with natural plant extracts.

Plant Extracts	Extraction medium (Solvents)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
<i>Eugenia jambolana</i>	Distilled water	42.33	10.36	2.25	35	4.26	0.99
	Ethyl alcohol	55.23	17.25	3.81	60	6.86	1.68
	Acetone	35.50	7.56	1.72	31	3.89	0.89
	Mean	44.35	11.72	2.59	42	5.00	1.19
<i>Punica granatum</i>	Distilled water	45.52	10.38	2.93	34	4.02	0.91
	Ethyl alcohol	47.56	11.16	2.55	30	4.86	1.09
	Acetone	44.83	10.25	2.28	25	5.86	1.28
	Mean	45.97	10.60	2.59	29.67	4.91	1.09
<i>Camellia sinensis</i>	Distilled water	45.40	12.23	2.86	37.5	5.42	1.21
	Ethyl alcohol	50.36	13.25	3.05	25	5.89	1.34
	Acetone	37.50	7.25	1.69	30	3.25	0.75
	Mean	44.42	10.91	2.53	30.83	4.85	1.10
Control (nematode only)		22.43	3.52	0.75	22.5	1.93	0.41
LSD _{0.05}							
Plant extracts (A) =		2.99	1.35	0.32	3.31	0.44	0.19
Solvents (B) =		1.72	1.02	0.23	4.59	0.47	0.13
Interactions (A×B) =		3.43	2.05	0.56	9.17	0.93	0.25

Data are averages of 3 replicates.

Chemical composition of leaf extract of *E. jambolana*.

The components of *E. jambolana*, leaf extract were identified by the GC-MS chromatogram, the active principles with their retention time (RT), and area sum %, are presented in Table (5). Thirty- three compounds were identified. The major compounds were 17-octadecynoic acid (10.23%) and hexadecanoic acid,1-(hydroxymethyl)-1.2-ethanediyl ester (9.58%). Other fatty acids and their esters have also been identified; they were 17-octadecynoic acid , Trans-2-Hexadecenoic acid, Hexadecanoic acid,1-(hydroxymethyl)-1.2-ethanediyl ester) and Phytanic acid.

Three terpenoidal compounds were also recorded; Phytol acetate, Serverogeninactate and Trans-Sesquisabinene hydrate. In addition, several oxygenated hydrocarbons and fatty alcohols were identified; they were 4- Penten-2ol,2-Butanol,3-chloro-,(R*,R*)-,2-Hexanol, 3-methyl-, 2-Heptadecanol,2-Undecanol, 2-Heptadecanol, 2-hexadecanol,14-octadecenol, 3.7.11.15-tetramethyl-2-hexadecen-1-ol. Moreover, there were more antioxidants, Oleic acid, Linolenic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester (Z,Z). Two steroidal compounds namely, Ouabagenin and ethyl iso-allocholate were also present.

Numerous flavonoids compounds were identified as Gardenin flavonoid, 5.6.7.3'.4' pentamethoxy flavone, Quercetin 3.5.7.3'.4'-pentamethyl ether, Baicalein trimethyl ether, Vitexin 5.7.2'-trimethoxy flavone, flavanone hydrazine, 3-(3.4-imethoxyphenyl) -4.6-dimethylcoumarin, 3-(3.4-Dimethoxyphenyl) -7-methyl-4-phenyloumarin, 4-hydroxy-2'.34'.6' tetramethoxychalcone, 2-hydroxychalcone, and 2'-hydroxy 2.4'.5 tetramethoxychalcone. Furthermore, olomoucine a purine derivative was also recorded in traces.

Table 5: Chemical composition of *Eugenia jambolana* leaf extract.

