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Research Article

# Nematotoxic potential of daikon, chinaberry and purslane herbal green manures against *Globodera rostochiensis in vitro* and microplot

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**Abstract:** The nematotoxic potential of water extracts and green manures prepared from three plant species, daikon *Raphanus sativus* var. *longipinnatus* L., purslane *Portulaca oleracea* L. and chinaberry *Melia azedarach* L., on *Globodera rostochiensis* was examined *in vitro* and in microplots. Significant alteration in J2 (second stage juvenile) activity and their hatching from eggs was observed with different exposure times by all plant species; more than 99% of the J2s were inactivated after 72h and the same degree of inhibition in hatching of the eggs occurred after exposure to the plant extracts. In microplots, the numbers of newly formed cysts and final nematode multiplication rates were reduced in unsterilized soil at 1, 3 and 5% (w:w) rates of amendment with fresh plant materials, and the infestation rates of potato plants did not differ significantly from those in soil treated with metham sodium 37%. The rate of emergence of J2 from cyst inocula declined by 36% in soil treated with chinaberry and purslane and by 71% in soil treated with daikon. The reduced availability of J2 in soil must be one of the reasons for decrease in nematode multiplication rates of 65% and 86% where soil was amended with chinaberry/purslane and daikon, respectively. In terms of plant growth improvement and nematode control, daikon amendment outperformed other treatments, including metham sodium.

**Keywords:** *Globodera rostochiensis,* potato, decline rate, fecundity, natural nematicide

## Introduction

The potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* are among the most destructive pests of potato plants worldwide and can cause substantial economic losses (Moens *et al*., 2018). In spite of quarantine efforts designed to impede their spread, these nematodes have been distributed to many potato producing countries (Pickup *et al*., 2018). South America is the original home of PCN, from where they have been spread to almost all other potato production regions (Hockland *et al*., 2012). It is estimated that annual losses of PCN can exceed 9% of European total potato production (Turner and Subbotin, 2013).

Soil disinfestation by metham sodium and long rotations are now the main available methods for decreasing PCN population density in Iran (Fatemy and Aghazade, 2016). However, both of these methods have drawbacks. In

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addition to their high costs, synthetic chemical compounds represent serious hazards to both environment and human health and should be replaced by other safer methods (Moosavi and Zare, 2015). Prolonged survival (up to 20 years) in the absence of a host (Jones *et al*., 2017) and possessing a dormant stage (second-stage juveniles (J2) in eggs that are in cysts) that is resistant to desiccation (Turner and Rowe, 2006) make PCN control by rotation difficult. Hence, intensive efforts have been made towards finding alternative practices that could efficiently manage PCN without associated risks.

Amending soil with organic matters, such as crop residues, green manures, plant by-products, and industrial or urban wastes, offers an alternative or complementary method for control of plant-parasitic nematodes (PPNs) (Akhtar, and Malik, 2000; McSorley, 2011). In addition to suppressing PPNs, amending soil with organic matter improves soil physical and nutritional condition and fertility, and its biological activity (Oka, 2010). Many constitutive or induced plant-derived compounds, such as repellents, attractants, hatching stimulants or inhibitors, and nematotoxicants, are all involved in determining how much damage plant-parasitic nematodes cause (Chitwood, 2002).

Some indigenous crops and trees with medicinal properties have also proved to have impressive nematicidal features and, based on this tendency, we selected three plant species from the herbal catalogue-daikon, purslane and chinaberry, all of which are endemic in Iran. We found no publication testing the efficacy of these plants against *G. rostochiensis* and, hence, we evaluated the possible ability of water extracts of fresh leaves of these plants to impede the movement of J2 and to decrease hatching from eggs of *G. rostochiensis* in *in vitro* tests.

We further tested the results of these tests in natural soil conditions by amending PCN-infested soil with different rates of fresh leaves of the plants and then measuring plant growth parameters, the degree of nematode suppression, and the amount of decline in egg hatching caused. All results were compared with those obtained by using treatment with metham sodium nematicide.

## Materials and Methods

### Preparation of potato root diffusate (PRD)

Sprouted tubers of *cv*. Marfona were individually planted in pots containing sterile soil and irrigated with distilled water as needed. Eight weeks later, watering was withheld for 24h followed by saturating the pots with distilled water. An additional 50 ml of distilled water was then added and the solution draining from each pot was passed through the pot twice more. The collected PRD was filtered through Whatman filter paper No. 1 and kept in dark glass bottles at 4 °C (Turner *et al*., 2009). The PRD solutions were diluted with distilled water to half strength before being used.

