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Resistance of ten common medicinal plants to the root-knot nematode *Meloidogyne javanica*

S. Ansari[1], H. Charehgani[1*] and R. Ghaderi[2]

Summary A preliminary survey indicated that the root-knot nematode *Meloidogyne javanica* is widely distributed in the rhizosphere of medicinal plants in Boyer-Ahmad region (Iran). Host suitability of ten species of medicinal plants to *M. javanica* was examined in a pot experiment under controlled greenhouse conditions: alkakengy (*Physalis alkekengi* L.), chamomile (*Matricaria chamomilla* L.), English plantain (*Plantago lanceolata* L.), fennel (*Foeniculum vulgare* Mill.), garden anchusa (*Anchusa italica* Retz.), horehound (*Marrubium vulgare* L.), lovage (*Levisticum officinale* L.), sorrel (*Rumex acetosella* L.), thistle (*Echinops adenocaulos* Boiss.) and woundwort (*Stachys pilifera* Benth.). According to the scheme of Canto-Saenz, seven species, namely garden anchusa, fennel, horehound, alkakengy, English plantain, woundwort and sorrel can be considered susceptible hosts with gall index (GI) > 2 and reproduction factor (RF) > 1, and thistle, lovage and chamomile, can be considered as hyper-susceptible with GI > 2 and RF ≤ 1.

Additional keywords: gall Index, hyper-susceptible, reproduction factor, susceptible

Introduction

Root exudates of plants contain chemical compounds which attract nematodes to the root or result in repulse, motility inhibition or even their death (Curtis *et al*., 2009). For example, chlorogenic acid which is subsequently oxidized by the action of host or nematode polyphenol oxidase might inhibit nematode activity and prevent root-knot nematode larvae from penetrating the endodermis into tissues suitable for giant cell production (Hung and Rohde, 1973). Three alkaloids namely sanguinarine, chelerytherine and allocryptopine have shown strong nematicidal activity (Wang *et al*., 2012). In addition, the phenolic acid compounds are potentially involved in resistance or tolerance of tall fescue (*Festuca* sp.) (Poaceae) to *Pratylenchus scribneri* (Pratylenchidae) (Bacetty *et al*., 2009).

Susceptibility of medicinal plants to parasitic nematodes vary between the species, from susceptible (Chinappen *et al.,* 1988; Rhoades, 1988; Mustika, 1992) to resistant (Mukhopadhyaya *et al.,* 1980; Tanda *et al.,* 1989; Haseeb and Butool, 1990; Haroon and Huettel, 1991) (Table 1). Studies by Sivakumar and Vadivelu (1997) on 46 medicinal and aromatic plants showed that *Meloidogyne hapla* (Heteroderidae) was the predominant nematode species followed by *Helicotylenchus indicus* (Hoplolaimidae), *Pratylenchus coffeae* (Pratylenchidae), *Tylenchorhynchus martini* (Belonolaimidae), *Xiphinema americanum* (Longidoridae), *Scutellonema conicephalum* (Hoplolaimidae) and *Hemicriconemoides mangiferae* (Criconematidae). Root-knot nematodes (*Meloidogyne* spp.) are serious pests of medicinal and ornamental plants (Haseeb *et al.,* 1984), which, in high population density can affect the quantity and quality of production (Haseeb *et al.,* 1996).

The objective of the present study was to determine the susceptibility of ten common medicinal plants as hosts to the root-knot nematode *Meloidogyne javanica* (Heteroderidae) under greenhouse conditions based on gall index (GA) and Reproduction Factor (RF), which are two important measures of nematode infestation (Sasser *et*

[1] Department of Plant Protection, College of Agriculture, Yasouj University, Yasouj, Iran.
[2] Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz, Iran.
* Corresponding author: h.charehgani@yu.ac.ir

al., 1984) and selection of resistant sources of different plants to root-knot nematodes (Talwana *et al.,* 1997; Cervantes-Flores *et al.,* 2008; Marchese *et al.,* 2010; Mudiope *et al.,* 2012; Gomes *et al.,* 2015; Karuri *et al.,* 2017).

