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RESEARCH ARTICLE

Evaluation of certain Hungarian plant extracts for their nematicidal properties against root-knot nematode, *Meloidogyne incognita* in-vitro.

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Abstract

The in-vitro nematicidal activity of aqueous extracts derived from different parts of nine Hungarian medicinal plants at two concentrations i.e. 1 and 2% on newly hatched juveniles mortality after 24 and egg hatching of *Meloidogyne incognita* after 72 hrs of exposure time revealed that larval mortality percentages generally increased with increase in plant extract concentrations tested at the exposure time. Using peppermint, tarragon and marjoram resulted in significantly higher larval mortality percentages at 1 and 2 % of concentrations that were amounted to 100 % at 24 hrs of exposure time; followed by Lemon balm at 2 % and 1 % with values of 77.7 and 66.7 %, respectively. Likewise, no hatching in eggs of *M. incognita* was happened when exposed to extracts obtained from these plants at the same concentrations for 72 hrs. However, observations were made on the use of valerian extract at all concentrations which showed the lowest larval mortality percentages and highest number of egg hatching.

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are the most economically important plant parasitic nematodes group that cause serious damage to most agricultural crops worldwide (Sasser et al., 1983). *Meloidogyne incognita* is the most common species of root-knot nematodes (RKN) and infects almost all cultivated plants, which makes it perhaps the most damaging of pathogens Sasser and Freckman (1987).

Nowadays, chemical nematicides are losing their popularity among farmers for protecting their crops from nematode infestations because of their harmful effects and environmental pollution. Plants appear to be a source of cheap effective pesticidal compounds, having low plant and human toxicity and being easily biodegradable (Prakash and Rao, 1997). Allelochemicals are plant-produced compounds that affect the activity of other organisms and are thought to be toxins and secondary metabolites that act as attractants or deterrents (Dodds, 1996; Brown and Morra, 1997). Consequently, a large number of plants/ plant parts have been screened for their nematicidal activities (Pandey, 1990; Nour El-Deen and Darwish, 2011 and Nour El-Deen et al., 2013).

A special feature of higher angiospermic plants is their capacity to produce a large number of organic chemicals of high structural diversity which called secondary metabolites (Evans et al., 1986). These metabolites are divided into different categories based on their mechanism of function like chemotherapeutic, bacteriostatic, bactericidal, antimicrobial and nematicidal (Purohit and Mathur, 1999 and Oka et al., 2000). Many compounds with nematicidal activity have been found in plants including alkaloids, diterpenes, fatty acids, glucosinolates,

isothiocyanates, phenols, polyacetylenes, sesquiterpenes and thienyls (Gommers, 1981; Chitwood, 2002; and Taba et al., 2009).

In Hungary, although most researchers have investigated the antifungal and antibacterial activities of certain medicinal plants, no or little attention has been given to nematicidal activities of extracts derived from such plants. Therefore, the aim of this research paper was to study the nematicidal activity of certain medicinal plant extracts on *Meloidogyne incognita* (J_2s) mortality and egg hatchability in-vitro.

Materials and Methods

This study was carried out in the laboratory of Plant Protection Institute, Georgikon Faculty, Pannonia University, Hungary to determine the nematotoxic effects of nine Hungarian plants at two concentrations on *Meloidogyne incognita* larval mortality and egg hatching in-vitro.

Plant Materials:

The plant parts of nine Hungarian medicinal plants used in this study are listed in Table (1). Plant materials were collected randomly from plants grown in the university experimental farm. The plant species were identified by referring the standard morphological characteristic features keys according to Király, 2009.

Plant Extracts Preparation:

Plant materials of the nine plants were washed thoroughly under running tap water, cut into small pieces, shade dried and used for extraction. Dried plant materials were homogenized to a fine powder and stored in airtight bottles. 4 g of each plant material powder were extracted with 200 ml of distilled water for 24 h to prepare 2% aoncentration, whereas, half of each plant material powder amount was extracted with 200 ml of distilled water for 24 h to prepare 1% concentration. The suspension was filtered and stored at 4^{o}C (Natarajan et al., 2006).

Table (1): Common, scientific and family names as well as parts of nine Hungarian plants used in this study.

