NEMATICIDAL EFFECT OF SOME HERBAL POWDERS AND THEIR AQUEOUS EXTRACTS AGAINST *MELOIDOGYNE JAVANICA*

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ABSTRACT

Moosavi, M.R. 2012. Nematicidal effect of some herbal powders and their aqueous extracts against *Meloidogyne javanica*. Nematropica 42:48-56.

Plant parasitic nematodes cause 12.3% losses to agricultural products worldwide, from which about 5% is attributed to root-knot nematodes (*Meloidogyne* spp.). Greenhouse data on some herbal powders and their aqueous extracts which increased plant growth, caused mortality of *Meloidogyne javanica* second-stage juveniles (J2s), egg hatch inhibition, and reduction in the infection rate, are presented with regard to elimination of the harmful consequences of chemical pesticides. Most of the herbal powders were effective in controlling *M. javanica*. Neem seeds powder and dried stem plus leaves of *Chrysanthemum coronarium* were the best treatments found in this study. The number of juveniles per 100 g soil was lower in soil amended with the seed powder of *Azadirachta indica*, the stem plus leaves of *C. coronarium* and the leaves of neem when compared with unamended soil. All extracts examined showed some level of nematicidal and/or nematostatic effect against J2s. Forty-eight hr exposure to extracts from the shoot plus leaves of *C. coronarium*, the seeds of *A. indica* and the shoot plus leaves of *Nerium oleander* resulted in death of 65, 62 and 53% of J2s, respectively. After 72 hr, the highest inhibition of egg hatch was 75% with neem seed extract. The seed extract of *A. indica* had the highest inhibition on penetration of J2s into the roots followed by the shoot plus leaves of *C. coronarium* and *N. oleander*. This research demonstrated that *A. indica* (seeds and leaves) and *C. coronarium* (shoots plus leaves) had better potential to control *M. javanica* compared with other treatments, and can be efficiently used as soil amendments.

Keywords: biocontrol, egg hatch inhibition, extract, herbal powder, infection rate, nematicidal activity

RESUMEN

Moosavi, M.R. 2012. Efecto nematicida de algunos polvos herbales y sus extractos acuosos sobre *Meloidogyne javanica*. Nematropica 42:48-56.

Los nematodos fitoparásitos causan pérdidas del 12.3% en la agricultura en el mundo, y cerca de 5% de las pérdidas se atribuye a nematodos agalladores (*Meloidogyne* spp.). En este trabajo se presentan datos que muestran actividad de polvos herbales y sus extractos acuosos sobre el crecimiento de las plantas, mortalidad de juveniles de segundo estadio (J2) de *Meloidogyne javanica*, inhibición de la eclosión de huevos, y reducción de la tasa de infección. La mayoría de los polvos evaluados fueron efectivos en el control de *M. javanica*. El polvo de semillas de neem y de tallos y hojas de *Chrysanthemum coronarium* fueron los mejores tratamientos en este estudio. La cantidad de juveniles por 100 g de suelo fue menor en suelo enmendado con polvo de semilla de *Azadirachta indica*, tallos y hojas de *C. coronarium* y hojas de neem comparados con suelo sin enmiendas. Todos los extractos evaluados mostraron algún nivel de efecto nematicida y/o nematostático sobre los J2. La exposición durante 48 horas a extractos de brotes y hojas de *C. coronarium*, semillas de *A. indica* y de brotes y hojas de *Nerium oleander* resultaron en mortalidad de 65, 62 y 53% de los juveniles, respectivamente. Después de 72 horas, la inhibición de eclosión más alta fue del 75% y se obtuvo con extracto de semilla de neem. El extracto de semilla de *A. indica* mostró la inhibición de penetración más alta, seguido de los extractos de brotes y hojas de *C. coronarium* y de *N. oleander*. Este estudio demuestra que *A. indica* (semillas y hojas) y *C. coronarium* (brotes y hojas) tienen mejor potencial de control de *M. javanica* comparados con otros tratamientos, y que se pueden usar eficientemente como enmiendas al suelo.

