RESEARCH/INVESTIGACIÓN

THE POTENTIAL OF SEPARATE AND COMBINED APPLICATION OF SOME PLANT EXTRACTS AND DEFENSE INDUCER MOLECULES FOR CONTROL OF *Meloidogyne javanica*

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ABSTRACT

Sholevarfard, A. R., and M. R. Moosavi. 2015. The potential of separate and combined application of some plant extracts and defense inducer molecules for controlling of *Meloidogyne javanica*. Nematropica 45:82-95.

The effect of aqueous extracts of *Nigella sativa*, *Ferula assa-foetidae*, *Peganum harmala*, and *Portulaca oleracea*, separately and in combination with salicylic acid (SA), β–aminobutyric acid (BABA), and jasmonic acid (JA) were examined for the control of *M. javanica* in the laboratory and greenhouse. Eggs and second-stage juveniles were exposed to different concentrations of the plant extracts and defense inducer (DI) molecules. In the greenhouse, the effect of treatments on the number of juveniles in roots, nematode reproduction, and plant growth were evaluated. The highest mortality of juveniles and lowest egg hatch occurred after exposure simultaneously to *F. assa-foetida* extract and BABA for 72 hr. Concomitant application of *F. assa-foetida* extract with 1.5 mM concentration of all DI molecules inhibited penetration of juveniles into roots as effectively as cadusafos which was used as a standard nematicide. Concurrent application of 1.5 mM BABA or SA with all of the plant extracts lowered nematode reproduction similar to the nematicide standard. Application of plant extracts and DI molecules did not affect fresh or dry shoot weight, but did affect shoot height and root fresh weight. Based on our data, the defence inducer molecules, in combination with all of the plant extracts that were tested, have potential for the control of *M. javanica*.

Key words: *Ferula assa-foetida*, jasmonic acid, nematode control, *Nigella sativa*, *Peganum harmala*, plant extract, *Portulaca oleracea*, root-knot nematode, salicylic acid, β–aminobutyric acid.

RESUMEN

Sholevarfard, A. R., y M. R. Moosavi. 2015. Potencial de aplicaciones separadas y combinadas de extractos vegetales y moléculas inductoras de defensa para el control de *Meloidogyne javanica*. Nematropica 45:82-95.

Se examinó el efecto de extractos acuosos de *Nigella sativa*, *Ferula assa-foetidae*, *Peganum harmala*, y *Portulaca oleracea*, separadamente y en combinación con ácido salicílico (SA), ácido β–aminobutírico (BABA), y ácido jasmónico (JA) para el control de *M. javanica* en el laboratorio y en invernadero. Se expusieron huevos y juveniles de segundo estadio a diferentes concentraciones de los extractos vegetales y moléculas inductoras de defensa (DI). En invernadero, se evaluó el efecto de los tratamientos sobre el número de juveniles en las raíces, la reproducción del nematodo y el crecimiento vegetal. La mayor mortalidad de juveniles y la menor eclosión de los huevos ocurrieron tras la exposición simultánea al extracto de *F. assa-foetida* y BABA durante 72 hr. Aplicaciones combinadas de extracto de *F. assa-foetida* con concentraciones 1.5 mM de todas las moléculas DI inhibieron la penetración de los juveniles en las raíces, tan efectivamente como el cadusafos, el cual fue usado como nematicida de referencia. Aplicaciones combinadas de 1.5 mM BABA o SA con todos los extractos vegetales redujeron la reproducción del nematodo de forma similar al nematicida de referencia. La aplicación de los extractos vegetales y moléculas DI no afectó al peso fresco o seco de la parte aérea de la planta, pero si afectó a su longitud y al peso fresco la raíces. De acuerdo a nuestros resultados, las moléculas inductoras de defensa en combinación con los extractos vegetales ensayados tienen potencial para el control de *M. javanica*.

Palabras clave: Ferula assa-foetida, ácido jasmónico, control de nematodos, *Nigella sativa*, *Peganum harmala*, extracto vegetal, *Portulaca oleracea*, nematodo agallador, ácido salicílico, ácido β–aminobutírico.

INTRODUCTION

About 5% of all plant losses worldwide are caused by root-knot nematodes (*Meloidogyne* spp.) either as yield loss or due to a loss in quality (Agrios, 2005). *Meloidogyne javanica* is a polyphagous parasite of approximately 2,000 different plant species including both monocotyledons and dicotyledons (Perry *et al.*, 2009). This nematode is the most prevalent species in Iran (Moosavi *et al.*, 2010). In many crops, the control of plant-parasitic nematodes, including *M. javanica*, is mainly based on chemical nematicide, although safe, environmentally appropriate, non-chemical methods would also be desirable (Moosavi and Zare, 2012). Perhaps the best approach to nematode control would be the simultaneous use of two or more practices.

Plants may resist pathogen infection via several different constitutive and induced defense mechanisms (Robert-Seilaniantz *et al.*, 2007; Schwessinger and Zipfel, 2008; Walters, 2011). Plant defense inducer molecules (DI) such as plant hormones or their derivatives are usually involved in commencing the inducible defense mechanisms. Some of the most well-known molecules for this purpose are salicylic acid (SA), jasmonic acid (JA), and β–aminobutyric acid (BABA) (Grant and Jones, 2009; Conrath, 2011; Walters, 2011; Takur and Sohal, 2013).

Numerous plant extracts have been reported to be efficacious in suppression of plant-parasitic nematodes (Ferris and Zheng, 1999; Zasada *et al.*, 2002; Kokalis-Burelle and Rodriguez-Kabana, 2006). Therefore, it is rational to test additional plants or indigenous varieties of plants for their efficacy in immobilizing, retarding development, or killing nematodes (Moosavi, 2012). Several plants in Iran have been reported as anthelmintics and have been traditionally used to treat human and animal diseases caused by nematodes, but there are few reports of their effects on plant-parasitic nematodes.

In this study the effect of aqueous extracts of black cumin (*Nigella sativa*), asafetida (*Ferula assa-foetidae*), harmal (*Peganum harmala*), and common purslane (*Portulaca oleracea*), separately and in combination with SA, BABA, and JA, were examined for their effects on *M. javanica* in the laboratory and greenhouse.

MATERIALS AND METHODS

Preparation of nematode inoculum

Nematodes were propagated on tomato plants (cv. 'Early-Urbana') starting from a single nematode egg sac previously identified as *M. javanica* (Moosavi *et al.*, 2011). To provide sufficient eggs for the tests, galled roots were cut into 0.5 to 1 cm pieces and agitated for 2 to 3 min in 0.5% sodium hypochlorite solution. The suspension was rinsed over 60- and 20-µm sieves (Hussey and Barker, 1973) to collect eggs that were suspended in sterile distilled water and adjusted to 100 eggs per ml. To prepare inoculum of second-stage juveniles (J2), eggs of *M. javanica* were extracted from tomato roots as described above and were put on a filter paper in a Whitehead tray (Whitehead and Hemming, 1965). The active second-stage juveniles (J2) were collected over 3 days and their population was estimated by average of three counts.