### Preparation of nematode inoculum

This nematode is an internal quarantine pest for Iran. Cysts of the golden nematode (*G. rostochiensis*) were obtained from a naturally infested field with a long history of infestation with this species. Soil samples collected were carefully and under extreme precaution transferred to Iranian Research Institute of Plant Protection, Tehran. Cysts were extracted from soil by the Fenwick can method (Fenwick, 1940) and stored at 4 °C for four months prior to using. A sample of intact cysts was gently crushed in sterile distilled water (Southey, 1986) and the numbers of released eggs were counted in three 1 ml subsamples and averaged.

To prepare the required second-stage juveniles (J2), cysts were disinfected by 0.5% sodium hypochlorite and rinsed in distilled water. Egg-containing cysts were kept in distilled water for 48h and then were put on a filter paper in a Whitehead tray (Whitehead and Hemming, 1965) containing PRD. Active J2s were collected over 3 days and their population was estimated.

The three plant species (Table 1) had been selected because of their adverse effects on human- and animal-parasitic nematodes (Zargari, 1988). Seeds of daikon and purslane were planted in microplots and, at flowering, aerial parts were cut and placed in freezer bags, kept at 5 °C and used within few days.

Different fresh parts of the plants were mixed with equal amounts of distilled water (1g: 1ml) and ground for one minute using a Waring blender. Dry fruits of chinaberry were first ground into fine powder before immersion in distilled water (1g powder: 10ml distilled water) at 10 °C for one day, followed by macerating in a blender. The macerates of all plant materials were coarsely filtered through cotton cloth, followed by filtering through Whatman No. 1 filter paper. All plant extracts were held in a refrigerator at 5 °C overnight to allow the liquid to clear (Fatemy and Aghazade, 2016). These stock solutions were used in *in vitro* experiments.

**Impact of plant extracts on J2 motility**

A completely randomized experiment was designed with threefold replication to assess the effect of different plant extracts on the motility of J2s in a laboratory test. Treatments consisted of the extracts of tissues shown in table 1 and distilled water was used as control. One hundred J2 of *G. rostochiensis* were placed in 3 ml of stock solutions of the respective plant extract in 5 cm diam. Petri dishes. The dishes were kept on a shaker at 20 ± 1 °C and motile or motionless J2 were recorded after 24, 48 and 72h (Ferris and Zheng, 1999). The J2s were touched with a fine mounted hair if there was uncertainty about their condition. At the end of the experiment the juveniles were transferred to distilled water for an additional 24h to check for possible recovery.

**Impact of plant extracts on egg hatch inhibition**

Another similar experiment was conducted to verify the inhibition effect of the same treatments on hatching of eggs within cysts of *G. rostochiensis*. Cysts of similar size were put in 0.5% NaOCl for one minute and rinsed three times with plenty of distilled water prior to immersion in distilled water for a week. For each of three replicates, 20 cysts (60 cysts for each treatment) were put in Petri dishes containing equal amounts (1: 1) of each plant extract and PRD. PRD was used as controls. Dishes were kept at 20 ± 1°C on a shaker in the dark and the solutions were replaced with fresh ones each week. The numbers of hatched J2s were counted weekly until the end of the seventh week. At the end of the experiment, the cysts were crushed and the numbers of unhatched eggs recorded. The percentage of egg hatch was calculated by dividing the number of hatched J2 by the total number of eggs (hatched and unhatched) in each replicate (Fatemy and Aghazade, 2016). Egg hatch inhibition was computed by dividing the number of J2 that hatched in each treatment by the number of J2 that hatched in the PRD control and multiplying by 100 (Sholevarfard and Moosavi, 2015). The experiment was conducted for 7 weeks, to mimic similar conditions to natural field which takes almost 8 weeks for J2s to hatch.

**Microplot experiment**

Fresh plant materials (leaves of chinaberry and aerial parts of daikon and common purslane) were cut into 0.5 cm pieces and mixed thoroughly with 1 kg unsterile loamy soil (pH 7.6) at rates of 1%, 3% and 5% w/w soil. Each 12.5 cm diam. plastic pot was initially filled to one third of its volume with green-manure-incorporated soil, then a mesh bag of cysts was put on the surface to give a final soil population density of 10 eggs/g soil. The remaining two-thirds volume of the pots was filled with the appropriate green-manure-incorporated soil. An additional 100g untreated soil was spread on the soil surface to reduce the escape of volatiles released by the green manures. Pots were buried in to their rims in microplots in a completely randomized design and at 50 cm spacing. After one month, a sprouted piece of potato tuber of cv. Marfona (susceptible to *G. rostochiensis*) was planted in each pot. The experiment had fourfold replication and was conducted at natural ambient temperature in June. Controls for the experiment were untreated soil infested with nematodes; nematode infested soil treated with Metham sodium; and sterilized soil (without nematodes). For metham sodium treatment, four kg of soil containing four bags of cysts was placed in a





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bucket and thoroughly mixed with 1 liter of water containing the appropriate amount of metham sodium (equivalent to 1500 kg/ha of 37% a.i.) and the bucket was then sealed with plastic for two weeks. The soil was then aerated for another two weeks before use in the experiment. The metham sodium treated cyst bags were recovered and used in the pots.