Common name	Scientific name	Family name	Part used
Basil	*Ocimum basilicum*	Lamiaceae	Leaf and Flower
Rosemary	*Rosmarinus officinalis*	Lamiaceae	Leaf
Marjoram	*Origanum vulgare*	Lamiaceae	Leaf and Flower
Sage	*Salvia officinalis*	Lamiaceae	Leaf
Lemon balm	*Melissa officinalis*	Lamiaceae	Leaf and Flower
Grapevine	*Vitis vinifera*	Vitaceae	Shoot
Valerian	*Valeriana officinalis*	Valerianaceae	Root and Rhizome
Tarragon	*Artemisia dracunculus*	Asteraceae	Leaf and Flower
Peppermint	*Mentha piperita*	Lamiaceae	Leaf and Flower

Nematode Inocula Preparation:

The root-knot nematode *M. incognita* eggs were extracted from infected sunflower roots using 0.5 % NaOCl solution and shaking for 2 minutes (Hussey and Barker, 1973), while second-stage juveniles (J_2) were extracted from infected roots by hatching. These eggs and J_2s were obtained from a pure culture established from single egg masses of *M. incognita* that previously identified according to the characteristics of its perineal pattern (Taylor and Sasser, 1978) and reared on tomato plants grown in greenhouse of Plant Protection Institute, Georgikon Faculty, Pannonia University, Hungary. Nematode inocula consisted of 100 viable eggs and 30 freshly hatched second stage juveniles of *M. incognita*.

Nematicidal Activity Test:

1- Juveniles mortality:

Approximately thirty freshly hatched second stage juveniles of the root-knot nematode, *M. incognita* in one ml distilled water was poured into wells of 24-well tissue culture plates over one ml of the tested concentrations. The plates were then covered with the lid and kept in an incubator at 25º C. A 2 mls of distilled water containing nematode larvae served as control. Each treatment was replicated three times. Dead nematodes were counted and recorded after 24 hours using Hawksely counting slide. Mobility was confirmed by touching the nematode with a fine needle. Nematodes that appeared no realistic movement were considered as dead. Percentages of the nematode mortality were then calculated and recorded for each concentration. Mortality percentages were transformed to arcsin (Bliss, 1937) values just before statistical analysis.

2- Egg hatching:

Fifty four wells of 24-well tissue culture plates were filled with (one milliliter) of one hundred of nematode eggs and extracts of different plants at concentrations of 1 and 2 %, each of which was replicated three times. Three wells which received only distilled water were used as control. The number of malforming eggs and (died or a live) hatched juveniles were recorded using Hawksely counting slide after 3 days.

Statistically, the obtained data were subjected to analysis of variance (ANOVA) as a factorial in complete block design (Gomez and Gomez, 1984) followed by Duncan's multiple range test to compare means (Duncan, 1955).

Results

The aqueous extracts of nine Hungarian medicinal plants and concentrations (1 and 2 %) on mortality percentage of newly hatched juveniles and egg hatching of *M. incognita* are depicted in tables 2 and 3, respectively. In general, larval mortality percentages significantly increased with increase in plant extract concentrations tested after 24 hrs of exposure duration. On the other hand, egg hatching decreased with increase in plant extract concentrations tested after 3 days of exposure duration.

Data in table (2) revealed that among treatments of the extracts of tested plants on *M. incognita* J_2s mortality, peppermint, tarragon and marjoram applications obviously ranked first for the highest values at 1 and 2 % of concentrations that were amounted to 100 % at 24 hrs of exposure time; followed by Lemon balm at 2 % and 1 % with values of 77.7 and 66.7 %, respectively. Treatment of valerian extract gave the lowest values of J_2 mortality at all concentrations those recorded 21 and 13.3 % of nematode mortality for 2 and 1 % concentrations, respectively (Table 2). It was clear that fifty percentages of newly hatched juveniles was dead when exposed to 2 % of sage extract. Moreover, extracts obtained from basil at 2 % and sage at 1 % gave 42.3 and 37.7 % of *M. incognita* mortality after 24 hrs of exposure.

Table (2): Effect of aqueous extracts of certain Hungarian plant extracts on mortality percentage of second stage juveniles of *M. incognita* under laboratory conditions.