Palabras clave: actividad nematicida, control biológico, extracto, inhibición de la eclosión, polvo herbal, tasa de infección

INTRODUCTION

Among plant-parasitic nematodes, the root-knot nematodes (RKNs) are the most economically important group. *Meloidogyne javanica*, one of the four most common species, is extremely polyphagous and attacks both monocotyledons and dicotyledons (Agrios, 2005; Perry *et al.*, 2009). At present, the major control method of these nematodes is based on the use of chemical nematicides, but alternative management strategies must be adopted due to the ban on soil fumigants, environmental and human health concerns and development of resistance to chemicals (Hallmann *et al.*, 2009).

Organic amendments offer an alternative or supplementing control tactic to chemical or cultural control of plant-parasitic nematodes (PPNs), but confirmation of their potential effect requires more detailed studies (Akhtar and Malik, 2000). Many types of organic amendments have been examined for their effect on suppressing PPN (Rodriguez-Kabana, 1986). Organic matter amendments have advantageous effects on soil physical condition, nutrients, fertility and biological activities (Kang *et al.*, 1981; Hungalle *et al.*, 1986; Akhtar and Malik, 2000). Several plant-derived compounds have specifically been involved in plant–nematode interactions. These compounds include repellents, attractants, hatching stimulants or inhibitors, and nematotoxicants, either constitutive or formed in response to nematode presence (Chitwood, 2002). Toxic substances to PPNs are seen in many plants and plant decomposition (Sukul, 1992; Akhtar and Malik, 2000; Oka, 2010).

Many plant extracts are effective in suppression of PPNs. These efficacious extracts belonged to 46 plant families including both annuals and perennials (Ferris and Zheng, 1999; Zasada *et al.*, 2002; Kokalis-Burelle and Rodriguez-Kabana, 2006). Therefore it is logical to examine new plants or new varieties of plants for their efficacy in immobilizing, retarding development or killing nematodes.

Meloidogyne javanica is predominant in Iran and causes serious problems for many plants (Moosavi *et al.*, 2010). However, there are various herbal plants and trees in Iran which have possibly nematicidal properties. Our objective was to test the efficacy of different components of six herbal amendments (total 12 treatments) against *M. javanica* in potted tomato. We also examined the potential toxicity of their extracts against *M. javanica* juveniles, egg hatch, and J2s infectivity.

MATERIALS AND METHODS

Production of nematode eggs

Inocula were increased on tomato plants (cv. 'Early-Urbana') starting from a single nematode egg mass previously identified as *M. javanica* (Moosavi *et al.*, 2010). To provide sufficient inoculum for the experiments, galled roots were cut into 0.5–1 cm pieces and agitated for 2–3 min in 0.5% sodium hypochlorite solution. The suspension was rinsed three times over 60 and 20 µm pore dia sieves. Eggs were transferred to a 250 ml beaker with 100 ml of sterilized distilled water (Elbadri *et al.*, 2009). The number of eggs were estimated by Peters' chamber and adjusted to 100 eggs per ml.

Herbal amendment

Six plant species were collected and divided into leaves, seeds and flowers. They were air-dried in the shade and finely ground into powder using a home blender. *Olea europaea* dried mill waste was also used as soil amendment. A total of 12 treatments (Table 1) were examined.

Pot experiment

Each herbal amendment was thoroughly mixed with sterilized sandy soil at a rate of 7.5% amendment: soil (w/w) and transferred into 12.5 cm diameter plastic pots. Each treatment was replicated five times, and pots were arranged in a completely randomized design in a greenhouse. A chemical nematicide, fosthiazate 5% G, was used at a rate of 0.3% w/w as a positive control and negative control pots were filled only with sterilized sandy soil. Two-week-old tomato seedlings (cv. 'Early-Urbana') were planted in each pot and after 2 weeks, 2000 eggs and second-stage juveniles of *M. javanica* were added to the soil around the roots of tomato seedlings (Elbadri *et al.*, 2009). All pots were kept under greenhouse conditions (27 ± 3 °C). After 8 weeks the plants