Preparation of plant extracts

The plant species (Table 1) were chosen according to the records of their nematicidal effect on human- and animal-parasitic nematodes (Zargari, 1988; Zare Karizi *et al.*, 2011; Fallah Hosseini *et al.*, 2011). They are either commercially available or can be collected from the field. The crushed seeds and asafetida oleo-gum resin were drenched in distilled water for 1 day prior to separating liquid from the macerated plant material using a cotton cloth. The liquid phase was centrifuged at 2,000 g for 20 min, and the supernatant was filtered through a Whatman No. 2 filter paper and a Millipore filter (0.22 µm) to obtain a clear liquid (Ferris and Zheng, 1999). The stock concentration of all extracts was 1 g of plant material per 10 g of distilled water. The required concentrations were prepared from stock solution by diluting it with distilled water.

Laboratory experiment

Two experiments were designed to evaluate the effect of plant extracts and DI molecules on J2. In one experiment, a completely randomized design was performed with five replications of each treatment. Treatments were three concentrations (500, 1,000 and 2,000 ppm) of each plant extract and two DI molecules (BABA and SA; Sigma-Aldrich), each at three concentrations (0.1, 0.7, 1.5 mM). A factorial experiment was also established in a completely

Table 1. List of plant species and their tissue used for aqueous extraction.

Common name	Scientific name	Tissue sampled
Black cumin	*Nigella sativa*	seed
Asafetida	*Ferula assa-foetida*	oleo-gum resin
Harmal	*Peganum harmala*	seed
Common purslane	*Portulaca oleracea*	seed

randomized design investigating four plant extracts (Table 1) each at 2,000 ppm and two DI molecules (BABA and SA) at 1.5 mM. One hundred J2 of *M. javanica* were placed in distilled water in each hole of an ELISA plate. After 2 hr when the nematodes settled down, most of the distilled water was drawn off with a 0.5-ml syringe (without needle) with the intake hole covered with a 20 mM aperture sieve. The removed water was replaced with 200 µl of the fresh plant extract, 200 µl of SA or BABA, or 100 µl of SA or BABA plus 100 µl of plant extract. The control treatment received distilled water. The plates were kept at 26 ± 2°C and nematode mortality was recorded after 24, 48, and 72 hr (Ferris and Zheng, 1999). Nematodes were considered dead when there was a complete lack of motion. The nematodes were poked with a needle when their status was uncertain.

Two similar experiments were also conducted to determine the effect of the same treatments on the viability of *M. javanica* eggs. In this study, 100 freshly extracted eggs were placed into holes in ELISA plates as with the previous trial. The plant extracts, DI molecules and their concentrations were the same as above. Distilled water was used for the control. The plates were kept at 26 ± 2°C in the dark, and the percentage of egg hatch was determined after 24, 48, and 168 hr (Zasada *et al.*, 2002). Egg hatch inhibition was calculated by dividing the number of hatched J2 in the treatments by the number of hatched J2 in the control and multiplying by 100.

Greenhouse experiment

The effects of the plant extracts and DI molecules on the infectivity of *M. javanica* were studied in sand. An experiment was established using a completely randomized design with a factorial treatment arrangement of the four plant extracts (2,000 ppm concentration) and the three DI molecules at concentrations of 0.1, 0.7, and 1.5 mM. In this study, 400 J2 were added to 200 g of sterile sand in 250-ml plastic pots. Thirty ml of plant extracts were added to the soil while the control treatment only received distilled water. The pots were kept in sealed plastic bags at 28°C for two days (Moosavi, 2012). Then a one-week-old tomato seedling (cv. 'Early-Urbana') was transplanted to each pot. In the pots that also included treatment with SA, JA (Sigma-Aldrich), or BABA, 2 ml of each DI molecule at appropriate concentrations were applied to the seedlings as a foliar spray with a perfume atomizer 24 hr before transplanting (Nandi *et al.*, 2002). The organophosphate insecticide/nematicide, cadusafos (FMC, USA), was used at a rate of 20 mg per kg soil as a control standard.

The experiment had four replications. After 14 days incubation at 28 ± 2°C, the plants were uprooted, their roots stained with acid-fuchsin (Byrd *et al.*, 1983), and the number of nematodes in the roots was counted. The corrected percent of J2 that penetrated into the roots was calculated by dividing the number of penetrated J2 in the treatments by the number of penetrated J2 in control and then multiplying by 100.

The effect of the plant extracts and DI molecules on the reproduction and pathogenicity of *M. javanica* was evaluated in the greenhouse. Experimental design was completely randomized in a factorial arrangement with five replications. Five tomato seeds (cv. Early-Urbana) were planted in 12.5- cm-diam plastic pots containing 1 kg non-sterile sandy soil. After one month, one seedling was selected, and others were eliminated from the pot. Five thousand eggs and J2 of *M. javanica* were added to the soil around the roots of tomato seedlings in 20-ml water. Ten milliliters of the appropriate concentrations of the DI molecules was applied as a foliar spray by an atomizer 24 hr before inoculation. Spraying was similarly repeated after 4 days (Nandi *et al.*, 2002). In the treatments that should have received plant extracts (separately or in combination with plant extracts), a total of 200 ml extract with 2,000 ppm concentration was applied per pot 1 hr after inoculation with nematode (Zasada *et al.*, 2002).

All pots were kept in a greenhouse at ambient temperature of 27 ± 3°C. After 8 wk, the plants were harvested and their fresh top and root weight, dry top weight, and top height were determined. Soil from each pot was mixed thoroughly, and nematodes were extracted from a 100-g soil sub-sample by the Baermann funnel technique over 48 hr. Juveniles were collected on 25-µm sieves and counted. To determine nematode multiplication, juveniles and eggs were extracted from the root systems by blender maceration in a 1% (v/v) NaOCl solution for 4 min (Bourne *et al.*, 1996). A reproduction factor (P_f/P_i) for each treatment was calculated by dividing total eggs and J2 that were collected at the end of the experiment (P_f) by the inoculation rate that was applied at the beginning (P_i). The efficacy of each treatment against *M. javanica* was calculated using the following formula ($\text{Control efficacy} = \frac{X-Y}{X} \times 100$) while X= (eggs+J2)/g soil of control and Y= (eggs+J2)/g soil of treatment (Moosavi *et al.*, 2010, 2011; Moosavi, 2012).

Statistical analysis

All experiments were repeated twice in time. Since the same trend was observed in the two experiments, the data were combined. Statistical analyses were performed using SAS (ver. 9.2) software, and the means were separated by Duncan's Multiple Range Test.

Where the control was not a part of the factorial experiments, two statistical analyses were performed to analyze the data. An analysis of variance was carried out for the factorial and another one-way analysis of variance was done to include all treatments. The means were separated by Duncan's Multiple Range Test in the second analysis.