Conc. / Plant extracts	% of nematode mortality		
	1%	**2%**	**Means**
Ocimum basilicum	33.3 ^{fg}	42.3 ^{de}	37.7 ^{d}
Rosmarinus officinalis	23.3 ^{hi}	33.3 ^{fg}	28.3 ^{e}
Origanum vulgare	100 ^{a}	100 ^{a}	100 ^{a}
Salvia officinalis	37.7 ^{ef}	50 ^{d}	44 ^{c}
Melissa officinalis	66.7 ^{c}	77.7 ^{b}	72.3 ^{b}
Vitis vinifera	16.7 ^{ij}	25.7 ^{gh}	21 ^{f}
Valeriana officinalis	13.3 ^{j}	21 $^{h-j}$	17.3 ^{f}
Artemisia dracunculus	100 ^{a}	100 ^{a}	100 ^{a}
Mentha piperita	100 ^{a}	100 ^{a}	100 ^{a}
N alone	0.0 ^{k}	0.0 ^{k}	0.0 ^{g}
Means	14.7 ^{b}	16.5 ^{a}	

*Each figure represents the mean of three replicates.

*Means in each column followed by the same letter did not differ at P< 0.05 according to Duncan's multiple range test.

N= 30 *M. incognita* J_2

Data presented in table (3) showed the efficacy of extracts derived from nine Hungarian medicinal plants at two concentrations on *M. incognita* egg hatching. An opposite trend was observed concerning egg hatching that was decreased as the concentrations increased. Likewise, no hatching in eggs of *M. incognita* was happened when exposed to extracts obtained from peppermint, tarragon and marjoram plants at 1 and 2 % for 72 hrs. However, number of hatched eggs in treatment of sage at 2 % was 3.3, whereas, lemon balm and basil extracts at 2 % gave the same value for egg hatchability (6.7). Valerian extract treatment significantly ranked first for the highest number of *M. incognita* egg hatching at 1 % with value of 93.3 as compared with those of other treatments; followed by

rosemary and grapevine at the same concentration with value of 76.7 for each in comparison with other treatments and nematode alone.

Table (3): Impact of aqueous extracts of certain Hungarian plant extracts on egg hatching of *M. incognita* under laboratory conditions.

Conc. / Plant extracts	No. of hatched juvenile (J_2)		
	1%	2%	Means
Ocimum basilicum	20 ^{e}	6.7 ^{ef}	13.3 ^{d}
Rosmarinus officinalis	76.7 ^{bc}	43.3 ^{d}	60 ^{c}
Origanum vulgare	0.0 ^{g}	0.0 ^{g}	0.0 ^{e}
Salvia officinalis	16.7 ^{ef}	3.3 ^{ef}	10 ^{d}
Melissa officinalis	10 ^{ef}	6.7 ^{ef}	8.3 ^{de}
Vitis vinifera	76.7 ^{bc}	66.7 ^{c}	71.7 ^{bc}
Valeriana officinalis	93.3 ^{ab}	73.3 ^{c}	83.3 ^{b}
Artemisia dracunculus	0.0 ^{g}	0.0 ^{g}	0.0 ^{e}
Mentha piperita	0.0 ^{g}	0.0 ^{g}	0.0 ^{e}
N alone	100 ^{a}	100 ^{a}	100 ^{a}
Means	37.3 ^{a}	32.0 ^{a}	

*Each figure represents the mean of three replicates.

*Means in each column followed by the same letter did not differ at P< 0.05 according to Duncan's multiple range test.

N= 100 *M. incognita* eggs.

Discussion

It is evident that the larval mortality percentages and egg hatching of *M. incognita* were affected to certain extent by all components and concentrations tested.

Apparently, *Meloidogyne incognita* J_2s mortality percentages increased as the concentrations of extracts derived from tested plants increased from 1 to 2 %. In general, it can be said that the highest percentages of *M. incognita* J_2 mortality as well as the lowest hatching in eggs were obtained from extracts derived from peppermint, tarragon and marjoram plants after 24 hrs of exposure time which amounted to 100 %. Moreover, treatment of lemon balm also gave a considerable larval mortality percentage either at 2 or 1 % with values of 77.7 and 66.7 %; however, the use of basil and sage plant extracts at 2 % also showed reliable larval mortality percentages 42.3 and 37.7 %, respectively.

The nematicidal effect of the tested extracts may possibly be attributed to their high contents of certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (Knoblock et al., 1989). The present findings are in accordance with those reported by Jourand et al., 2003 who found that leaf extracts of *Crotalaria virgulata* subsp. *grantina* had a nemostatic effect on the J_2s of *M. incognita* at low concentration. Insunza et al. (2001) recorded that extracts of the roots of *Melissa officinalis* were nematicidal or nematostatic to *Xiphinema americanum* after 24 h at the standard and 25% of S concentrations. In addition, strong nematicidal activity at the standard concentration was shown by top of *Ocimum basilicum* and the root extracts of *Mentha citrate*.