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

Common name	Scientific name	Tissue sampled	
Neem	*Azadirachta indica*	leaves	seed
Asian thorn apple	*Datura metel*	leaves	seeds
Olive	*Olea europaea*	mill waste	leaves
Glaucous star thistle	*Carthamus glaucus*	leaves	seed
Oleander	*Nerium oleander*	Shoot plus leaves	flower
Crown daisy	*Chrysanthemum coronarium*	shoot plus leaves	flower

were harvested and the fresh tops and roots weighed (Moosavi *et al.*, 2010). Root galling was immediately rated on a scale of 0–10, where 0 = complete and healthy root system and 10 = plant and root are dead (Sikora and Fernandez, 2005). The number of galls on roots of each treatment was counted and divided by their weight and presented as the number of galls and egg masses root-g. In the case of compound galls (multiple infections), the number of extra egg masses was added to the number of galls (Moosavi *et al.*, 2010). Soil from each pot was mixed thoroughly and nematodes were extracted from a 100 g soil sub-sample using Jenkins centrifugation (1964) method. Second-stage juveniles (J2) were collected on 25 µm sieves and counted microscopically.

Preparation of plant extracts

The dried material (Table 1) were soaked in distilled water for 1 day prior to processing in a blender for 2 minutes and the liquid separated from the macerated plant material using a cotton cloth. The liquid mixture was centrifuged at 2000g for 20 min and the supernatant was filtered through a Whatman No. 2 filter paper to obtain a clear liquid (Ferris and Zheng, 1999). The stock concentration of each extract was 1 g of plant material/10 g of distilled water.

Preparation of second stage juveniles (J2s)

Eggs of *M. javanica* were extracted from tomato roots as described above and were put on a filter paper in a Baermann funnel. The J2s were collected over two days, were placed in a beaker, counted microscopically at 40X and their population was estimated by average of three counts.

Effect of extracts on juveniles

About 200 J2s were placed in distilled water in a BPI dish, with five replications for each treatment. The surplus water surrounding the J2s was removed with a pipette, and one ml of plant extract stock solution was placed into each dish. After the nematodes settled to the bottom, the solution was removed and replaced with 1 ml of fresh plant extract. Distilled water was used for the control. The dishes were kept at 28°C. Nematode activity was recorded at 24 and 48 hr after addition of the plant extracts. Then J2s were transferred to distilled water for an additional 24 hr to check for recovery (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. Those nematodes that responded to touching were counted as immotile (Cayrol *et al.*, 1989).

Inhibition effect of extracts on egg hatching

Ten egg masses with the same age and size were put in each BPI dish containing 0.3 ml of extracts. Distilled water was used for the control. Dishes were kept at 28°C in the dark, and the number of hatched J2s was counted after 24 and 72 hr (Zasada *et al.*, 2002). The percentage of egg hatch inhibition was calculated by dividing subtraction of hatched J2s in control from hatched J2s in treatments by those in the control and multiplied by 100. The egg masses were then transferred to distilled water and incubated for another 7 days at 28°C for the reversibility tests. Reduction in hatching rate after removal of the extracts was calculated similarly. There were four replications for each treatment.

Effect of extracts on infectivity

Approximately 1000 J2s were added to 200 g of sterile sand per 250 ml plastic cup and this was supplemented with 30 ml of plant extract. Distilled water was used for the control. To reduce evaporation, the cups were kept in plastic bags at 28°C for two days. At the end of this time, a one-week-old tomato seedling (cv. 'Early-Urbana') was transplanted to each cup. The plants were incubated for three days at 28°C. The plants were uprooted, their roots stained with acid-fuchsin and the number of J2s in the roots were counted (Tsai, 2008). Infection rate was calculated by dividing the number of penetrated J2s by the number of J2s in the inoculum multiplied by 100. There were four replications for each treatment.

Data analysis

All experiments were repeated twice in time. The same trend was observed in the two experiments and the data in the experiments were combined. Data were analyzed by ANOVA (SPSS ver. 15 for windows) and means were separated by Tukey's Studentized Range Test.