RESULTS

Laboratory experiment

Significant difference was observed in the mortality of J2 when they were exposed to different concentrations of plant extracts and DI molecules for 24 hr (F=62.1, df=18, P<0.0001); 48 hr (F=333.1, df=18, P<0.0001); and 72 hr (F=418.4, df=18, P<0.0001). The highest mortality of J2 occurred with 2,000 ppm of *F. assa-foetida* extract regardless of the exposure time (Table 2). After 72 hr, the highest mortality occurred when the J2 were exposed to 2,000 ppm of *F. assa-foetida* extract (38.4%) or 2,000 ppm of *P. oleracea* extract (33.2%). Exposure of J2 to 500 ppm of *P. oleracea* for 72 hr resulted in the least mortality (18.6%) among all plant extracts (Table 2). BABA was more effective against J2 at all concentrations and exposure times compared with SA. When J2 were exposed to 0.1 mM SA for 72 hr, their mortality was lower than mortality in the water control. The percent of dead J2 in all

Table 2. The effect of different plant extracts and defense inducer (DI) molecules on mortality of *Meloidogyne javanica* second-stage juveniles (J2).

Plant extract or DI molecule	Concentration	% dead J2 (mean ± SE) [y,z]		
		24 hr	48 hr	72 hr
Ferula assa-foetida	500 ppm	7.6 (± 0.5) f A	23.2 (± 0.6) c B	27.6 (± 0.5) d C
	1000 ppm	9.8 (± 0.4) cd A	28.2 (± 0.4) b B	31.6 (± 0.4) c C
	2000 ppm	13.2 (± 0.4) a A	33.4 (± 0.5) a B	38.4 (± 0.5) a C
Portulaca oleracea	500 ppm	2.4 (± 0.5) I A	15.4 (± 0.5) f B	18.6 (± 0.5) j C
	1000 ppm	4.6 (± 0.5) h A	18.6 (± 0.5) e B	24.2 (± 0.4) fg C
	2000 ppm	11.4 (± 0.5) b A	28.2 (± 0.9) b B	33.2 (± 0.7) b C
Peganum harmala	500 ppm	4 (± 0.3) h A	12.2 (± 0.4) g B	21 (± 0.7) hi C
	1000 ppm	6.2 (± 0.4) g A	14.4 (± 0.5) f B	21.4 (± 0.5) h C
	2000 ppm	8 (± 0.3) ef A	21.6 (± 0.5) d B	25.6 (± 0.5) e C
Nigella sativa	500 ppm	3.4 (± 0.5) hi A	9 (± 0.3) ij B	20 (± 0.5) i C
	1000 ppm	7.6 (± 0.2) d A	15 (± 0.5) f B	23.2 (± 0.4) g C
	2000 ppm	9.8 (± 0.4) cd A	18.6 (± 0.4) e B	24.6 (± 0.5) ef C
Salicylic acid	0.1 mM	4 (± 0.4) h A	4.2 (± 0.8) m A	5.2 (± 0.4) o A
	0.7 mM	3.4 (± 0.5) hi A	6 (± 0.3) l B	7.4 (± 0.2) n C
	1.5 mM	6 (± 0.3) g A	7.4 (± 0.2) k B	10.2 (± 0.4) m C
β-amino butyric acid	0.1 mM	8 (± 0.3) ef A	8.6 (± 0.5) jk A	11 (± 0.3) m B
	0.7 mM	9.2 (± 0.4) de A	10.2 (± 0.2) hi B	13 (± 0.3) l C
	1.5 mM	10.6 (± 0.9) bc A	10.6 (± 0.5) h A	15 (± 0.3) k B
Control		2.2 (± 0.4) i A	3.8 (± 0.4) m B	6.8 (± 0.4) n C

[y] Mean values followed by different uppercase letters on the same row, or followed by different lowercase letters in the same column are significantly different according to Duncan's test (P < 0.05).
[z] Each treatment had ten replications (five replications in two successive experiments).

Table 3. The effect of different plant extracts and defense inducer (DI) molecules on inhibition of egg hatch.

Plant extract or DI molecule	Concentration	% dead J2 (mean ± SE)[y,z]		
		24 hr	48 hr	72 hr
Ferula assa-foetida	500 ppm	21 (± 0.7) de A	27.8 (± 0.9) cd B	52.4 (± 0.9) c C
	1000 ppm	25 (± 0.8) bc A	33.4 (± 0.9) b B	54.6 (± 0.8) bc C
	2000 ppm	27 (± 0.7) ab A	36.4 (± 0.8) a B	62.4 (± 1.3) a C
Portulaca oleracea	500 ppm	12.6 (± 0.9) g A	22 (± 0.7) e B	44.2 (± 1) e C
	1000 ppm	19.6 (± 1) ef A	28.2 (± 0.9) c B	54 (± 0.8) bc C
	2000 ppm	25.2 (± 0.6) bc A	34.2 (± 1) ab B	57 (± 0.7) b C
Peganum harmala	500 ppm	19.4 (± 0.8) ef A	26 (± 1) cd B	47 (± 1) de C
	1000 ppm	23 (± 0.8) cd A	29.2 (± 1.2) bc B	48 (± 0.9) d C
	2000 ppm	27.8 (± 1) a A	36.8 (± 1) a B	62.2 (± 0.9) a C
Nigella sativa	500 ppm	18.2 (± 1) f A	25.2 (± 0.9) d B	48.2 (± 1.3) d C
	1000 ppm	21.2 (± 0.9) de A	28.6 (± 1) c B	53.8 (± 1) bc C
	2000 ppm	24.4 (± 1.2) bc A	34.6 (± 0.8) ab B	56.6 (± 0.9) b C
Salicylic acid	0.1 mM	2.6 (± 0.4) h A	4 (± 1) f AB	5.6 (± 1) g B
	0.7 mM	3 (± 0.7) h A	4.2 (± 0.9)f A	7.4 (± 0.9) fg B
	1.5 mM	3.2 (± 1) h A	5 (± 0.8) f A	5.6 (± 1.3) g A
β-amino butyric acid	0.1 mM	3.6 (± 0.9) h A	6.4 (± 1.4)f AB	8.4 (± 1.4) fg B
	0.7 mM	2.2 (± 0.7) h A	5.6 (± 0.9) f B	9.6 (± 1.2) f C
	1.5 mM	2.8 (± 0.8) h A	5.6 (± 1) f A	10 (± 1) f B

[y] Mean values followed by different uppercase letters on the same row, or followed by different lowercase letters in the same column are significantly different according to Duncan's test ($P < 0.05$).
[z] Each treatment had ten replications (five replications in two successive experiments).

treatments increased with increased exposure time or concentration; however, the rate of increase was lower for the DI molecules than for the plant extracts (Table 2).

All of the plant extracts and DI molecules significantly inhibited egg hatching of *M. javanica* after 24 hr (F=131.3, df=17, P<0.0001); 48 hr (F=170.5, df=17, P<0.0001); and 1 wk (F=467.7, df=17, P<0.0001) relative to the water control (Table 3). The highest level of hatch inhibition occurred when the eggs were exposed for 1 wk to 2,000 ppm of *F. assa-foetida* extract (62.4%) or 2,000 ppm of *P. harmala* extract (62.2%). Exposure of nematode eggs to 500 ppm of *P. oleracea* for one week resulted in the least hatch inhibition (44%) among all plant extracts. The inhibitory effect of the plant extracts on egg hatching was more than of the DI molecules. The lowest hatch inhibition of the plant extracts was more than the maximum amount of hatch inhibition by the DI molecules (Table 3).