Our results are in line with several reports that claimed that these plants contain several biologically active compounds which are a source of nematotoxic effect. Some of the reported biologically active compounds included α-thujene (26.8%), limonene (22.9%), linalyl acetate (17.4), ocimene (10.7%), linalool (5.7%), myrcene (3.6%) and α-pinene (2.5%) in leaves, stem and flowers of *Salvia officinalis*; carvacrol (61%), thymol (21.8%), δ-terpinene (4%), p-cymene (5.5%), myrcene (1.2%) and α-terpinene (1.3%) in leaves of *Origanum syriacum*; and piperitenone (54.2%), pulegone (10.7%), piperitenone oxide (11.3%), menthone (3.3%) and 1,8 cineole (2.8%) in leaves and flowers of *Mentha microphylla*. Over 20 major compounds of the essential oils were identified, but the most toxic against *M. incognita* J_2s were carvacrol, linalool, thymol and menthone. At very low concentrations (1 mg l-1)

several oils immobilized the juveniles and some also reduced hatching. Essential oils from spearmint and oregano showed a very high nematicidal activity (Oka et al., 2000).

Some pure essential oils (carvacrol, linalool, thymol and menthone) exhibited nematode-suppressive characteristics equivalent to that of cadusafos, a synthetic pesticide (Ibrahim and Haydock, 1999). Moreover, these compounds have also antifungal (Ankri and Mirelman, 1999 and Silva et al., 2001), antibacterial and insecticidal properties, they are easily biodegraded and broken down into products that aren't harmful to plant, human, animals or to the environment.

In the present work, treatment of valerian extract did not act as strong nematicide on nematodes, since it was found to be the least effective to kill larvae as well as stimulate the hatching of *M. incognita* eggs. More than 75% of the eggs were hatched in the extracts of rosemary and grapevine at low concentration. Furthermore, un-hatched eggs contained motile larvae, suggesting that substance in the rosemary and grapevine did not inhibit hatching; rather they prolonged the time interval required for hatching.

Results obtained from the present study clearly suggested that aqueous extracts of some native Hungarian plants especially *O. vulgare*, *M. piperita* and *A. dracunculus*, one of the most important cultivated spice plant showed great potential in the inhibition of *M. incognita* egg hatching and mortality of larval stage. Other plant species tested in this study showed low degrees of nematode activities for the previous criteria. The nematicidal activity of the plant extracts used in this study may serve as leads for development of plant-based agrochemicals. Nevertheless, it is not known whether the nematicidal activity was due to a single compound or to a complex of compounds, or other mechanisms and/or interactions. Ongoing research is evaluating methods to use some of these materials as alternative methods for root-knot nematode control in-vivo in the future.

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References

Ankri, S., Mirelman, D. (1999). Antimicrobial properties of allicin from garlic. Microbes and Infection, 2: 125-129.

Bliss, C. I. (1937). Table A. 10. Transformation of bionomial percentages (Arcsin). This table appeared in Plant Protection (Leningrad), 12 (67): 448-449.

Brown, P.D. and Morra, M.J. (1997). Control of soil-borne plant pests using glucosinolate containing plants. In: Advances in Agronomy. Vol. 61. (D.L. Sparks, ed.), Academic Press, San Diego, CA, USA, 167–215.

Chitwood, D. J. (2002). Phytochemical based strategies for nematode control. Annual Review of Phytopathology, 40: 221-249.

Dodds, C.J. (1996). The control of slug damage using plant derived repellents and antifeedants. In: Slug and Snail Pests in Agriculture, (I.F. Henderson, ed.). British Crop Protection Council, Farnham, UK, Monograph No. 66: 335–340.

Duncan, D.B. (1955). Multiple rang and multiple,F-test Biometrics,11: 1-42.

Evans, J. S., Pattison, E. and Morris, P. (1986). Antimicrobial agents from plant cell culture, in: secondary metabolites in plant cell culture. Cambridge University, London. p.12.

Gomez, K.A. and Gomez, A.A. (1984). Statistical Procedures for Agricultural Research. 2nd Ed., John Wiley&Sons. Inc. New York.