RESULTS

Pot experiment

Some of herbal amendments used in this study were promising in controlling of *M. javanica* and in reducing the level of J2s in the soil (Table 2). All soil amendments increased the shoot weight and decreased the root weight compared with the control ($P<0.0001$), but the best result were related to chemical nematicide (fosthiazate). The gall index was lower in soils amended with the seeds and the leaves of *A. indica*, the shoot plus leaves of *C. coronarium* and *N. oleander* compared with other herbal amended treatments ($P<0.0001$) (Table 2). Except for fosthiazate treatment, pots amended with the powdered seed of *A. indica* had the least number of galls and egg masses in 1 g root (Table 2). The number of juveniles per 100 g soil considerably decreased in soil amended with the powdered seed of *A. indica*, the

Table 2. Effect of herbal amendments on shoot and root weight, gall index, number of galls and egg masses, and J2s of *M. javanica* in 100 g soil of tomato plants 8 weeks after inoculation with nematodes.

Herbal amendment	Shoot weight (g)	Root weight (g)	Gall index	Gall & EM[y] (g root)	No. of J2 (100 g soil)
A. indica-leaves	16.9 (± 0.33)[x] b	8.1 (± 0.23) bcd	2.5 (± 0.27) c	11.1 (± 0.79) cd	342 (± 9.4) g
A. indica-seeds	18 (± 0.21) a	8 (± 0.26) cd	2 (± 0.21) cd	6.6 (± 0.55) e	199 (± 9.6) i
Datura metel-leaves	14.7 (± 0.25) d	8.7 (± 0.2) bcd	4.2 (± 0.20) b	18.6 (± 0.56) b	681 (± 10.4) de
Datura metel-seeds	15.4 (± 0.22) cd	8.8 (± 0.16) bc	3.7 (± 0.26) b	16.4 (± 0.74) b	643 (± 11.7) e
Olea europaea-mill waste	15.3 (± 0.24) d	8.8 (± 0.14) bcd	3.9 (± 0.23) b	16.7 (± 0.69) b	671 (± 10.7) de
Olea europaea-leaves	14.4 (± 0.2) d	9 (± 0.18) b	4.5 (± 0.22) b	19.6 (± 0.68) b	791 (± 15.5) b
Carthamus glaucus-leaves	15.5 (± 0.17) cd	8.7 (± 0.23) bcd	3.7 (± 0.26) b	16.9 (± 0.42) b	659 (± 9.2) e
Carthamus glaucus-seeds	14.97 (± 0.2) d	8.9 (± 0.18) bc	4.3 (± 0.21) b	18.2 (± 0.54) b	752 (± 11.4) bc
N. oleander-shoot&leaves	16.5 (± 0.16) bc	8.1 (± 0.23) bcd	2.6 (± 0.22) c	12.7 (± 0.78) c	471 (± 10.7) f
N. oleander-flowers	15.6 (± 0.24) cd	8.7 (± 0.2) bcd	4 (± 0.21) b	16.7 (± 0.87) b	644 (± 11.7) e
C. coronarium-shoot & leaves	17.6 (± 0.18) ab	8.2 (± 0.17) bcd	2.4 (± 0.22) c	8.7 (± 0.53) de	268 (± 11.8) h
C. coronarium-flowers	15.1 (± 0.14) d	8.8 (± 0.2) bcd	4.1 (± 0.31) b	17.8 (± 0.72) b	717 (± 15.4) cd
Control	12.4 (± 0.7) e	9.9 (± 0.24) a	6.3 (± 0.26) a	26.9 (± 1.29) a	1225 (± 31.7) a
Nematicide (fosthiazate)	18.1 (± 0.21) a	7.8 (± 0.28) d	1.4 (± 0.16) d	2.6 (± 0.15) f	36.8 (± 2.5) j

[x] Values are means ± SE of ten replicated pots per treatment (five replications in two successive experiments). Values in the same column followed by different lower-case letter(s) are significantly different ($P < 0.05$).
[y] EM= egg mass

shoot plus leaves of *C. coronarium*, and the leaves of *A. indica* (P<0.0001).

Effect of extracts on juveniles

All tested plant extracts showed some level of nematicidal and nematostatic effect against J2s of *M. javanica* (P<0.0001) (Table 3). All extracts had higher mortality rates following 48 hr of exposure as compared with 24 hr. About 63 and 58% of J2s were killed after 24 h by exposure to the extracts from the shoot plus leaves of *C. coronarium* and the seeds of *A. indica*, respectively. In addition to these two treatments the extracts from the shoot plus leaves of *N. oleander* and leaves of *A. indica* were found to be highly toxic to juveniles of *M. javanica* and killed more than half of the J2s following 48 hr of exposure. The mortality and immobility rates of juveniles treated with each of these extracts are presented in Table 3. After transferring the juveniles from the extracts into distilled water, some of them recovered. The highest recovery rate was 5.5% which related to the seeds extract of *Datura metel* (Table 3).