Materials and Methods

Preparation of nematode inoculum

Eggs of *M. javanica* were extracted from galled root of tomato (*Solanum lycopersicum* cv. Early-Urbana) using the sodium hypoclorite method (NaOCl) (Hussey and Barker, 1973). Infected roots collected from the greenhouse of Boyer-Ahmad region were chopped to 2-3 cm pieces and were shaken in the 0.5 % sodium hypochlorite (NaOCl) for 90 seconds and poured into a stack of two sieves, with a 75 µm aperture size at top followed by a 25 µm aperture size. Eggs retained on 25 µm aperture size sieve, which were washed quickly to remove all NaOCl and were counted under a stereomicroscope.

Pot experiments

The experiments were conducted under greenhouse conditions at 28 ± 4ºC under 16:8 h (light : dark) photoperiod. Seeds of *Physalis alkekengi* L. (alkakengy) (Solanaceae), *Matricaria chamomilla* L. (chamomile) (Asteraceae), *Rumex acetosella* L. (sorrel) (Polygonaceae), *Plantago lanceolata* L. (English plantain) (Plantaginaceae), *Foeniculum vulgare* Mill. (fennel) (Apiaceae), *Anchusa italica* Retz. (garden anchusa) (Boraginaceae), *Marrubium vulgare* L. (horehound) (Lamiaceae), *Levisticum officina-*

Table 1. Degree of resistance (DR) of medicinal plants to the root-knot nematodes *Meloidogyne javanica* and *Meloidogyne incognita* (Walker, 1995; Baida *et al*., 2011).

Medicinal plants	DR to *M. incognita* race 3 (Walker, 1995)	DR to *M. incognita* (Baida *et al*., 2011)	DR to *M. javanica* (Baida *et al*., 2011)
Anethum graveolens L.	H	H	H
Artemisia absinthium L.	H	-	-
Erurca vesicaria L.	H	-	-
Foeniculum vulgare Mill.	H	-	-
Hyssopus officinalis L.	H	H	H
Lavandula augustifolia Mill.	H	-	-
Nepeta cataria L	H	-	-
Ocimum basilicum L.	H	H	H
Salvia officinalis L.	H	-	-
Thymus vulgaris L.	H	H	H
Mikania glomerata Sprengel.	-	H	H
Pimpinella anisum L.	-	R	H
Coriandrum sativium L.	S	-	-
Matricaria recutita L.	S	S	S
Melissa officinalis L.	S	-	-
Mentha piperita L.	-	-	-
Origanum majorana L.	S	R	R
Origanum vulgare L.	S	-	-
Rosmarinus officinalis L.	S	-	-
Ruta graveolens L.	S	R	R
Satureja hortensis L.	S	-	-
Tanacetum vulgare L.	S	-	-
Tomato (control)	-	S	S
Mentha pulegium L.	-	R	R
Plectranthus barbatus Andr.	-	R	R
Commiphora myrrha (Nees) Engl.	-	R	R
Carpobrotus edulis (L.) N.E. Br	-	R	R
Plectranthus neochilus Schltr.	-	R	R

S = Susceptible; H = Hypersusceptible; R=Resistance

le L. Koch (lovage) (Apiaeae), *Echinops adenocaulos* Boiss. (thistle) (Asteraceae) and *Stachys pilifera* Benth. (woundwort) (Lamiaceae) were sown in plastic pots (13 cm diameter and 10 cm height) containing 1.5 kg steam-sterilized sandy loam soil. After 45 days, each plant was inoculated with 5000 eggs + second stage juveniles (J_2) of *M. javanica* as the initial population (Pi). Inoculation was done by pipetting the egg + J_2 suspension into 3 holes around the plant root system. The experiment was conducted in a completely randomized design with four replications. The plants were watered daily and were harvested 60 days after inoculation.

The roots were gently washed with tap water and number of eggs in one gram of root were counted according to the procedure developed by Hussey and Barker (1973). One gram of root was stained with acid fuchsine according to the procedure developed by Byrd *et al.* (1983). The total number of eggs, galls and egg-masses per plant root system was determined by multiplying with the root weight per plant. The number of second stage juveniles (J_2)/100 cm^3 of soil was counted after extraction using the modified Baermann pie-pan method (Coyne *et al.,* 2014) and the total number of nematodes in soil was computed by extrapolating the number in 100 cm^3 to the volume of soil (1.5 kg).