Gommers, F. J. (1981). Biochemical interactions between nematodes and plants and the irrelevance to controlling review. Helminthol. Abstr., 50:9-24.

Hussey, R.S. and Barker, K.R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter, 57: 1925-1928.

Ibrahim, S.K. and Haydock, P.P.J. (1999). Cadusafos inhibits hatching, invasion and movement of the potato cyst nematodes *Globodera pallida*. Journal of Nematology, 31: 201–206.

Insunza, V., Aballay, E. and Macaya, J. (2001). In vitro nematicidal activity of aqueous plant extracts on chilean populations of *Xiphinema americanum* sensu lato. Nematropica, 31(1): 47-54.

Jourand, P., Rapior, S., Fargette, M. and Mateille, T. (2003). Nemostatic effects of a leaf extract from *Crotolaria virgulata* subsp. *gratiana* on *Meloidogyne incognita* and its use to protect tomato roots. Nematology, 6: 79–84.

Király, G. (2009). Új magyar füvészkönyv: Magyarország hajtásos növényei. Aggteleki Nemzeti Park Igazgatóság. (in English: New Hungarian Botanical Guide: Cormophyta). Aggtelek National Park Directorate Press.

Knoblock, K., Weis, N. and Wergant, R. (1989). Mechanisms of antimicrobial activity of essential oils. 37th Ann. Cong. Med. Plant Res. Braunschweig, pp. 5-9.

Natarajan, N., Cork, A., Boomathi, N., Pandi, R., Velavan, S and Dhakshnamoorthy, G. (2006). Cold aqueous extract of African marigold *Tagetes erecta* for control tomato root knot nematode, *Meloidogyne incognita*. Crop Protection, 25: 1210-1213.

Nour El-Deen, A. H. and Darwish, Hadeer Y. (2011). Nematicidal activity of certain Egyptian weeds and bald cypress callus extracts against *Meloidogyne incognita* infecting eggplant under greenhouse conditions. Egypt. J. Agronematol. 10 (2). 242-254.

Nour El-Deen, A.H., Omaima M. Abdel-Kafie and Naira M. El-Ghareb (2013). Evaluation of seaweed extract and various plant products against *Meloidogyne incognita* on basil. Georgikon for Agriculture, 16 (1): 29-34. Proceedings of XXIII Keszthelyi Növényvédelmi Fórum. Keszthely, Hungary, January 23-25, 2013.

Oka, Y., Necar, S., Putievesky, E., Ravid, V., Yaniv, Z. and Spiegel, Y. (2000). Nematicidal activity of essential oils and their components against the root-knot nematode. Phytopathol., 90(7): 710-715.

Pandey, R. (1990). Studies on phytonematotoxic properties in the extract of some medicinal and aromatic plants. Int. Nematol. Net-work Newslett., 7: 19-20.

Prakash, A. and Rao, J. (1997). Botanical pesticides in agriculture. CRC. Lewis Publishers, Boca Raton, New York, London, Tokyo.

Purohit, S. S. and Mathur, S. K. (1999). Drugs in biotechnology fundamentals and applications. Purohit SS. Maximillan Publishers, India. p. 576.

Sasser, J. N., Eisenback, J. D. and Carter, C. C. (1983). The international *Meloidogyne* project- its goals and accomplishments. Annu. Rev. Phytopathol., 21: 271- 278.

Sasser, J.N. and Freckman, D.W. (1987). A world prospective on nematology: The role of the Society. In: Vistas on Nematology (Veech J.A., D.W. Dickson, ed.), Society of Nematologists Inc., Hyattsville, USA, 7–14.

Silva, L., Filho, M., Bonifácio, K. and Ito, I. (2001). In Vitro Evaluation of the Antimicrobial Activity of a Castor Oil-Based Irrigant. Journal of Endodontics, 27(12): 717-719.

Taba, S., Sawada, J. and Moromizato, Z. (2008). Nematicidal activity of Okinawa island plants on the root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood. Plant Soil, 303: 207-216.

Taylor, A.L. and Sasser, J.N. (1978). Biology, identification and control of root-knot nematodes (*Meloidogyne* species). Coop. Publ, Dep. Plant Pathol., North Carolina State Univ. and U.S. Agency Int. Dev., Raleigh, NC., 111 pp.