Pots were irrigated as required and fed with liquid fertilizer containing essential elements.

**Data gathering**

After three months the plants were harvested and their fresh and dry top and root weights were determined. During the process of harvesting, roots were shaken so the dead females attached would fall back to the soil; then followed by placing roots on a 150µm sieve, washing was carried out with tap water ending with blotting dry the roots. Soil and debris from each replicate of each treatment remaining on sieve were then added to original pot. The number, weight and diameter of tubers were also recorded. Soil from each pot was mixed thoroughly, and cysts were extracted from a 200-g soil sub-sample by the Fenwick can method (Fenwick, 1940). The J2 were extracted from another 100-g soil sub-sample (Whitehead and Hemming, 1965). The cysts in each mesh bag were crushed and the remaining unhatched eggs were counted. Hatching percentage was calculated by dividing the number of hatched eggs by the total number of eggs. The mean number of eggs in each cyst and reproduction index of each treatment were computed by dividing the final population of nematodes (Pf) by the number of new cysts and by dividing the final population (total eggs and J2) of nematodes at the end of the experiment (Pf) by the initial nematode population (Pi), respectively.

**Data analysis**

The normality and homogeneity of the raw data was examined by the Shapiro–Wilk normality test. An analysis of variance was performed using Minitab statistical software (Minitab 16, Minitab Inc., State College, PA), and means were separated by Tukey's studentized range test ($P \leq 0.05$).

## Results

**Impact of plant extracts on J2s**

Significant differences were observed in the motility of J2 among the different treatments after 24h ($F = 323.6$, $df = 5$, $P < 0.0001$), 48h ($F = 1063.6$, $df = 5$, $P < 0.0001$) and 72h ($F = 1248.8$, $df = 5$, $P < 0.0001$). However, the effects of different aqueous extracts on motility of J2 after the same exposure time were not statistically different between plant species. With chinaberry extract, the percent of immotile J2 increased with increasing exposure time (Table 2). No recovery was observed when the immotile J2 from any plant extract were transferred to distilled water.

**Impact of plant extracts on hatching from eggs**

All of the plant extracts significantly and strongly inhibited hatching from eggs of *G. rostochiensis* ($F = 1291$, $df = 5$, $P < 0.0001$), such that the hatching percentage of nematode eggs was below 1% for all of the treatments after seven weeks of exposure. This degree of hatching was very low compared with hatching in the control treatment (78%). The greatest egg hatch inhibition was seen in chinaberry fruit (dry and fresh) treatments ($F = 20.5$, $df = 4$, $P < 0.0001$). However, all treatments inhibited egg hatching by more than 98% (Table 3).

**Microplot experiment**

Total fresh ($F = 38.06$, $df = 11$, $P < 0.0001$) and dry ($F = 21.55$, $df = 11$, $P < 0.0001$) weights of potato plants were significantly affected by the different treatments. Other plant growth characteristics, such as the number of tubers ($F = 3.04$, $df = 11$, $P < 0.006$), tuber weight ($F = 4.21$, $df = 11$, $P < 0.001$), and tuber diameter ($F = 2.54$, $df = 11$, $P < 0.017$) were also influenced significantly. The highest and lowest total fresh weights of potato plants were seen in sterilized soil (without nematodes) and untreated infested soil, respectively. Except for the treatments to incorporate 1% and 3% of chinaberry fresh leaves in the soil, the total dry weights of treatments were not significantly different from the untreated nematode-infected treatment. Among treatments





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to incorporate plant fresh fragments in the soil, daikon (regardless of its concentration) increased the total fresh weight of potato plants more than other treatments. Daikon 5% also increased tuber number to values on par with sterilized soil. However no significant difference was observed for tuber diameter and total dry weight of potato plants among the treatments that received plant fresh fragments. Adding daikon (3% and 5%) to soil could significantly increase the tuber weight more than untreated nematode-infected treatment. Treatment of soil with metham sodium did not increase potato plant growth sufficiently to warrant its use (Table 4).