Salicylic acid had the least inhibitory effect on egg hatching regardless of the exposure time. At a similar time of exposure, the lowest egg hatching occurred at the highest concentration of each plant extract or DI molecule. Egg hatch inhibition increased as the time of exposure increased (Table 3).

Analysis of variance for the effect of the plant extracts, DI molecules, and their interaction on J2 mortality and egg hatch inhibition is demonstrated in Table 4. The ability of the plant extracts to kill J2 was significantly different except for the first 24 hr. After 48 and 72 hr, the extracts of *F. assa-foetida* and *P. harmala* were more effective than the other extracts. BABA was more effective in killing J2 for

Table 4. Analysis of variance for *Meloidogyne javanica* second-stage juvenile (J2) mortality and egg hatch inhibition when they were exposed to the plant extracts and defense inducer (DI) molecules in a factorial design.

Source[y]	Mean squares					
	% J2 mortality			% Egg hatch inhibition		
	24 hr	48 hr	72 hr	24 hr	48 hr	1 week
E	4.3 ns[z]	58.3**	181.7**	246.5**	643.7**	2935.5**
DI	27.2	11	3 ns	122.5**	140.6**	348.1**
E×DI	42.5**	384.3**	798.1**	59.3**	15 ns	17.1 ns
Error	68.4	82.4	39.7	120.8	126.4	149.2
C.V. (%)	24.12	18.47	21.26	17.33	27.69	12.83

[y] E = plant extract, DI = defence inducer molecule.
[z] ns = Not significant, * = significant at 5% probability and ** = significant at 1% probability.

Table 5. The effect of the plant extracts and defense inducer (DI) molecules on mortality of *Meloidogyne javanica* second-stage juveniles (J2).

DI molecules	Plant extract (2,000 ppm)	% dead J2 (mean ± SE) [z]		
		24 hr	48 hr	72 hr
β-amino butyric acid (1.5 mM)	*Ferula assa-foetida*	11.2 (± 0.6) a A	26.8 (± 0.7) a B	54.4 (± 0.9) a C
	Portulaca oleracea	9 (± 0.7) b A	16.2 (± 0.7) e B	44.2 (± 0.9) d C
	Peganum harmala	9.8 (± 0.6) ab A	21.2 (± 0.9) cd B	53.6 (± 1) ab C
	Nigella sativa	8.8 (± 0.6) b A	19.6 (± 0.5) d B	39 (± 0.7) e C
Salicylic acid (1.5 mM)	*Ferula assa-foetida*	6 (± 0.7) c A	17.4 (± 0.5) e B	46.4 (± 0.8) cd C
	Portulaca oleracea	8.8 (± 0.6) b A	22.6 (± 0.8) bc B	47.6 (± 1.2) c C
	Peganum harmala	9 (± 0.8) b A	23.6 (± 0.9) b B	43.8 (± 0.7) d C
	Nigella sativa	8.4 (± 0.6) b A	24.4 (± 0.7) b B	51.2 (± 0.9) b C
Control		2.2 (± 0.4) d A	3.8 (± 0.4) f B	6.8 (± 0.4) f C

[z] Mean values followed by different uppercase letters on the same row, or followed by different lowercase letters in the same column are significantly different according to Duncan's test ($P < 0.05$). Each treatment had ten replications (five replications in two successive experiments).

Table 6. The effect of the plant extracts and defense inducer (DI) molecules on inhibition of egg hatch of *Meloidogyne javanica*.

DI molecules	Plant extract (2,000 ppm)	% dead J2 (mean ± SE) [z]		
		24 hr	48 hr	72 hr
β-amino butyric acid (1.5 mM)	*Ferula assa-foetida*	45.2 (± 0.7) a A	56.8 (± 0.9) a B	82 (± 0.7) a C
	Portulaca oleracea	35.2 (± 0.7) d A	44 (± 1) ef B	65.4 (± 0.8) d C
	Peganum harmala	42.4 (± 0.9) b A	51.2 (± 1) b B	72 (± 1.1) c C
	Nigella sativa	39.6 (± 0.9) c A	49 (± 1) bcB	60.2 (± 0.9) e C
Salicylic acid (1.5 mM)	*Ferula assa-foetida*	38.8 (± 0.6) c A	51.6 (± 0.8) b B	78 (± 1) b C
	Portulaca oleracea	35.2 (± 1) d A	42.2 (± 0.7) f B	57.8 (± 0.9) e C
	Peganum harmala	37.4 (± 1) cd A	47.2 (± 0.9) cd B	65.6 (± 1.3) d C
	Nigella sativa	37 (± 0.9) cd A	45 (± 0.5) de B	54.6 (± 0.9) f C

[z] Mean values followed by different uppercase letters on the same row, or followed by different lowercase letters in the same column are significantly different according to Duncan's test ($P < 0.05$). Each treatment had ten replications (five replications in two successive experiments).

the first 24 hr, but SA was more effective after 48 hr. No significant difference in J2 mortality was seen when they were exposed to the DI molecules for 72 hr. The interaction between the plant extracts and DI molecules significantly affected J2 mortality at all exposure times (Table 4).

Egg hatch inhibition at all exposure times significantly differed according to the plant extracts and DI molecules. *Ferula assa-foetida* extract was the most effective plant extract in reducing egg hatching rate regardless of the exposure time. The inhibitory effect of the interaction between the plant extracts and DI molecules on egg hatching was only significant when the exposure time was 24 hr (Table 4).

Table 5 shows the effects of the DI molecules (1.5 mM) and plant extracts (2,000 ppm) on J2 mortality. The maximum mortality of J2 after 24 and 72 hr was seen when *F. assa-foetida* extract and *P. harmala* extract was used in combination with BABA. After 48 hr, the highest mortality (26.8 %) occurred when the J2 were exposed simultaneously to *F. assa-foetida* extract and BABA.

In most cases, simultaneous exposure of the nematode eggs to the DI molecules and plant extracts resulted in greater inhibition of egg hatching than with the separate use of the DI molecules or plant extracts. The most effective combination in suppressing egg hatch was BABA with *F. assa-foetida* extract (Table 6).

Greenhouse experiment

The plant extracts, DI molecules and their interaction affected the penetration rate of J2 into the tomato roots (Table 7). The lowest J2 penetration occurred when the *F. assa-foetida* extract (30.1%) was applied to soil or when 1.5 mM SA (34%) was sprayed onto the seedlings. Among the DI molecules, the second lowest J2 penetration occurred when 1.5 mM BABA (39.4%) and 1.5 mM JA (41.1%) were sprayed onto the seedlings.

The nematicide standard (cadusafos) resulted in the highest inhibition of J2 penetration (88.2%). The *F. assa-foetida* extract in combination with 1.5 mM concentration of all DI molecules or 0.7 mM SA and BABA inhibited J2 penetration more effectively than the other treatments. The inhibition of J2 penetration by the *P. harmala* extract in combination with 1.5 mM SA was similar to the effect of a combination of *P. harmala* extract with 1.5 mM BABA. The *N. sativa* extract was the least effective in inhibiting J2 penetration when it was applied either separately or simultaneously with the DI molecules (Table 9).