No.	Retention time/min	Names of compounds	Area sum%
1	4.53	4- Penten-2ol	2.42
2	4.83	2-Butanol,3-chloro-,(R*,R*)-	3.12
3	7.22	2-Hexanol,3-methyl-	1.17
4	7.77	2-Heptadecanol	4.2
5	8.77	2-Undecanol	3.18
6	9.81	2-Heptadecanol	4.34
7	10.64	Trans-Sesquisabinene hydrate	1.6
8	11.31	Ouabagenin	5.66
9	11.67	Gardenin	1.22
10	12.51	Flavanone hydrazine	2.07
11	12.76	Oleic acid	1.85
12	12.91	17-octadecynoic acid	10.32
13	13.07	3.7.11.15-tetramethyl-2-hexadecen-1-ol	3.84
14	13.2	Phytol, acetate	5.65
15	13.37	14 -octadecanol	0.51
16	13.45	5.6.7.3'.4' pentamethoxy flavone	0.52
17	13.75	3-(3.4-imethoxyphenyl)-4.6-dimethylcoumarin	1.38
18	13.92	Hexadecanoic acid,1-(hydroxymethyl)-1.2-ethanediyl ester	9.58
19	14.46	Ethyl iso-allocholate	0.96
20	14.94	Quercetin 3.5.7.3'.4'-pentamethyl ether	2.36
21	15.11	Trans-2-Hexadecenoic acid	1.84
22	15.53	Baicalein trimethyl ether	0.67
23	15.91	Serverogeninactate	0.67
24	16.17	Linolenic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester	2.21
25	16.78	(Z,Z)	0.74
26	16.83	2'-hydroxy 2.4'.5-tetramethoxychalcone	0.66
27	20.61	2-hexadecanol	6.34
28	21.72	Phytanic acid	1.4
29	21.87	4-hydroxy-2'.34'.6'- tetramethoxychalcone	0.47
30	22.08	Olomoucine	0.47
31	22.47	Vitexin	1.22
32	22.72	2-hydroxychalcone	0.44
33	22.91	3-(3.4-Dimethoxyphenyl)-7-methyl-4-phenyloumarin 5.7.2'-trimethoxy flavone	7.86

Biochemical changes in soybean plants infected with *M. javanica* and treated with some natural plant extracts under greenhouse conditions

All of the tested plant extracts significantly increased the activity of peroxidase enzyme in soybean plants, compared to the control treatment (Table 6). Plants treated with ethyl alcohol and acetone leaf extracts of *E. jambolana* recorded the highest percentages increases (187.85% and 163.15%, respectively) in peroxidase activity, while, the lowest percentage increase (101.59%) in peroxidase activity was recorded in plants treated with the aqueous leaf extract of *C. sinensis*.

Table 6: Peroxidase (PO), and catalase (CA) enzymes activities in soybean plants infected with *Meloidogyne javanica* and treated with natural plant extracts under greenhouse conditions.

Plant Extracts	Extraction medium (Solvents)	Enzyme activity			
		Enzyme unit min ⁻¹ (mg protein) ⁻¹ .			
		Peroxidase (PO)		Catalase (CA)	
		Activity	% increase	Activity	% increase
<i>E. jambolana</i>	Distilled water	1.235	146.02	11.245	49.42
	Ethyl alcohol	1.445	187.85	15.428	105.00
	Acetone	1.321	163.15	13.502	79.40
	Mean	1.334	165.67	13.392	77.94
<i>P. granatum</i>	Distilled water	1.085	116.14	9.862	31.04
	Ethyl alcohol	1.251	149.20	11.248	49.46
	Acetone	1.182	135.46	10.002	32.90
	Mean	1.173	133.66	10.371	37.80
<i>C. sinensis</i>	Distilled water	1.012	101.59	10.002	32.90
	Ethyl alcohol	1.096	118.33	10.371	37.80
	Acetone	1.069	112.95	8.368	11.19
	Mean	1.059	110.96	9.580	26.57
Control (nematode only)		0.502	0.0	7.526	0.0
LSD _{0.05}					
Plant extracts (A) =		0.03		0.23	
Solvents (B) =		0.04		0.3	
Interactions (A×B) =		0.01		0.9	

Data are average of 3 replicates.