The number of newly formed cysts ($F$ = 148.98, $df$ = 10, $P$ < 0.0001), J2 in soil ($F$ = 120.91, $df$ = 10, $P$ < 0.0001), hatch percentage ($F$ = 14.22, $df$ = 10, $P$ < 0.0001) and reproduction index ($F$ = 66.25, $df$ = 10, $P$ < 0.0001) were reduced significantly in all plant-amended treatments compared with the values in untreated infested soil (Table 5). The mean number of eggs in each cyst ($F$ = 2.24, $df$ = 10, $P$ < 0.041) was greatest numerically in metham sodium treated soil, but not significantly different from all other treatments except for that in which purslane was added to soil at a rate of 1% (Table 5).

**Table 1** Plant species and the tissues used for aqueous extraction.

| Common name | Scientific name | Family | Tissue sampled |
|---|---|---|---|
| Daikon | *Raphanus sativus* var. *longipinnatus* L. | Brassicaceae | fresh aerial part |
| Purslane | *Portulaca oleracea* L. | Portulacaceae | fresh aerial part |
| Chinaberry | *Melia azedarach* L. | Meliaceae | fresh leaf; fresh fruit; dry fruit |

**Table 2** The effect of different plant extracts (1g/10ml) on the motility of *Globodera rostochiensis* second-stage juveniles (J2).

| Treatments | % immotile J2[1] | | |
|---|---|---|---|
| | 24 h | 48 h | 72 h |
| Control (distilled water) | 6.3 ± 0.9b (B) | 11.3 ± 2.3b (AB) | 14.7 ± 2.2b (A) |
| Chinaberry dry fruit | 94.2 ± 0.6a (C) | 96.7 ± 0.3a (B) | 99.7 ± 0.3a (A) |
| Chinaberry fresh fruit | 87.7 ± 2.2a (B) | 94.7 ± 0.8a (A) | 98.7 ± 0.9a (A) |
| Chinaberry fresh leaf | 88.8 ± 0.7a (B) | 98.5 ± 0.8a (A) | 99.7 ± 0.3a (A) |
| Daikon | 96.3 ± 1.9a (A) | 99.7 ± 0.3a (A) | 100a (A) |
| Purslane | 95.0 ± 3.6a (A) | 99.7 ± 0.3a (A) | 100a (A) |

[1] Means (± SE) allocated different uppercase letters on the same row or lowercase letters in the same column are significantly different according to Tukey's studentized range test ($P$ < 0.05).

**Table 3** Effect of aqueous extracts of different plant extracts (1g/10ml) on hatching of encysted J2 of *Globodera rostochiensis* after 7 weeks of exposure.

| Treatments[1] | % egg hatching[2] | % egg hatch inhibition[2] |
|---|---|---|
| Control (PRD) | 78.2 ± 2.20a | -- |
| Chinaberry dry fruit | 0.4 ± 0.05b | 99.5 ± 0.06a |
| Chinaberry fresh fruit | 0.5 ± 0.04b | 99.4 ± 0.05ab |
| Chinaberry fresh leaf | 0.6 ± 0.02b | 99.2 ± 0.02bc |
| Daikon | 0.8 ± 0.06b | 98.9 ± 0.08cd |
| Purslane | 0.8 ± 0.04b | 98.9 ± 0.06d |

[1] Each treatment contained equal amount (1: 1) of each plant extract and PRD (potato root diffusate). Distilled water and PRD (1: 1) were used as the control. [2] Means (± SE) followed by different letters are significantly different according to Tukey's studentized range test ($P$ < 0.05).





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**Table 4** The effects of the plant fresh fragments on potato plant growth three months after inoculation with *Globodera rostochiensis*.