Inhibition effect of extracts on egg hatching

All tested herbal extracts were found to decrease the hatching rate in egg masses of *M. javanica* (P<0.0001) (Table 4). The leaf extract of *O. europaea* had the lowest level of inhibition on hatch of nematode eggs (14 and 16% after 24 hr and 72 hr, respectively). The highest inhibition of hatching was 75 % after 72 hr with extract of *A. indica* seeds. Data suggests the effect of all extracts on inhibition of egg hatching persisted even after the egg masses were transferred into distilled water (Table 4).

Effect of extracts on infectivity

When the herbal extracts were applied in the soil, the infection rate of J2s on tomato roots was significantly reduced (P<0.0001) (Fig. 1). There were significant but low level of inhibition on the penetration of J2s into roots by the leaf extract of *O. europaea*, while the seeds extract of *A. indica* had the highest inhibition. The efficacy of extracts from the shoot plus leaves of *C. coronarium* and *N. oleander* were similar and placed in the second high rank (Fig. 1).

DISCUSSION

All of the herbal amendments improved plant growth. Plant fresh top weight was significantly (P<0.0001) increased compared to the untreated control (Table 2). The greatest shoot weight was in pots supplemented with the seed powder of *A. indica*. Higher shoot weight can be the consequence of better nematode control

Table 3. The effect of different herbal extracts on mortality, immobility and recovery of *M. javanica* second-stage juveniles.

	% Mortality (mean ± SE)		% immobility (mean ± SE)		% Recovery[x]
Herbal extract	24 h	48 h	24 h	48 h	24 h
A. indica-leaves	47.9 (± 1.37) c	52.7 (± 1.4) c	21.9 (± 0.79) b	24.9 (± 0.67) b	4.7 (± 0.4) ab
A. indica-seeds	58.5 (± 0.96) b	62.3 (± 0.84) b	25.8 (± 0.53) a	27.5 (± 0.67) a	4 (± 0.45) abcd
Datura metel-leaves	31.1 (± 0.82) e	33.9 (± 1.15) e	13 (± 0.73) d	16.2 (± 0.9) d	4.5 (± 0.3) abc
Datura metel-seeds	23.7 (± 0.7) f	26.1 (± 0.79) f	11.9 (± 0.53) d	13.6 (± 0.52) e	5.5 (± 0.54) a
Olea europaea-mill waste	13.2 (± 0.71) h	16 (± 0.71) g	5.5 (± 0.58) fg	7.4 (± 0.75) gh	3 (± 0.4) bcde
Olea europaea-leaves	9.6 (± 0.52) i	11 (± 0.36) h	4 (± 0.47) g	5.3 (± 0.42) h	1.8 (± 0.36) e
Carthamus glaucus-leaves	21.1 (± 0.78) f	23.7 (± 0.86) f	6.9 (± 0.78) ef	8.2 (± 0.76) fg	1.7 (± 0.3) e
Carthamus glaucus-seeds	17.2 (± 0.59) g	19.1 (± 0.64) g	9 (± 0.47) e	10 (± 0.58) f	2.5 (± 0.43) de
N. oleander-shoot&leaves	50.3 (± 1.2) c	52.9 (± 0.97) c	16.1 (± 0.57) c	17.9 (± 0.59) d	2.8 (± 0.39) cde
N. oleander-flowers	42.6 (± 0.87) d	44.1 (± 0.77) d	11.4 (± 0.45) d	13.8 (± 0.39) e	2.9 (± 0.46) bcde
C. coronarium-shoot & leaves	63.3 (± 1.1) a	65.5 (± 0.78) a	20.6 (± 0.79) b	22.2 (± 0.73) c	4.1 (± 0.43) abcd
C. coronarium-flowers	33.2 (± 0.76) e	35.7 (± 0.73) e	16.8 (± 0.7) c	18.3 (± 0.65) d	3.9 (± 0.48) abcd
Control	0.3 (± 0.1) j	0.6 (± 0.22) i	0 (± 0.0) h	0 (± 0.0) i	0 (± 0.0) f

Each treatment had ten replications (five replications in two successive experiments). Values in the same column followed by different lower-case letter(s) are significantly different ($P < 0.05$).
[x] After 48 h from beginning of the experiment, J2s were transferred to distilled water for an additional 24 hours to check for recovery.