Plants treated with the same previously extracts showed the highest catalase (CA) activities (105.00 and 79.40%, respectively). While, the lowest percentage increase was recorded in the acetone leaf extract of *C. sinensis* (11.19%). All of the tested plant extracts significantly increased the activity of chitinase enzyme compared to the control treatment (Table 7). The highest percentage increases were recorded with the ethyl alcohol extract of *P. granatum*, *E. jambolana*, and *C. sinensis* (201.53, 157.43, and 130.12%, respectively). However, its lowest percentage increase in the activity was recorded by aqueous leaf extract of *C. sinensis* (70.60%). For β -1, 3 glucanase enzyme activity, the ethyl alcohol leaf extract of *E. jambolana* treatment recorded the highest increase (79.19%), compared to the control treatment. On the other hand, the aqueous peel extract of *P. granatum* treatment recorded the lowest increase in the activity of β -1, 3 glucanase enzyme (7.53%), compared with other treatments.

Table 7: Chitinase, and β -1, 3 glucanase enzymes activities in soybean plants infected with *Meloidogyne javanica* and treated with natural plant extracts under greenhouse conditions.

Plant Extracts	Extraction medium (Solvents)	Enzyme activity			
		Enzyme unit min^{-1} (mg protein) $^{-1}$.			
		Chitinase activity		β -1, 3 glucanase activity	
		Activity	%Increase	Activity	% Increase
<i>E.jambolana</i>	Distilled water	2.596	108.51	3.968	21.42
	Ethyl alcohol	3.205	157.43	5.856	79.19
	Acetone	2.695	116.47	4.528	38.56
	Mean	2.832	127.47	4.784	46.39
<i>P.granatum</i>	Distilled water	2.865	130.12	3.514	7.53
	Ethyl alcohol	3.754	201.53	4.521	38.34
	Acetone	3.105	149.40	4.015	22.86
	Mean	3.241	160.32	4.017	22.91
<i>C.sinensis</i>	Distilled water	2.124	70.60	3.869	18.39
	Ethyl alcohol	2.865	130.12	4.361	33.45
	Acetone	2.632	111.41	3.856	17.99
	Mean	2.540	104.04	4.029	23.28
Control (nematode only)		1.245	-	3.268	-
LSD _{0.05}					
Plant extracts (A) =		0.06		0.12	
Solvents (B) =		0.08		0.17	
Interactions (A×B) =		0.01		0.01	

Data are average of 3 replicates.

Effect of natural plant extracts on the total phenolic compounds (TPC).

The effect of some natural plant extracts on the phenolic compounds in soybean plants infected with *M. javanica* was determined under greenhouse conditions. Data in Table (8) revealed that all treatments significantly increased the total phenolic contents, compared to the control treatment. The highest percentage increase was recorded with the ethyl alcohol leaf extract of *E. jambolana* (186.66%), followed by acetone extract treatment (153.18%). The minimal percentage increase was recorded by the aqueous leaf extract of *C.sinensis* (50.12%), compared to the other treatments.

DISCUSSION

Many plants have been known for their medicinal and antimicrobial properties against plant-parasitic nematodes, many years ago (Suberu, 2004). Approximately, 2400 plant species are known to possess biologically active compounds that control various plant pests and pathogens effectively. Our results showed that ethyl alcohol extracts of *E. jambolana*, *P. granatum* and *C. sinensis* were the most potent in controlling *M. javanica* compared to the aqueous extracts. These results are in agreement with previous results showing that methanol extracts of pomegranate fruit rinds inhibited egg hatching and viability of second-stage juveniles J2 of *M. incognita* in an in vitro assay (Meyer et al., 2016).

Table 8: Effect of some natural plant extracts on the total phenolic compounds of soybean plants infected with *Meloidogyne javanica* under greenhouse conditions.

Plant Extracts	Extraction medium (solvents)	Total Phenolic contents	
		TPC	% Increase
<i>E.jambolana</i>	Distilled water	3.253	87.82
	Ethyl alcohol	4.965	186.66
	Acetone	4.385	153.18
	Mean	4.201	142.55
<i>P.granatum</i>	Distilled water	3.012	73.90
	Ethyl alcohol	4.215	143.36
	Acetone	3.651	110.80
	Mean	3.626	109.35
<i>C.sinensis</i>	Distilled water	2.6	50.12
	Ethyl alcohol	3.12	80.14
	Acetone	2.905	67.73
	Mean	2.875	65.99
Control (nematode only)		1.732	-
LSD _{0.05}			
Plant extracts (A)	=		0.06
Solvents (B)	=		0.08
Interactions (A×B)	=		0.24

Data are average of 3 replicates.