| Treatments [1] | Total Fresh Weight [2] | Total dry weight [2] | Tuber No. [2] | Tuber weight [2] | Tuber diameter [2] |
|---|---|---|---|---|---|
| Chinaberry 1% | 57.2 ± 3d | 13.2 ± 0.7b | 3.0 ± 0.4ab | 45.7 ± 3abc | 2.3 ± 0.24ab |
| Chinaberry 3% | 57.5 ± 3.5d | 12.5 ± 1.5b | 2.7 ± 0.2ab | 49.2 ± 3.9abc | 2.4 ± 0.2ab |
| Chinaberry 5% | 61.5 ± 1.3cd | 11.5 ± 0.9bc | 3.2 ± 0.2ab | 48.2 ± 3.1abc | 2.7 ± 0.2ab |
| Daikon 1% | 71.7 ± 1.7bc | 10.0 ± 0.7bc | 2.7 ± 0.5ab | 44.2 ± 3.4bc | 2.7 ±0.27ab |
| Daikon 3% | 75.5 ± 3.2b | 9.5 ± 1.3bc | 3.2 ± 0.7ab | 52.5 ± 4.7ab | 2.8 ± 0.21ab |
| Daikon 5% | 80.0 ± 1.2b | 11.5 ± 1.2bc | 3.7 ±0.7a | 54.7 ± 3.5ab | 2.8 ± 0.25ab |
| Purslane 1% | 54.5 ± 1.8d | 10.2 ± 1.6bc | 2.2 ± 0.5ab | 42.0 ± 4.1bc | 2.4 ± 0.22ab |
| Purslane 3% | 58.5 ± 1.8d | 9.7 ± 0.8bc | 2.2 ± 0.2ab | 43.2 ± 3.7bc | 2.1 ± 0.17ab |
| Purslane 5% | 60.2 ± 2.9cd | 11.7 ± 1.7bc | 2.7 ± 0.5ab | 45.0 ± 4.3abc | 2.5 ± 0.25ab |
| Metham sodium | 61.2 ± 2.9cd | 11.0 ± 1.5bc | 3.2 ± 0.5ab | 47.7 ± 3.7abc | 2.5 ± 0.21ab |
| Gr-infested control [3] | 35.2 ± 3.1e | 5.7 ± 0.8c | 1.2 ± 0.5b | 26.0 ± 9.2c | 1.6 ± 0.57b |
| Uninfested control | 96.2 ± 1.5a | 29.5 ± 1.4a | 4.2 ± 0.5a | 68.0 ± 6.3a | 3.4 ± 0.25a |

[1] Fresh plant material (leaf of chinaberry and aerial parts of daikon and common purslane) fragments were mixed thoroughly with 1 kg of unsterile loamy soil (pH 7.6). [2] Means (± SE) followed by different letters are significantly different according to Tukey's studentized range test ($P < 0.05$). [3] Gr-infested control = untreated *Globodera rostochiensis* infected control.

**Table 5** The effect of the plant fresh fragments on *Globodera rostochiensis* reproduction traits three months after inoculation.

| Treatments [1] | Cyst/200g soil [2] | J2/100g soil [2] | Eggs/cyst [2] | %egg hatch [2] | Reproduction factor [3] |
|---|---|---|---|---|---|
| Chinaberry 1% | 14.2 ± 0.8b | 348.0 ± 10.7c | 65.7 ± 3.5ab | 57.3 ± 1.3b | 0.5 ± 0.02b |
| Chinaberry 3% | 12.0 ± 0.4b | 337.0 ± 15.3cd | 66.5 ± 4.0ab | 57.3 ± 0.8b | 0.4 ± 0.03bc |
| Chinaberry 5% | 12.7 ± 0.5b | 323.0 ± 6.2cd | 66.5 ± 3.1ab | 52.3 ± 2.2bc | 0.4 ± 0.04b |
| Daikon 1% | 5.5 ± 0.3c | 125.5 ± 13.3e | 73.2 ± 3.7ab | 30.0 ± 2.6cd | 0.2 ± 0.01cd |
| Daikon 3% | 4.5 ± 1.0c | 107.5 ± 10.3e | 70.7 ± 3.5ab | 30.1 ± 1.7cd | 0.2 ± 0.04d |
| Daikon 5% | 2.5 ± 0.9c | 89.0 ± 6.3e | 81.7 ± 6.4ab | 15.2 ± 2d | 0.1 ± 0.04d |
| Purslane 1% | 15.2 ± 1.0b | 431.0 ± 17.6b | 62.2 ± 3.3b | 59.7 ± 0.7b | 0.5 ± 0.03b |
| Purslane 3% | 14.5 ± 0.6b | 383.0 ± 18.1bc | 69.0 ± 5.2ab | 55.6 ± 2.8b | 0.5 ± 0.04b |
| Purslane 5% | 13.2 ± 0.6b | 357.0 ± 7.2bc | 67.0 ± 3.2ab | 42.8 ± 14.2bc | 0.4 ± 0.02b |
| Metham sodium | 12.0 ± 0.4b | 268.0 ± 23.9d | 83.0 ± 3.9a | 44.9 ± 2.1bc | 0.5 ± 0.03b |
| Gr-infested control | 40.2 ± 1.5a | 648.0 ± 22.2a | 71.2 ± 6.3ab | 85.1 ± 1.5a | 1.4 ± 0.10a |

[1] Fresh plant materials (leaf of chinaberry and aerial parts of daikon and common purslane) fragments were mixed thoroughly with 1 kg of unsterile loamy soil (pH 7.6). [2] Means (± SE) followed by different letters are significantly different according to Tukey's studentized range test ($P < 0.05$). [3] Reproduction factor = final/initial population. [4] Gr-infested control = untreated *Globodera rostochiensis* infested control.