The final nematode population (Pf) per pot (the total number of nematodes per plant root and the number of J_2 in soil per pot) were computed and finally, the reproductive factor (RF) of nematode was calculated by dividing the Pf by Pi (5,000 eggs + J_2). Gall index (GI) was estimated on a scale of 0 to 5, where 0 = no galls; 1 = 1 to 2 galls; 2 = 3 to 10 galls; 3 = 11 to 30 galls; 4 = 31 to 100 galls; and 5 = more than 100 galls in the root system (Taylor and Sasser 1978). The degree of resistance of medicinal plant species was allocated according to the modified scheme of Canto-Saenz (Sasser *et al.,* 1984), which is based on GI and RF as follows: resistant (GI ≤ 2, RF ≤ 1); tolerant (GI ≤ 2, RF > 1); hypersusceptible (GI > 2, RF ≤ 1); susceptible (GI > 2, RF > 1).

Statistical analysis

The SAS system V9.1 (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses. Statistical analyses were performed using a one-way analysis of variance ANOVA and the significant difference between means was determined by Duncan's multiple range test (DMRT) ($p < 0.1$).

Results and Discussion

Sorrel and horehound had significantly higher number of galls and egg-masses, than the other plant species ($p < 0.01$) (Table 2). The lowest number of galls and egg-masses were recorded in chamomile, garden anchusa and thistle, being significantly lower than those on other plants ($p < 0.01$). The number of eggs in the sorrel root system and the number of J_2 per pot of garden anchusa were significantly higher than those on the other tested plants. Reproduction factor ranged from 0.05 (in chamomile) to 39.61 (in sorrel) but there was no significant difference among the RFs of chamomile and thistle, lovage, woundwort, english plantain and chamomile ($p < 0.01$) (Tables 2 and 3). Therefore, according to our results in Table 3, the resistance of tested medicinal plants to infection by *M. javanica* can be ranked as follows, according to the Canto-Saenz's scheme (Sasser *et al.,* 1984): thistle (*E. adenocaulos*), lovage (*L. officinale*) and chamomile (*M. chamomilla*) are classified as hypersusceptible, showing significant damage (GI > 2) while the RF remains below 1. Garden anchusa (*A. italica*), fennel (*F. vulgare*), horehound (*M. vulgare*), alkakengy (*P. alkekengi*), english plantain (*P. lanceolata*), woundwort (*S. pilifera*) and sorrel (*R. acetosella*) are ranked as susceptible, with heavy galling (GI = 5) and high reproduction factors (RF > 2). Sorrel and horehound are the most susceptible hosts with high reproduction factors (RF = 39.61 and 20.08, respectively).

Our findings on chamomile, *M. chamomilla,* to *M. javanica* (hyper-susceptible host) were similar to those by Baida *et al.* (2011) on susceptibility of *Matricaria recutita* L., while

Table 2. Mean population indices of the root-knot nematode *Meloidogyne javanica* on ten species of medicinal plants, 60 days after inoculation under greenhouse conditions.

Medicinal plants	Number of J_2 in soil	Number of eggs/root	Number of egg-masses/root	Number of galls/root
Physalis alkekengi L.	9525 ± 35.18^{c}	19514 ± 356^{c}	148 ± 5.10^{cd}	161 ± 6.03^{de}
Matricaria chamomilla L.	56 ± 7.18^{f}	184 ± 2.73^{e}	14 ± 1.79^{e}	21 ± 1.64^{f}
Rumex acetosella L.	37958 ± 859^{b}	176719 ± 6948^{a}	786 ± 82.61^{a}	801 ± 82.85^{a}
Plantago lanceolata	3143 ± 42.65^{e}	9306 ± 59de	344 ± 5.67^{b}	365 ± 5.43^{b}
Foeniculum vulgare Mill.	6619 ± 35.91^{d}	5104 ± 94.58^{e}	258 ± 6.57^{bc}	287 ± 6.69^{bc}
Anchusa italica Retz.	72380 ± 244^{a}	241 ± 8.95^{e}	98 ± 2.81^{de}	109 ± 4.32^{def}
Marrubium vulgare L.	5670 ± 66.24^{d}	99109 ± 1901^{b}	688 ± 20.14^{a}	734 ± 27.78^{a}
Levisticum officinale L.	640 ± 36.51^{f}	3411 ± 64^{e}	156 ± 6.51^{cd}	165 ± 5.09^{de}
Echinops adenocaulos Boiss.	0 ± 0^{f}	364 ± 10.27^{e}	16 ± 3.20^{e}	63 ± 10.26^{ef}
Stachys pilifera Benth.	919 ± 35.9^{f}	15506 ± 487^{cd}	190 ± 7.55^{cd}	197 ± 8.12^{cd}