Though nematode reproduction was significantly affected by the plant extracts, DI molecules and their interactions (Table 7), the growth of the tomato plants was less influenced (Table 8).

Considering the DI molecules as a main factor, the highest nematode control occurred when the plants were sprayed with a 1.5 mM concentration of all DI molecules (88.4, 88.4, and 86.8% for BABA, SA and JA, respectively). The lowest final population of the nematode was seen in the pots that were sprayed with 1.5 mM of all DI molecules (24.1, 24.1, and 27.5 eggs and J2/g soil for SA, BABA, and JA, respectively) or 0.7 mM BABA (29.6 eggs and J2/g soil).

The extracts of *F. assa-foetida* (85.5%) and *P. harmala* (84.3%) had the best efficacy in nematode control. The lowest reproduction rate occurred when the extract of *F. assa-foetida* (5.9 fold) and *P. harmala* (6.7 fold) were applied to soil relative to the treatments that did not receive any plant extract (10.9 fold).

Among the treatments that only received one of the plant extracts or DI molecules, the plants that were sprayed with 1.5 mM SA had the lowest final population (25 egg and J2 /g soil) of nematode (Table 9). This treatment was comparable with cadusafos (17.2 egg and J2 /g soil), 1.5 mM BABA (31.5 egg and J2 /g soil) or 1.5 mM JA (32.6 egg and J2 /g soil) (Table 9).

There were some promising treatments in controlling of *M. javanica* and in reducing the level of the nematode reproduction rate when the extracts and DI molecules were applied simultaneously (Table 9). Combined use of 1.5 mM BABA with all of the plant extracts reduced nematode reproduction rate similar to cadusafos. Similarly, the extract of *F. assa-foetida* with 1.5 mM SA and JA or with 0.7 mM BABA decreased the nematode reproduction rate to the same degree as cadusafos (Table 9).

The effect of the plant extracts as a main factor was only significant on the root fresh weight, while the DI molecules and the interaction of the DI molecules and plant extracts significantly affected shoot height and root fresh weight (Table 8). A significant increase in shoot height was seen in the plants that were sprayed with 0.7 mM JA (16. cm) relative to the plants that were not sprayed with any DI molecules (12.5 cm).

Generally, plant height was significantly greater in the treatments that were sprayed with a 1.5 mM concentration of all DI molecules relative to the water control (Table 10). When the DI molecules were applied at 1.5 mM concentration, in combination with or without the plant extracts, the root fresh weight was the same as in the chemical standard (cadusafos). The root weight of the treatments that only received the plant extract (and no DI molecules) was significantly higher than the root weight in the

Table 7. Analysis of variance for the corrected percent of second-stage juveniles (J2) in roots and nematode reproduction on tomato when they were exposed to four plant extracts and three defense inducer (DI) molecules at three concentrations (0.1, 0.7, and 1.5 mM).

Source[w]	Mean squares			
	Corrected percent of root penetration [x]	Total egg and J2 /g soil [y]	Pf/Pi [y]	Control efficacy [y]
E	23110.8**[z]	13266.6**	533.2**	320.8**
DI	8512.5**	55674.4**	2831.72**	2872.9**
E×DI	4190**	71702.7**	3040.5**	2801.3**
Error	2518.4	7789	530.5	1464.6
C.V. (%)	27.38	22.53	25.61	8.72

[w] E = plant extract, DI = defense inducer molecules
[x] The J2 penetration percent of treatments was corrected according to the water control. Each treatment had eight replications (four replications in two successive experiments).
[y] Each treatment had ten replications (five replications in two successive experiments).
[z]ns, *, **: Non significant, significant at 5% and 1% probability levels, respectively.

Table 8. Analysis of variance for growth of nematode-infected tomato when exposed to four plant extracts (E) and three defense inducer (DI) molecules at three concentrations (0.1, 0.7, and 1.5 mM).

Source[x]	Mean squares			
	Shoot height[y]	Shoot fresh weight[y]	Shoot dry weight[y]	Root fresh weight[y]
E	47.2 ns[z]	7.6 ns	0.06 ns	0.65**
DI	228.5*	82.7 ns	0.89 ns	15.87**
E×DI	798.6*	292.5 ns	6.37 ns	3.38**
Error	2714	1535.3	28.33	4.85
C.V. (%)	22.28	37.84	34.46	18.63

[x] E = plant extract, DI = defense inducer molecules.
[y] Each treatment had ten replications (five replications in two successive experiments).
[z] ns, *, **: Non significant, significant at 5% and 1% probability levels, respectively.

Table 9. The effect of the plant extracts and defense inducer (DI) molecules on invasion of tomato roots by second-stage juveniles (J2) of *Meloidogyne javanica* 14 days after inoculation and on nematode reproduction 8 weeks after inoculation.

Plant extract (2,000 ppm)	DI molecule and concentration (mM)[x]	Corrected percent of root penetration[y]	Total egg and J2 /g soil[z]	Pf/Pi[z]	Control efficacy[z]
–	–	100 (± 3.8) z	208.5 (± 10.9) a	41.7 (± 2.2) a	–
	BABA 0.1	74.4 (± 3.9) x	42.7 (± 2.1) c–f	8.5 (± 0.4) e–j	79.5 (± 1) k–o
	BABA 0.7	58.9 (± 2.7) s–w	36.5 (± 2.9) e–l	7.3 (± 0.6) h–m	82.5 (± 1.4) g–l
	BABA 1.5	47.1 (± 2.2) k–r	31.5 (± 2.8) i–o	6.3 (± 0.5) j–o	84.9 (± 1.3) e–j
	JA 0.1	83.7 (± 3.9) y	49.6 (± 2.8) c	9.9 (± 0.6) b–g	76.2 (± 1.3) o
	JA 0.7	66.3 (± 3.8) w	39 (± 3.1) d–j	7.8 (± 0.6) g–k	81.3 (± 1.5) j–n
	JA 1.5	52.7 (± 3.3) q–u	32.6 (± 4.5) h–n	6.5 (± 0.9) i–n	84.4 (± 2.2) f–j
	SA 0.1	77.5 (± 4.3) xy	42.1 (± 4.5) c–h	8.4 (± 0.9) f–j	79.8 (± 2.1) k–o
	SA 0.7	62 (± 4.2) uvw	35.8 (± 1.8) e–l	7.2 (± 0.4) h–m	82.8 (± 0.8) g–l
	SA 1.5	44.6 (± 3.2) i–q	25.3 (± 1.4) m–r	5.1 (± 0.3) l–r	87.9 (± 0.7) a–f

Table 9. (Continued) The effect of the plant extracts and defense inducer (DI) molecules on invasion of tomato roots by second-stage juveniles (J2) of *Meloidogyne javanica* 14 days after inoculation and on nematode reproduction 8 weeks after inoculation.