Similarly, Abdel-Monaim et al. (2017) showed that ethyl alcohol leaf extract of *E. jambolana* was more effective in controlling damping-off and root rot diseases caused by *Fusarium solani* and *Rhizoctonia solani* on faba bean plants under *in vitro* and *in vivo*. Different solvent kinds have been previously used for the extraction of biologically active compounds from plants. As we found in our results, it was also shown that organic solvents are the most effective in the extraction of antimicrobial compounds when compared to water extracts (Parekh et al., 2005).

In the greenhouse experiment, it was found that the organic solvent extracts of all tested plants were generally more efficient than the aqueous extracts in suppressing the nematode reproduction, compared to the control treatment. As per the *in vitro* experiment, the ethyl alcohol leaf extract of *E. jambolana*, was more effective in suppressing gall formation and number of egg masses/plant (root system) developed by *M. javanica* on soybean roots, and number of J₂s in soil. This might be due to the bio-active compounds in the plant extracts that might have a nematotoxic effect against *M. javanica* (Knoblock et al., 1989). Actually, there were some bio-active compounds in the GC-MS analysis of leaf extract *E. jambolana*, which can act singly or in combination against *M. javanica*. These compounds included the flavonoids, which can play multiple defense roles against nematodes involving the inhibition of egg hatching (Wuyts et al., 2006), causing quiescence by slowing down the nematode movement, causing reversible inactivity of the nematodes, repelling the nematode away, or killing them (Birt et al., 2001; Neuhouser, 2004).

Growth of the soybean plants treated with the tested plant extracts significantly increased, compared to the non-treated control plants. Organic solvent extracts were also the most effective compared to the aqueous extract in this respect. Soybean plants treated with ethyl alcohol leaf extract of *E. jambolana* exhibited the highest shoot and root weights and lengths in general. This could be explained by the assumption that

the plant extracts substantially reduced population and damage of *M. javanica* to the infected plant and enhanced plant growth due to their fertilizing ability (Akpheokhai et al., 2012).

Resistance induction in plants is created in activating latent defense mechanisms by means of localized infection by pathogens or as a response to treatment with different biotic agents or abiotic factors (Roberti et al., 2015). Our results revealed that all of the tested plant extracts increased the activities of defense-related enzymes viz. peroxidase, catalase, β -1,3-glucanases, and chitinases. Ethyl alcohol leaf extract of *E. jambolana* also registered the highest activities of oxidative enzymes, as well as total phenolic contents in soybean plants compared to the other treatments.

The synthesis and accumulation of defense-related enzymes have been frequently found to be associated with plant defense mechanisms against various pathogens (Lebeda et al., 1999). Catalase occurs in peroxisomes and decomposes hydrogen peroxide to water and oxygen. Higher concentrations of H_2O_2 regulate programmed cell death, but lower concentrations of H_2O_2 participate in many resistance mechanisms, including reinforcement of the plant cell wall, phytoalexin production, and enhancement of resistance to various stresses. The role of peroxidase in plant defense systems is to remove the toxic effect of hydrogen peroxide from tissues and to participate in the synthesis of phenolic compounds and the building of intermolecular bonds to fortify cell walls at the sites of pathogen invasions (Passardi et al., 2004). Both β -1,3-glucanase and chitinase are well known as PR proteins (Van Loon, 1999). They accumulate in the plants as a response to wounding or infection by pathogens, and there is compelling evidence that β -1,3-glucanase and chitinase are acting alone and/or particularly in combination to contribute in the plant defenses against fungal infection (Leubner-Metzger and Meins, 1999).

As shown from the results of this study, all of the tested plant extracts increased the levels of total phenolics, compared to the control treatment. Also, the ethyl alcohol leaf extract of *E. jambolana* achieved the highest increase in the levels of total phenolics, compared to other treatments. These results are in agreement with previous reports (Abdel-Monaim et al., 2017). Generally, plants containing high amounts of polyphenols are mostly being resistant to several plant diseases (Malik and Singh, 1980). This increase in total phenols could be attributed to their role in enhancing the defense capabilities of the plants to infectious diseases and development of the pathogens. The toxic phenolic compounds in plant cells were found to act through: 1- the structure of the bond form with cell wall components of plant tissues (Mahadevant and Sridhar, 1986), and 2- enhancing the host resistance by stimulating host defense mechanisms (Subba-Rao et al., 1988).