Values in the same column followed by the same letter(s) are not significantly different ($p < 0.01$) based on Duncan's multiple range test (DMRT). Values are means ± standard error.

Table 3. Designation of resistance of ten species or aromatic plants to the root-knot nematode *Meloidogyne javanica* based on reproduction factor and gall index.

Medicinal plants	Reproduction factor	Gall Index	Resistance
Physalis alkekengi L.	5.56 ± 0.08^{c}	5	S
Matricaria chamomilla L.	0.05 ± 0.002^{d}	3	H
Rumex acetosella L.	39.61 ± 2.92^{a}	5	S
Plantago lanceolata	2.44 ± 0.006^{cd}	5	S
Foeniculum vulgare Mill.	2.28 ± 0.03^{cd}	5	S
Anchusa italica Retz.	5.19 ± 0.018^{c}	5	S
Marrubium vulgare L.	20.08 ± 0.38^{b}	5	S
Levisticum officinale L.	0.30 ± 0.0042^{d}	5	H
Echinops adenocaulos Boiss.	0.07 ± 0.002^{d}	4	H
Stachys pilifera Benth.	3.16 ± 0.10^{cd}	5	S

Values in the same column followed by the same letter(s) are not significantly different ($p < 0.01$) based on Duncan's multiple range test (DMRT). Values are means ± standard error.
Resistance based on Sasser *et al.* (1984): S = Susceptible; H = Hypersusceptible

Walker (1995) considered chamomile a susceptible host. Fennel was found to be a susceptible host whereas according to Walker (1995) is hyper-susceptible to *M. incognita* race3.

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Αντοχή δέκα κοινών φαρμακευτικών φυτών στον κομβονηματώδη *Meloidogyne javanica*

S. Ansari, H. Charehgani και R. Ghaderi

Περίληψη Προκαταρκτική επισκόπηση έδειξε ότι ο κομβονηματώδης *Meloidogyne javanic*a είναι ευρέως διαδομένος στη ριζόσφαιρα φαρμακευτικών φυτών στην περιοχή Boyer-Ahmad (Ιράν). Εξετάστηκε η καταλληλότητα δέκα ειδών φαρμακευτικών φυτών ως ξενιστές του *M. javanica* σε πείραμα υπό ελεγχόμενες συνθήκες θερμοκηπίου: φυσαλίς (*Physalis alkekengi* L.), χαμομήλι (*Matricaria chamomilla* L.), πεντάνευρο (*Plantago lanceolata* L.), μάραθο (*Foeniculum vulgare* Mill.), αγχούζα (*Anchusa italica* Retz.), σκυλόχορτο (*Marrubium vulgare* L.), λουίζα (*Levisticum officinale* L.), ξινάκι (*Rumex acetosella* L.), εχίνοπας (*Echinops adenocaulos* Boiss.), στάχυς (*Stachys pilifera* Benth.). Σύμφωνα με το πρωτόκολλο του Canto-Saenz, επτά από τα είδη, η αγχούζα, ο μάραθος, το σκυλόχορτο, η φυσαλίς, το πεντάνευρο, ο στάχυς και το ξινάκι, μπορούν να θεωρηθούν ευαίσθητοι ξενιστές με δείκτη παρουσίας κόμβων του νηματώδη στις ρίζες (gall index - GI) > 2 και συντελεστή αναπαραγωγής (RF) >1, ενώ ο εχίνοπας, η λουίζα και το χαμομήλι, μπορούν να θεωρηθούν υπερ-ευαίσθητοι με GI> 2 και RF ≤ 1.

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