Plant extract (2,000 ppm)	DI molecule and concentration (mM)[x]	Corrected percent of root penetration[y]	Total egg and J2 /g soil[z]	Pf/Pi[z]	Control efficacy[z]
	–	62 (± 2.7) uvw	60.9 (± 3.7) b	12.2 (± 0.6) b	70.8 (± 1.6) p
	BABA 0.1	60.8 (± 1.2) t–w	42.1 (± 1.7)c–h	11 (± 0.3) bcd	79.8 (± 0.8) k–o
	BABA 0.7	55.2 (± 2.7) r–v	31.7 (± 2) i–o	5.9 (± 0.4) k–q	84.8 (± 1) e–j
	BABA 1.5	49.6 (± 3.3) n–s	25 (± 1.3) m–r	4 (± 0.3) n–r	88 (± 0.6) a–f
	JA 0.1	62.6 (± 2.7) vw	43.1 (± 3.9) c–f	11.8 (± 0.7) b	79.3 (± 1.8) k–o
Nigella sativa	JA 0.7	58.9 (± 2.7) s–w	35.7 (± 3) e–l	7.8 (± 0.6) g–k	82.9 (± 1.4) g–l
	JA 1.5	51.5 (± 2.6) p–t	29.9 (± 1.9) i–p	5.5 (± 0.4) k–r	85.6 (± 0.9) d–i
	SA 0.1	50.2 (± 3.9) o–s	44.1 (± 2.1) c–f	10.9 (± 0.4) b–e	78.8 (± 1.7) l–o
	SA 0.7	43.4 (± 2.2) i–q	36 (± 1.7) e–l	7.3 (± 0.3) h–m	82.7 (± 0.8) g–l
	SA 1.5	37.8 (± 3.3) e–k	23 (± 2.7) n–r	3.9 (± 0.5) o–r	88.9 (± 1.3) a–e
	–	45.3 (± 2.8) j–q	46.7(± 3.3) cd	9.3 (± 0.7) c–h	77.6 (± 1.8) no
	BABA 0.1	44 (± 2.2) i–q	39.4 (± 0.9) d–i	8.5 (± 0.2) e–j	81.1 (± 0.4) j–n
	BABA 0.7	38.4 (± 2.2) e–l	27.7 (± 3.8) l–r	5 (± 0.8) l–r	86.7 (± 1.8) a–g
	BABA 1.5	32.2 (± 2.3) c–h	20.8 (± 1.1) pqr	3.2 (± 0.2) qr	90 (± 0.5) abc
	JA 0.1	48.4 (± 2.8) m–r	44 (± 3.1) c–f	10.9 (± 0.6) b–e	78.9 (± 1.5) l–o
Peganum harmala	JA 0.7	45.3 (± 2.7) j–q	28 (± 2.1) k–r	5.4 (± 0.4) k–r	86.6 (± 1) a–g
	JA 1.5	35.3 (± 3.9) d–i	29.4 (± 2) j–q	5.1 (± 0.4) l–r	85.9 (± 0.9) c–i
	SA 0.1	40.9 (± 2.1) h–o	34.7 (± 2.6) f–m	9.2 (± 0.5) d–h	83.4 (± 1.2) g–k
	SA 0.7	38.4 (± 2.7) e–l	32.8 (± 1.4) g–n	6.7 (± 0.3) i–m	84.3 (± 0.7) f–j
	SA 1.5	30.4 (± 3.3) c–f	24.1 (± 0.8) n–r	4 (± 0.2) n–r	88.4 (± 0.4) a–f
	–	51.5 (± 2.7) p–t	58.3 (± 5) b	11.6 (± 1) bc	72 (± 2.4) p
	BABA 0.1	55.2 (± 3.3) r–v	42.1 (± 1.7)c–h	9.9 (± 0.3) b–g	79.8 (± 0.8) k–o
	BABA 0.7	47.7 (± 2.2) l–r	29 (± 2.7) k–r	5.4 (± 0.5) k–r	86.1 (± 1.3) c–i
	BABA 1.5	42.8 (± 1.9) i–p	23.7 (± 1.4) n–r	3.8 (± 0.3) o–r	88.6 (± 0.7) a–e
	JA 0.1	48.4 (± 2.8) m–r	41.9 (± 2.8) c–h	10.3 (± 0.6) b–f	79.9 (± 1.3) j–o
Portulaca oleracea	JA 0.7	45.3 (± 3.3) j–q	28.6 (± 2.6) k–r	5.4 (± 0.5) k–r	86.3 (± 1.2) b–h
	JA 1.5	40.3 (± 1.6) g–n	23.2 (± 1.6) n–r	3.7 (± 0.3) pqr	88.9 (± 0.7) a–e
	SA 0.1	49.6 (± 2.2) n–s	45.45 (± 4.1) cde	12 (± 0.8) b	78.2 (± 1.9) mno
	SA 0.7	42.8 (± 2.8) i–p	31.6 (± 0.3) i–o	6.1 (± 0.05) j–p	84.9 (± 0.1) e–j
	SA 1.5	36 (± 2.7) d–j	28 (± 3.5) k–r	4.8 (± 0.7) m–r	86.6 (± 1.6) a–g
	–	32.9 (± 1.6) c–h	42.4 (± 3.2) c–g	8.5 (± 0.6) e–j	79.7 (± 1.5) k–o
	BABA 0.1	36.6 (± 4) d–j	37.3 (± 3.7) d–l	7.5 (± 0.7) g–l	82.1 (± 1.8) h–m
	BABA 0.7	29.8 (± 2.1) b–e	22.9 (± 2.4) n–r	4.1 (± 0.5) n–r	89 (± 1.1) a–e
	BABA 1.5	24.8 (± 2.7) bc	19.6 (± 1.2) r	3 (± 0.2) r	90.6 (± 0.6) a
	JA 0.1	39.7 (± 2.8) f–m	37.2 (± 2.2) d–l	8.9 (± 0.4) d–i	82.1 (± 1) h–m
Ferula assa-foetida	JA 0.7	31 (± 2.7) c–g	37.8 (± 4.2) d–k	8.3 (± 0.8) f–j	83.8 (± 2) i–m
	JA 1.5	25.4 (± 1.6) bc	22.3 (± 3) o–r	3.7 (± 0.6) o–r	89.3 (± 1.5) a–d
	SA 0.1	31 (± 1.6) c–g	34.5 (± 3.3) h–n	6.6 (± 0.6) i–m	84.4 (± 1.7) f–j
	SA 0.7	27.9 (± 2.8) bcd	29.4 (± 1.2) j–q	5.4 (± 0.3) k–r	85.9 (± 0.5) c–i
		21.1 (± 1.6) b	20.1 (± 0.9) qr	3 (± 0.1) r	90.4 (± 0.4) ab
Nematicide (cadusafos)	0.2%	11.8 (± 2.6) a	17.2 (± 1.7) r	3.4 (± 0.3) qr	91.8 (± 0.8) a

Values in the same column followed by different letter(s) are significantly different according to Duncan's test ($P < 0.05$).
[x] DI = defense inducer molecules; SA = salicylic acid; JA = jasmonic acid; BABA = β-amino butyric acid
[y] The percent of penetrated J2 into roots was corrected according to water control. Each treatment had eight replications (four replications in two successive experiments). Values in parenthesis show ± standard error.
[z] Each treatment had ten replications (five replications in two successive experiments). Values in parenthesis show ± standard error.