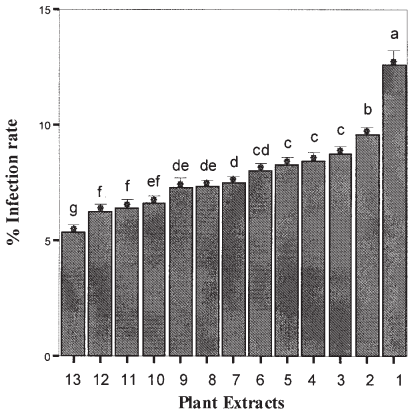

Fig. 1. The effect of some herbal extracts on the infection rate of J2s on tomato roots. Means with the same letters are not significantly different. The bars correspond to the standard errors which represent mean ± 2 SE. Each treatment had eight replications (four replications in two successive experiments).

1- control and the rest are the extract of

2- leaves of *Olea europaea* 3- leaves of *Datura metel* 4- seeds of *Carthamus glaucus*

5- flowers of *Chrysanthemum coronarium* 6- leaves of *Carthamus glaucus* 7- mill waste of *Olea europaea*

8- seeds of *Datura metel* 9- flowers of *Nerium oleander* 10- leaves of *Azadirachta indica*

11- shoot plus leaves of *N. oleander* 12- shoot plus leaves of *C. coronarium* 13- seeds of *Azadirachta indica*

(Moosavi *et al.*, 2010). Organic matter amendment to soil has been shown to have beneficial effects on soil nutrients, soil physical conditions, soil biological activity and crop viability (Addabdo, 1995; Oka, 2010). Root weight also increased in untreated infected plants compared with those amended with herbal powder (Table 2) due to the formation of galls and giant cells (Perry *et al.*, 2009).

Maximum inhibition of gall formation was observed in pots amended with the seed powder of *A. indica*, except for fosthiasate treatment (Table 2). In pots amended with herbal powder, the population of J2s decreased significantly ($P<0.0001$). The highest decrease in J2 population was seen in soil supplemented with the seed powder of *A. indica*.

Neem (*A. indica*) has the reputation for being a

Table 4. The effect of different herbal extracts on inhibition of egg hatch of *M. javanica*.

	% Inhibition of hatching (mean ± SE)		
Herbal extract	24 h [x]	72 h [x]	7 days [y]
A. indica-leaves	64.4 (± 2.18) b	60.9 (± 1.8) b	65 (± 1.9) b
A. indica-seeds	72.7 (± 1.98) a	74.9 (± 1.3) a	73.6 (± 1.2) a
Datura metel-leaves	31.3 (± 2.52) ef	33.1 (± 3.5) ef	24.4 (± 2.5) ef
Datura metel-seeds	40 (± 2.12) d	38.2 (± 1.7) de	34.7 (± 2) d
Olea europaea-mill waste	30.5 (± 2.16) ef	29.2 (± 2.1) fg	30.5 (± 2) de
Olea europaea-leaves	14.2 (± 2.2) h	16.1 (± 1.9) h	19 (± 2) f
Carthamus glaucus-leaves	36.7 (± 1.63) de	33.1 (± 1.7) ef	36 (± 2) d
Carthamus glaucus-seeds	19.3 (± 1.9) g	22.7 (± 1.5) gh	24.4 (± 1.1) ef
N. oleander-shoot&leaves	51.3 (± 2) c	51.9 (± 1) c	54.7 (± 1.7) c
N. oleander-flowers	40.4 (± 1.8) d	43 (± 1.3) d	35.5 (± 1.6) d
C. coronarium-shoot & leaves	67.6 (± 1.6) ab	63 (± 2) b	70.2 (± 1) ab
C. coronarium-flowers	27.6 (± 1.6) f	28.6 (± 1.3) fg	29.5 (± 1.5) de

Each treatment had eight replications (four replications in two successive experiments). Values in the same column followed by different lower-case letter(s) are significantly different ($P < 0.05$).
[x] Inhibition rate of egg hatching while the egg masses were immersed in the extracts.
[y] Inhibition rate of egg hatching after the egg masses were transferred to distilled water from the extracts.