The greatest number of newly formed cysts was recorded from the untreated nematode-inoculated control. Amending soil with daikon (1, 3 and 5%) decreased the number of cysts and J2 in soil very strongly. Combining daikon with soil also decreased the hatch percentage and reproduction index of *G. rostochiensis* more than all other treatments (Table 5). The greatest final nematode population was recorded in untreated infested soil but amending soil with daikon at 3% and 5% could reduce nematode density 7 and 14 times, respectively. The smallest reproduction factors were also obtained in these treatments (Table 5).

## Discussion

PCN is an economically important pathogen whose management is very challenging. Chemical and cultural practices are currently the main





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controlling methods employed against PCN. Increasing public awareness of the side effects of synthetic nematicides and the drawbacks of adopting a single nematode management practice create needs for the incorporation of multiple and innovative strategies into customized nematode management programs.

Amending soil with plant-derived materials seems to be a safe alternative tool against phytopathogenic nematodes that is currently attracting considerable interest. Although the plants selected for use as soil supplements in this experiment were chosen based on previous records of their nematicidal effects, none of them to our knowledge has previously been tested against *G. rostochiensis*.

In the current research, egg hatching of *G. rostochiensis* in potato root diffusate increased for the first 3 weeks and then declined (Figure 1). Several chemical and physical factors, such as temperature, moisture and host plant root diffusates, have effects on egg hatch from cysts. Dependence on host plant root diffusates to initiate egg hatch varies among the different cyst-forming genera and species (Sharma and Sharma, 1998). Complete reliance on host diffusates for hatch is seen in species with restricted host ranges, such as PCN, whose host plants are limited to the Solanaceae (Masler and Perry, 2018). Though the J2 that had hatched during the first three weeks after planting potatoes in the field were responsible for most of the root invasion (LaMondia and Brodie, 1986), egg hatching and J2 emergence of PCN takes place over about 8 weeks in field conditions (Trudgill *et al*., 1996). Lower energy reserves in late-hatching J2 can reduce the rate of root invasion (Robinson *et al*., 1985).

All of the plant extracts were able strongly to inhibit egg hatching. Except for the first week when the eggs were kept in distilled water, egg hatching was very slow for the next 7 weeks. The inhibition effect of the extracts became greater with increase in time of exposure (Figure 2).

The adverse effects of extracts on J2 motility tended to increase numerically (but not necessarily significantly) with exposure time. All of the extracts tested could rapidly and strongly immobilize (paralyze) the J2 of *G. rostochiensis*. More than 99% of J2s became inactive after being exposed for 72h to the extracts. The effects of daikon and purslane extracts were greater, reaching high levels after 24h (Figure 3).

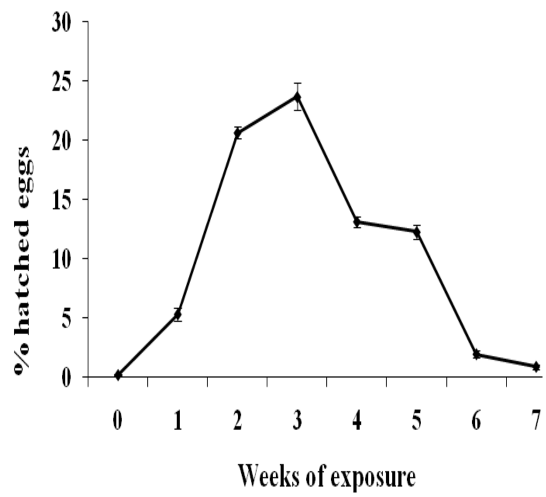


**Figure 1** Percent of hatched eggs of *Globodera rostochiensis* during seven weeks of exposure to potato root diffusate. Cysts were soaked for a week in distilled water and then transferred to potato root diffusate for another seven weeks. Each data point represents the mean ± SE of weekly percentage.