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الملخص العربي

تأثير بعض المستخلصات النباتية الطبيعية في مكافحة نيماتودا تعقد الجذور *Meloidogyne javanica* على فول الصويا تحت ظروف المعمل والصوبة

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امكانية استخدام بعض المستخلصات النباتية لها تأثير في مكافحة النيماتودا المتطفلة على النباتات كبديل للمكافحة الكيماوية. في هذه الدراسة تم استخدام المستخلص المائي والعضوى (كحول- اسيتون) لثلاثة انواع من النباتات الطبية وهى اوراق البامبوزيا - قشور الرمان- الشاي الاخضر تحت ظروف المعمل والصوبة في مكافحة نيماتودا تعقد الجذور *Meloidogyne javanica* على فول الصويا صنف جيزة 35. اوضحت النتائج ان المستخلص الكحولى لكلا من اوراق البامبوزيا وقشور الرمان ادى الى تثبيط كلى قى فقس بيض النيماتودا كليا. اما نسبة تثبيط فقس البيض للمستخلص الكحولى لأوراق الشاي الاخضر كانت 60% فى الوقت ذاته حدث زيادة تدريجية فى نسبة موت اليرقات بزيادة فترة التعرض للمستخلصات من 24 الى 48 ساعة. ايضا اشارت النتائج أن المستخلصات العضوية لجميع النباتات المختبرة كانت أكثرفاعلية في تقليل تكاثر نيماتودا تعقد الجذور على فول الصويا داخل الصوب، وان المستخلص الكحولى لنبات البامبوزيا ادى الى تثبيط تكوين العقد وكذلك كتل البيض على جذور نباتات فول الصويا وكذلك الطور اليرقى الثانى فى التربة، ايضا المستخلص الكحولى لقشور الرمان له تأثير فعال فى تثبيط تكوين كتل البيض على جذور نبات الصويا وكذلك الطور اليرقى الثانى فى التربة. يعتبر المستخلص المائى لقشور الرمان اقل المعاملات كفاءة فى تقليل اعداد العقد النيماتودية وكتل البيض على الجذور وكذلك اعداد الطور اليرقى الثانى فى التربة. ادت معاملة النباتات بالمستخلص الكحولى لأوراق نبات البامبوزيا الى زيادة فى نمو نباتات فول الصويا حيث ادت الى زيادة فى كل من اوزان اطوال المجموع الخضرى بالاضافة الى زيادة طول ووزن الجذر مقارنة بالمعاملة الكنترول. اوضح التحليل الكيماوى لمستخلص الكحول لأوراق نبات البامبوزيا باستخدام التحليل الكروموتوجرافى وجود 33 مركب نشط حيويًا منها "الفلافونويد ومضادات الأكسدة والهيدروكربونات المؤكسدة والأحماض الدهنية غير المشبعة وإستر الأحماض الدهنية وغيرها". اوضحت الدراسات البيوكيميائية ان المعاملة بالمستخلصات النباتية ادت الى زيادة نشاط الانزيمات المرتبطة بمقاومة النبات للأمراض مثل انزيمات البيروكسيديز، كاتاليز، شيتينييز، إنزيمات 1،3 بيتا جلوكاناز بالاضافة الى محتوى النباتات من الفينولات الكلية فى نباتات فول الصويا المصابة بنيماتودا تعقد

الجنور فى الصوبة. بشكل عام كانت المستخلصات العضوية للنباتات المختبرة اكثر كفاءة من المستخلصات المائية فى زيادة نشاط تلك الانزيمات والفينولات الكلية. سجل المستخلص الكحولى لأوراق نبات البامبوزيا اعلى نشاط فى جميع الإنزيمات المختبرة بالاضافه لمحتوى الفينولات الكلية على نباتات فول الصويا.

الكلمات الدالة: *Glycine max*، تأثير مبيدات نيماتودية، انزيمات المقاومة، المركبات الفينولية ، المستخلصات النباتية.