''miracle'' tree mainly due to its medicinal and pesticidal properties (Oka, 2010). Many neem preparations are shown to effectively control several nematode species including RKN (Akhtar, 2000; Akhtar and Malik, 2000; Chitwood, 2002; Agbenin *et al.*, 2005; Bharadwaj and Satyawanti, 2006; Javed *et al.*, 2007a,b; Oka *et al.*, 2007; Bharadwaj and Sharma, 2007; Javed *et al.*, 2008a,b; Ntalli *et al.*, 2009; Oka, 2010).

However, the active components and their concentrations in the aqueous extracts of the plant sources are not known. Aqueous extracts were used in this study in the belief that they most closely resemble the chemistry of soil-incorporated plant residues. All of the plant extracts were found to exhibit some level of toxicity toward juveniles of *M. javanica*. Generally, the mortality rates of juveniles increased with an increase in exposure time. A similar result was reported by Elbadri *et al.* (2008). The extract from the shoot and leaves of *C. coronarium*, the seeds and the leaves of *A. indica* were found to be extremely toxic to juveniles of *M. javanica* and could inactivate (kill and immobile) more than 78% of J2s after 48 hr.

There are several reports on high mortality of RKNs by using different aqueous extracts of neem formulations (Akhtar, 2000; Aziz *et al.*, 1995; Khurma and Singh, 1997; Javed *et al.*, 2008b). Aqueous extracts of *C. coronarium* plants reportedly had nematostatic activity against several life-stages (egg-masses, separated eggs and J2) of RKNs and the effect was irreversible (Bar-Eyal *et al.*, 2006).

The shoot and leaf powder of *C. coronarium* was the second best soil amendment in reducing galls (Table 2). Applying different parts of *C. coronarium* (flowers, leaves, roots or seeds) as soil amendments significantly reduced the reproduction rates of *M. artiellia* (Perez *et al.*, 2003). It is reported that in soil amended with *C. coronarium* the populations of *Tylenchorhynchus brassicae* (Tiagi and Wani, 1992), *Rotylenchulus reniformis* (Tiagi *et al.*, 1988), *M. incognita* (Tsay *et al.*, 2004), *M. javanica*, *Heterodera avenae* and *Pratylenchus mediterraneus* (Bar-Eyal *et al.*, 2006) were significantly reduced, but there was no effect on beneficial entomopathogenic nematodes (Bar-Eyal *et al.*, 2006). It has been also seen that only mature, flowering *C. coronarium* plants, exhibit nematicidal activity (Bar-Eyal *et al.*, 2006). Perez *et al.* (2003) reported that the nematicidal effect of *C. coronarium* against *M. artiellia* was highest in the flowers, while Bar-Eyal *et al.* (2006) demonstrated that shoots and leaf application were more effective than the flowers. Our results are similar to that obtained by Bar-Eyal *et al.* (2006).

The most effective herbal extracts that reduced egg hatch rate were extracts from the seeds of *A. indica*, the shoot plus leaves of *C. coronarium*, the leaves of *A. indica* and the shoot plus leaves of *N. oleander* (Table 4). It is demonstrated that fresh latex obtained from *N. oleander* was toxic to juveniles and reduced egg

hatch of *M. javanica* (Zureen and Khan, 1984) but was phytotoxic when applied at high concentration (Zasada *et al.*, 2002).