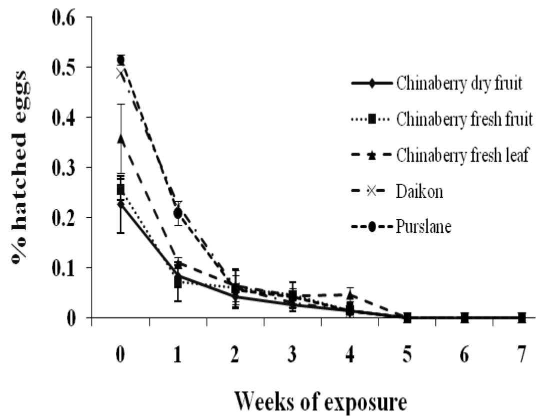


**Figure 2** The percentage of *Globodera rostochiensis* egg hatch during 7 weeks of holding cysts in plant extracts under test. The cysts were soaked for a week in distilled water and then transferred to potato root diffusate for another seven weeks. Bars represent error bars and each treatment had 3 replicates.

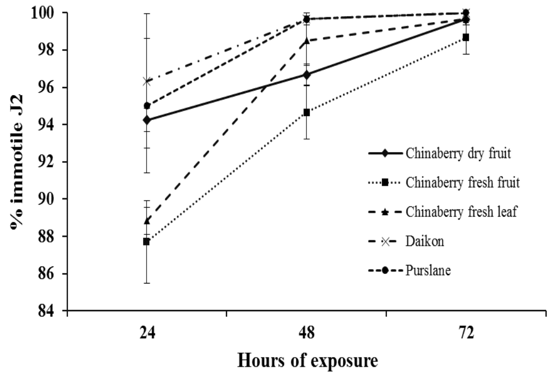


**Figure 3** The changes in percentage of immotile J2 of *Globodera rostochiensis* that were kept in different aqueous plant extracts over time. Bars represent error bars and each treatment had 3 replicates.

European plant breeders developed two radish varieties with the names "Carwoodi Nematode Control Radish" and "Image Nematode Control Radish" for controlling *Meloidogyne chitwoodi* and *H. schachtii*, respectively. Carwoodi Nematode Control Radish produces high levels of glucosinolates in its top parts that, when mulched and incorporated into the soil, break down and serve as a biofumigant. The control efficacy of Carwoodi Radish is at least 90% on *M. chitwoodi* and *G. rostochiensis* (Young-Mathews, 2016) and reduced *M. incognita* egg mass production by 93-99% (Ros *et al*., 2016). It is reported that Japanese (Kaiware) daikon *Raphanus sativus* seeds and sprouts released 4-methylthio-3-butenyl isothiocyanate and 4-methylsulfinyl-3-butenyl isothiocyanate, which had selective cytotoxic/apoptotic activity (Barillari *et al*., 2008).

Using of purslane against phytonematodes has resulted in contradictory results. In spite of an old record of good nematicidal activity of *P. oleracea* against *M. incognita* (Abivardi, 1971), later research was not so promising against *M. javanica* (Sholevarfard and Moosavi, 2015). Stepanyan and Ploeg (2001) reported that purslane was a moderately good host for *M. incognita* but placing adults and juveniles of *Xiphinema americanum* in aqueous extract of purslane (1:4 w/v) could immobilize 100% of the nematodes (Insunza *et al*., 2001).

Some bioactive ingredients such as alkaloids, flavonoids and catecholamines were identified as constitutive components of purslane plants (Zhang *et al.*, 2002; Zhu *et al.*, 2010) and it is reported that *P. oleracea* possesses antifungal activity (Bongoh and Jun, 2000).

Incubation of the J2 of *M. incognita* in different doses of polar and non-polar extracts of chinaberry fruit showed that doses higher than 0.08% (w/w) were nematicidal while lower doses were nematostatic. Complete control of *M. incognita* occurred in potted tomato plants (cv. Belladonna) when doses higher than 2.5% (w/w) were applied (Ntalli *et al*., 2010).

The oilseed and alcoholic extract of chinaberry at 1000 ppm concentration was more effective on *M. incognita* motility than castor bean and rapeseed extract and, after 72 h, could make approximately 75% of J2 immotile. The seed oil of chinaberry at 1000 ppm concentration reduced nematode egg hatch by one fifth. Chinaberry oil also caused the greatest gall reduction on cucumber roots in a pot experiment (Katooli *et al*., 2010).

The antifungal potential of hexanic and ethanolic extracts from fruit, seed kernels, and senescent leaves of chinaberry were also tested in a serial agar dilution method against several selected phytopathogenic fungi. These extracts had fungistatic or fungicidal activity at different concentrations. The active compounds with antifungal activity were characterized as vanillin; 4-hydroxy-3-methoxycinnamaldehyde; and (±)-pinoresinol (Carpinella *et al*., 2003).