Table 10. The effect of the plant extracts and defense inducer molecules on plant growth 8 weeks after inoculation with nematodes.

Plant extract (2,000 ppm)	DI molecule and concentration (mM)[x]	Shoot [y, z]			Root fresh weight (g) [y, z]
		height (cm)	fresh weight (g)	dry weight (g)	
–	–	6.1 (± 0.6) c	3.7 (± 0.4) c	0.5 (± 0.04) de	2.2 (± 0.13) a
	BABA 0.1	15.7 (± 2.9) abc	6.5 (± 1.8) bc	0.8 (± 0.26) a–e	1.2 (± 0.09) d–j
	BABA 0.7	12.3 (± 0.4) bc	5.3 (± 0.7) bc	0.6 (± 0.11) cde	0.9 (± 0.07) k–t
	BABA 1.5	18.1 (± 1.6) ab	8.8 (± 1.5) ab	0.94 (± 0.18) a–e	0.7 (± 0.1) q–t
	JA 0.1	13.8 (± 1.2) abc	4.1 (± 0.6) c	0.5 (± 0.14) de	1.5 (± 0.13) bc
	JA 0.7	15.5 (± 1.4) abc	5.3 (± 0.5) bc	0.6 (± 12) cde	0.9 (± 0.07) k–t
	JA 1.5	14.1 (± 1.3) abc	6.9 (± 1.5) bc	0.94 (± 0.29) a–e	0.7 (± 0.11) st
	SA 0.1	17.4 (± 1.1) ab	7.7 (± 0.7) bc	1.1 (± 0.08) a–d	1.3 (± 0.1) c–g
	SA 0.7	15 (± 1) abc	5.3 (± 1) bc	0.6 (± 0.16) b–e	1.1 (± 0.08) e–k
	SA 1.5	18.1 (± 2.6) ab	6.6 (± 1.7) bc	0.7 (± 0.21) a–e	0.8 (± 0.09) n–t
Nigella sativa	–	13.8 (± 0.8) abc	5.1 (± 1.5) bc	0.6 (± 0.21) cde	1.3 (± 0.1) c–g
	BABA 0.1	14.6 (± 0.7) abc	5.7 (± 0.7) bc	0.7 (± 0.1) b–e	1.3 (± 0.1) c–f
	BABA 0.7	12.7 (± 0.8) bc	3.8 (± 0.8) c	0.6 (± 0.1) b–e	0.9 (± 0.03) l–t
	BABA 1.5	15.8 (± 2.2) abc	5.4 (± 1.1) bc	0.7 (± 0.1) a–e	0.7 (± 0.02) q–t
	JA 0.1	17.2 (± 1.5) ab	6.8 (± 1.1) bc	0.8 (± 0.1) a–e	1.4 (± 0.01) bcd
	JA 0.7	19.9 (± 3.3) a	9.1 (± 2) ab	1.2 (± 0.3) abc	1.1 (± 0.03) e–l
	JA 1.5	16.5 (± 1.2) abc	6.8 (± 0.8) bc	0.8 (± 0.1) a–e	0.9 (± 0.03) k–t
	SA 0.1	17.4 (± 2.4) ab	6.3 (± 1.4) bc	0.7 (± 0.1) b–e	1.3 (± 0.07) c–h
	SA 0.7	14.2 (± 2) abc	5.5 (± 1.1) bc	0.6 (± 0.1) b–de	1 (± 0.04) h–o
	SA 1.5	16.1 (± 0.9) abc	6.7 (± 08.) bc	0.9 (± 0.1) a–e	0.8 (± 0.04) o–t
Peganum harmala	–	14.8 (± 0.9) abc	5.6 (± 1.3) bc	0.8 (± 0.15) a–e	1.3 (± 0.1) c–f
	BABA 0.1	12.7 (± 1.7) bc	4.9 (± 1.2) bc	0.6 (± 0.1) b–e	1.1 (± 0.03) f–m
	BABA 0.7	16.6 (± 4.1) abc	6.9 (± 1.4) bc	0.9 (± 0.2) a–e	0.9 (± 0.05) l–t
	BABA 1.5	14.2 (± 0.7) abc	6.1 (± 1.1) bc	0.6 (± 0.1) b–e	0.7 (± 0.02) st
	JA 0.1	13.5 (± 3) bc	5.3 (± 1.4) bc	0.7 (± 0.2) b–e	1.3 (± 0.04) c–g
	JA 0.7	16.3 (± 1.8) abc	6.3 (± 1.4) bc	0.7 (± 0.2) b–e	0.9 (± 0.08) k–r
	JA 1.5	14.5 (± 1.8) abc	7.1 (± 1.6) bc	0.9 (± 0.2) a–e	0.8 (± 0.02) m–t
	SA 0.1	14.4 (± 3.3) abc	7 (± 1.6) bc	0.7 (± 0.2) b–e	1.3 (± 0.03) cde
	SA 0.7	16.8 (± 1.7) abc	6.5 (± 1.5) bc	0.7 (± 0.2) a–e	1 (± 0.03) i–p
	SA 1.5	13.2 (± 2.7) bc	7.2 (± 1.1) bc	0.9 (± 0.2) a–e	0.8 (± 0.04) p–t
Portulaca oleracea	–	13.2 (± 0.7) bc	5 (± 1.3) bc	0.6 (± 0.19) b–e	1.6 (± 0.07) b
	BABA 0.1	16.1 (± 1.2) abc	5.9 (± 1.2) bc	0.7 (± 0.1) b–e	1.2 (± 0.06) d–j
	BABA 0.7	16.4 (± 2) abc	7.1 (± 1.5) bc	0.8 (± 0.2) a–e	0.9 (± 0.03) k–s
	BABA 1.5	15.4 (± 1.2) abc	5.4 (± 1.1) bc	0.6 (± 0.1) b–e	0.8 (± 0.03) q–t
	JA 0.1	11.7 (± 2) bc	6.4 (± 1.8) bc	0.8 (± 0.2) a–e	1.3 (± 0.04) c–h
	JA 0.7	14.1 (± 1.6) abc	5.8 (± 1.3) bc	0.7 (± 0.2) b–e	0.9 (± 0.04) k–s
	JA 1.5	14.8 (±1.9) abc	6.7 (± 2.4) bc	0.8 (± 0.3) a–e	0.7 (± 0.02) q–t
	SA 0.1	16.9 (± 1.7) abc	6.6 (± 1.1) bc	0.9 (± 0.2) a–e	1.3 (± 0.15) c–g
	SA 0.7	14.7 (± 3.1) abc	3.7 (± 1.2) c	0.5 (± 0.2) cde	1 (± 0.01) j–q
	SA 1.5	17.2 (± 2.6) ab	7.7 (± 1.9) bc	1 (± 0.3) a–d	0.8 (± 0.02) o–t

Table 10. (Continued) The effect of the plant extracts and defense inducer molecules on plant growth 8 weeks after inoculation with nematodes.