Extract from the leaves of *Olea europaea* showed the least egg hatch inhibition (14%). The olive mill waste was more effective but still only slightly inhibited egg hatch (30%). These results are contradictory to reports that sterile water extracts of two-phase olive mill waste strongly inhibited egg hatch and J2 motility of *M. incognita* (Cayuela *et al.*, 2008). Root galling and egg masses on tomato roots induced by *M. incognita* and *M. javanica* were significantly reduced by addition of waste water from the olive-oil industries to the soil (Vouyoukalou and Stefanoudaki, 1998). The same effect was also seen when fresh olive pomace was incorporated into the soil (Rodriguez-Kabana *et al.*, 1992, 1995), but in an *in vitro* larval migration inhibition assay, extracts from *O. europaea* did not significantly inhibit *Haemonchus contortus* larvae migration (Manolaraki *et al.*, 2010).

Dried materials were used in this experiment, and perhaps the fresh portions of olive plants were more effective than dried portions; similarly the organic extract was more effective compared with the aqueous extract.

Improved effects of fresh portions of olive plant was previously documented where olive green chopped leaves had a superior effect in reducing the number of *Meloidogyne* females than dried leaves (Amin and Youssef, 1999). Amending soil with the 1:1 mixture of composted dry-olive marc and dry-rice husk did not reduce the root galling and final populations of *M. incognita* and *M. javanica* (Nico *et al.*, 2004). But, when olive green leaves were tested in Greece, it caused significant reduction in root galling and the total number of J2s (Vouyoukalou, 1994). The methanol extract of the olive leaves inhibited hatching of the eggs almost completely (Vouyoukalou, 1994). Olive green leaves as soil amendment reduced *M. javanica* and *R. reniformis* in another study (Amin and Youssef, 1999).

Unlike our results, there were also some reports on effective control of nematodes with dried olive portions. It was demonstrated that olive dried parts caused significant reduction in galls and egg masses of *M. incognita* and *R. reniformis* (EL-Nagar *et al.*, 1993; Abadir *et al.*, 1994).

The infection rate of tomato roots was reduced by half when the seeds extract of *A. indica* were applied to soil. Extract of *Carthamus glaucans* had a modest effect on the infection rate of J2s. The genus *Carthamus*, like *Tagetes*, belongs to the family Asteraceae and is composed of 15 species of east Mediterranean origin (Vilatersana *et al.*, 2005). Species of *Tagetes* are one of the most reported antagonistic plants which have been used as a source of nematicidal substances (Chitwood, 2002; Ferraz and de Freitas, 2004). Except for one report in which *C. tinctorius* was used as green manure (Bar-Eyal *et al.*, 2006), there is no information about the ability of the species of this genus to control nematodes. Relation of *Carthamus* to *Tagetes* could suggest a potential ability of controlling nematodes; therefore this study was performed to evaluate the nematicidal ability of *C. glaucans*, an indigenous Iranian species, against *M. javanica*. Supplementing soil with *C. tinctorius* caused a moderate effect on both increasing the fresh top weight and decreasing the gall index of tomato plants infected with *M. javanica* (Bar-Eyal *et al.*, 2006). A noticeable antibacterial activity was also reported for CH_2Cl_2 extract from *C. lanatus* (Taskova *et al.*, 2002).

Except for negligible better effect on mortality of J2s, the seeds of *Datura* were more effective than the leaves. The leaf extract of *D. metel* was reported to be toxic to oriental leafworm moth, *Spotoptera litura* (Murugan *et al.*, 1999) and sheep gastrointestinal nematodes, *Haemonchus contortus* (Kamaraj *et al.*, 2010). The aqueous extract of its leaves inhibited the egg hatching of *M. incognita* (Goswami and Vijayalakshmi, 1990) and the *D. metel* plants which were grown adjacent to tomato plants showed nematicidal effect against *M. javanica* (Oduor-Owino, 1993; Oduor-Owino, 2003). *Datura* dry leaves cause the highest reduction (86.4%) in the number of females of *M. javanica* (Amin and Youssef, 1999); however it was not a promising treatment in this research.

In this research, some plant sources were screened for their ability to suppress *M. javanica*, and it is demonstrated that *A. indica* (seeds and leaves), *C. coronarium* (shoot plus leaves), *N. oleander* (shoot plus leaves) and *D. metel* (seeds) have the potential of controlling RKN and can be efficiently used as soil amendments. Future research should be focused on the microplot and field experiments, along with assessment the combination use of these amendments with other controlling methods.

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