Leaf extract of *Melia azedarach* was able to significantly reduce *M. incognita* reproduction and increase chickpea growth compared with untreated plants (Rehman *et al*., 2012). Keeping *Bursaphelenchus xylophilus* in 500 ppm extract of Korean *M. azedarach* inactivated 34% of the nematodes (Elbadri *et al*., 2008).

The nematicidal components of chinaberry were identified as acetic acid, butyric acid, hexanoic acid, decanoic acid, furfural, 5-hydroxymethylfurfural and furfurol (Ntalli *et al*., 2010; Ntalli and Caboni, 2012); however, other researchers have mentioned other





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compounds. When the J2 of *M. incognita* were immersed for 1h and 1 day in water extracts of the Italian and Algerian chinaberry, the Italian extract showed a significant effect on nematode activity. The nematicidal properties of the extract were attributed to its high phenolic content. The extract was fractionated and its nematicidal components were identified as *p*-coumaric acid and *p*-hydroxybenzoic acid (Aoudia *et al*., 2012).

*Meloidogyne incognita* reproduction traits on cucumber were reduced by a similar degree to the effect of fenamifos (0.02g a.i. $kg^{-1}$) when the crushed fruit of *M. azedarach* were applied to soil at the rates of 30 and 60 g $kg^{-1}$. Nematicidal effects of chinaberry water extract were attributed to its aldehyde, alcohol and carboxylic content. It has also been proved that chinaberry extract can induce plant defence mechanisms and increase plant resistance against nematode infection (Cavoski *et al*., 2012).

According to our results, while chinaberry fruit (fresh & dry) extracts caused the greatest egg hatch inhibition, daikon and purslane extracts immobilised J2 more rapidly in laboratory conditions. Furthermore, in the microplot experiment, the rate of J2 emergence from cysts in soil was reduced by 36% in chinaberry and purslane and 71% in daikon amended soils compared to untreated nematode infested soil. These effects might partly explain the observed inhibitory effects caused by these plants on nematode infestation level, as chinaberry and purslane soil amendment suppressed nematode multiplication by 65%, comparable to that of metham sodium nematicide application, and daikon amendment caused suppression by approximately 86%. The incorporation of daikon (regardless of its concentration) into soil resulted in better plant growth and nematode control. Therefore, application of daikon as green manure shows promise for *G. rostochiensis* management and may have potential for commercial use.

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# خاصیت نماتدکشی کود سبز ترب‌سفید، زیتون تلخ و خرفه روی *Globodera rostochiensis* در شرایط آزمایشگاهی و میکروپلات

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**چکیده:** خاصیت نماتدکشی عصاره آبی و کودهای سبز ترب‌سفید *Raphanus sativus* var. *longipinnatus* L.، خرفه *Portulaca oleracea* L. و زیتون تلخ *Melia azedarach* L. روی نماتد سیست سیب‌زمینی *Globodera rostochiensis* در شرایط آزمایشگاهی و میکروپلات بررسی گردید. تغییرات قابل‌توجهی در فعالیت و تفریخ لارو سن دو (J2) در زمان‌های مختلف مواجهه با عصاره‌های گیاهی مشاهده شد. در تست آزمایشگاهی، پس از ۷۲ ساعت مواجهه با عصاره گیاهان، عدم‌تحرک لاروها و تفریخ تخم بیش از ۹۹ درصد بود. در میکروپلات، تعداد سیست‌های جدید و میزان تولید مثل نهایی در خاک غیراستریل دارای ۱، ۳ و ۵ درصد کود سبز گیاهان کاهش یافت، و سطوح آلودگی سیب‌زمینی در این تیمارها مشابه با خاک ضدعفونی شده با متام سدیم بود. میزان کاهش خروج J2 از سیست‌های اینوکولوم در خاک دارای زیتون تلخ و خرفه نزدیک به ۳۶ درصد و در خاک ترب سفید معادل ۷۱ درصد بود. محدودیت جمعیت J2 در خاک شاید یکی از دلایل کاهش نرخ تولید مثل ۶۵ و ۸۶ درصدی مشاهده شده در خاک تیمار شده به‌ترتیب با زیتون تلخ/خرفه و ترب‌سفید باشد. ترب‌سفید از نظر ارتقاء بهبود رشد گیاه و کنترل نماتد، در مقایسه با سایر تیمارهای گیاهی و متام سدیم بیش‌ترین کارآیی را داشت.

**واژگان کلیدی**: *Globodera rostochiensis*، سیب‌زمینی، نرخ کاهش جمعیت، تولید مثل، نماتدکش طبیعی