Plant extract (2,000 ppm)	DI molecule and concentration (mM)[x]	Shoot [y, z]			Root fresh weight (g) [y, z]
		height (cm)	fresh weight (g)	dry weight (g)	
Ferula assa-foetida	–	14.8 (± 1.5) abc	3.5 (± 0.6) c	0.58 (± 0.06) b–e	1.3 (± 0.2) cde
	BABA 0.1	14.4 (±1.6) abc	5.2 (± 2.3) bc	0.7 (± 0.2) b–e	1 (± 0.06) j–q
	BABA 0.7	15.5 (± 1.67) abc	7.6 (± 1.6) bc	0.8 (± 0.2) a–e	0.9 (± 0.02) l–t
	BABA 1.5	15.5 (± 1.2) abc	7.3 (± 0.8) bc	0.9 (± 0.1) a–e	0.6 (± 0.03) t
	JA 0.1	16.8 (± 1.9) abc	6.3 (± 1.4) bc	0.6 (± 0.2) b–e	1.2 (± 0.03) d–i
	JA 0.7	17.4 (± 1.1) ab	6.4 (± 1.4) bc	0.7 (± 0.2) b–e	1.1 (± 0.06) f–m
	JA 1.5	16.5 (± 1.2) abc	4.5 (± 1.2) bc	0.3 (± 0.1) e	0.8 (± 0.04) o–t
	SA 0.1	12.8 (± 1.5) bc	3.4 (± 0.9) c	0.5 (± 0.2) de	1 (± 0.04) g–n
	SA 0.7	18.1 (± 3) ab	7.4 (± 1) bc	1.2 (± 0.2) ab	0.9 (± 0.03) k–t
		10.8 (± 0.6) bc	5.4 (± 1.4) bc	0.6 (± 0.2) b–e	0.7 (± 0.02) rst
Nematicide (cadusafos)	0.2%	17.6 (± 2.1) ab	10.3 (± 1.1) a	1.4 (± 0.1) a	0.6 (± 0.05) t

[x] DI = defense inducer molecules; SA = salicylic acid; JA = jasmonic acid; BABA = β-amino butyric acid
[y] Values in the same column followed by different letter(s) are significantly different according to Duncan's test ($P < 0.05$). Each treatment had ten replications (five replications in two successive experiments).
[z] Values in parenthesis show ± standard error.

nematicide standard (Table 10).

DISCUSSION

Growing social awareness of the health and ecological concerns of some chemical pesticides has stimulated a search for alternative methods of pest control (Moosavi and Zare, 2012). In the last few decades, extracts or products of many plants have been examined for their efficacy in the management of plant-parasitic nematodes. Although there are several promising examples, few have been commercially used (Oka, 2010). The plants were chosen for study in this experiment according to local convenience, low price, and reputed efficacy against nematode and other micro-invertebrate pests of humans or animals.

Systemic plant defense inducing compounds that exploit natural defense mechanisms of plants could be an alternative approach for plant protection. Systemic acquired resistance (SAR), induced systemic resistance (ISR), the resistance provided by symbiotic fungi and β-aminobutyric acid-induced resistance (BABA-IR) are all examples of approaches that have been suggested for pathogen control (Bakker *et al.*, 2006; Conrath, 2011).

Ethanol extract of *F. assa-foetida* at different concentrations significantly increased the J2 mortality of *M. incognita* and *M. javanica*. When the extract was used at 0.25% concentration (2,500 ppm), the mortality of *M. incognita* and *M. javanica* J2 after 48 hr was 19% and 25%, respectively (Zia ul Haq *et al.*, 2010). However in another experiment, the aqueous extract of *F. assa-foetida* caused 100% mortality of *M. incognita* after 48 hr and 73% egg hatch inhibition after 1 wk (El-Sherbiny and Zen-El-Dein, 2012).

Ferula assa-foetida possesses antimicrobial and antioxidant activities and has been used as a cure for nematodes and other intestinal parasites (Iranshahy and Iranshahi, 2011). This plant contains antiviral and cytotoxic agents (Lee *et al.*, 2009) as well as sesquiterpene coumarins, which possess biological activity (Nazari and Iranshahi, 2011). Inhibitory effects of its essential oils on growth of *Fusarium* have also been reported (Sitara *et al.*, 2008). Soil amendment with *F. assa-foetida* could significantly reduce the infection of root colonizing fungi as well as *M. incognita* (Baloch *et al.*, 2013).

Peganum harmala is a comon medicinal plant with anthelmintic properties (Shalaby, 2013). The extracts of this plant have been used as a remedy against tapeworm infection in man and animals (Akhtar *et al.*, 2000) as well as for gastrointestinal cestodes in goats (Akhtar and Riffat, 1986). Antibacterial, insecticidal, and molluscicidal activity has also been reported for this plant (Edziri *et al.*, 2010; Sallam and El-Wakeil, 2012; Benzara *et al.*, 2013). The biological activity of *P. harmala* was attributed to its alkaloidal constituents (Moloudizargari *et al.*, 2013). Evaluation of various *P. harmala* based-products against *M. javanica* demonstrated that the emulsified oil was more effective than other methods. The aqueous extract of *P. harmala* at a 2,000-ppm concentration reduced root galling by 69% (Mayad *et al*., 2013).

Portulaca oleracea contains several alkaloids, flavonoids and catecholamines that have received attention as major bioactive components (Zhang *et al.*, 2002; Zhu *et al.*, 2010). An antifungal property for this plant has also been reported (Bongoh and Jun, 2000).

Nigella sativa seed oil is mainly made of thymoquinone, dithymquinone, thymohydroquinone, and thymol that showed pronounced anthelmintic activity against tapeworms, earthworms, nematodes and cestode (Fallah Hosseini *et al.*, 2011). Anti-bacterial and anti-viral effects have also been reported for this plant (Salem, 2005; Kamal *et al.*, 2010; Shalaby *et al.*, 2012). Aqueous extract of *N. sativa* resulted in hatching inhibition and J2 mortality of *M. javanica* after 72 hr of 95 and 36%, respectively, while the effect of methanol extract with 1,000-ppm concentration was 95 and 35% (Abbas *et al.*, 2009). However, its ethanolic extracts with 2,500 ppm concentration respectively killed 7 and 9% of *M. incognita* and *M. javanica* after 48 hr (Zia-ul-Haq *et al.*, 2010).

Plants contain an extensive range of hormones that are necessary for the regulation of growth, development, reproduction, and survival. Infection with pathogens may cause fluctuations in the amounts of different hormones, in some cases inducing plant defense responses (Bari and Jones, 2009). Although the mechanisms involved are not well understood, the role of SA, JA, and ethylene as primary signals in the regulation of local and systemic plant defense responses has been well documented (Spoel and Dong, 2012).

According to our results, artificial induction of plant defense could play a significant role in the integrated management of root-knot nematodes on tomato. When the extracts or DI molecules were applied separately, the best results were seen with the DI molecules. However, combined use of the DI molecules and plant extracts resulted in better control of the nematode. The most effective concentration of the DI molecules was 1.5 mM either alone or in combination with the plant extracts. Each of the DI molecules in combination with each of the tested plant extract show promise for nematode control, but should be studied under field conditions to confirm the efficacy seen in these greenhouse tests.

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