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Effect of some aromatic leaf extracts on egg hatching of root-knot nematode, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Nematoda: Meloidogynidae)

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Abstract
Root-knot nematodes are one of the most important limiting factors affecting plant growth and productivity. Currently, synthetic pesticides are the main chemicals used to control nematodes, but natural products can provide a safer alternative. Nematicidal compounds have been identified in a wide variety of plant species. A research was conducted to evaluate potential of aromatic leaf extracts from *Tagetes patula*, *Eucalyptus globulus*, *Rosmarinus officinalis*, *Thymus vulgaris*, *Mentha piperita* and *Laurus nobilis*, in controlling the hatch of eggs of the root-knot nematode, *Meloidogyne javanica*. Five concentrations of water soluble leaf extracts from the six plant species were filtered, added to petri dishes and infested with eggs of *M. javanica*. The best results for all concentration values were obtained with *T. patula* and *E. globulus* with no hatching within 48 h. At the end of this period, the control trial value was found to be 44.04 %.

Keywords: Bio-control, *Meloidogyne javanica*, plant extract, root-knot nematode

Introduction

Nematodes belong to the animal kingdom. Sometimes called eelworms, nematodes are wormlike in appearance but quite distinct taxonomically from the true worms. Most of the several thousand species of nematodes live feely in fresh or salt waters or in the soil and feed on microscopic plant and animals. Numerous species of nematodes attack and parasitize humans and animals, in which they cause various diseases. Several hundred species, however, are known to feed on living plants, causing a variety of plant diseases (Karakas and Bolukbasi, 2019) [1].

Root-knot nematodes (RKNs), (*Meloidogyne* spp.) occur throughout the world but are found more frequently and in greater numbers in areas with warm or hot climates and short or mild winters. Root-knot nematodes are also found in greenhouses everywhere when non-sterilized soil is used. They attack more than 2000 species of plants, including almost all cultivated plants.

Root-knot nematodes damage plants by devitalizing root tips and either stopping their growth or causing excessive root production, but primarily by causing formation of swelling of the roots, which not only deprive plants of nutrients but also disfigure and reduce the market value of many root crops. When susceptible plants are infected at the seedling stage, losses are heavy and may result in complete destruction of the crop. Infections of older plants may have only slight effects on yield or they may reduce yields considerably (Perry and Moens, 2006) [2].

Meloidogyne javanica (Treub, 1885) Chitwood, 1949 is a species of plant-parasitic nematodes (PPNs). It is one of the tropical RKNs and a major agricultural pest in many countries. It has many hosts. This species is a nematode pathogen that affects over 770 species of plants. The hosts of this pathogen include both weeds and crops of economic importance (Kheir *et al*., 1979; Volvas *et al*., 2005) [3, 4]. Those of economic importance include tea, grapevine, vegetables, fruit trees, cereals, an ornamentals. *Meloidogyne javanica* is considered an agricultural pest, as it is extremely abundant and damaging (Alford, 2012) [5].

Synthetic pesticides have been considered the most effective and accessible means to control of agricultural pests (Soltani *et al*., 2013) [6]. These chemicals are associated with undesirable effects on the environment due to their slow biodegradation in the environment and some toxic residues in the products for vertebrates especially for mammalian health (Sahebani and Hadavi, 2008) [7].

The negative effects of synthetic pesticides have increased the need for effective and biodegradable pesticides. Natural products are an excellent alternative to synthetic pesticides as a way to reduce negative effects on human health and the environment (Thomas *et al*., 2002) [8]. The popularity of botanical pesticides is growing once again and some plant products are used globally as green pesticides (Hamza *et al*., 2016) [9]. Among the various natural ingredients that are particularly noticeable as natural ingredients for insect management are essential oils and extracts from aromatic plants.

Therefore, the present study was initiated to find and recommend possibly the most effective aromatic plant leaf extracts on egg hatching of RKN, *M. javanica* under laboratory conditions.

2. Materials and Methods

2.1 Sampling

A total of 40 samples were collected from vegetable growing areas of Antalya-Turkey (29° 20’-32°35’ E; 36° 07’-37° 29’ N). Samples consisted of about 10% open fields and 90% greenhouses. Between 5 and 10 plants were uprooted and average seven root systems and soil samples were collected at each area.

2.2. Nematode culture

Galled roots were gently washed with tap water and an egg-mass was collected using needle and placed in Eppendorf tubes under a stereomicroscope. These egg-masses were surface-sterilized for a short period in 0.5% sodium hypochlorite (NaOCl), rinsed in tap water three times and prepared for inoculation. Susceptible cucumber (*Cucumis sativus* L.) seedlings with 5-6 true leaves were transplanted into 250 ml pots containing a mixture of 68% sand, 21% silt and 11% clay soil autoclaved at 121°C for 40 min. Single egg-masses were inoculated in to a hole 2-3 cm deep near each cucumber seedlings five days after the transplantation. The assay was conducted at 26±1°C and 60±5% RH, with a 16:8 h L:D photoperiod in a controlled environment chamber. Five single egg-mass cultures were established for tests. Eight weeks after the inoculation, plants were uprooted and the most developed selected for multiplication in pure culture.

Uprooted infected plants carefully washed in running tap water and egg-masses collected in to Petri dishes containing distilled water. For this process, mature females of RKNs were removed from galled cucumber roots using needles and forceps under a stereomicroscope.

2.3. Identification

Species level identification was made according to Jepson (1987) [10] and Karssen (2002) [11].

2.4 Test plants

French marigold-*Tagetes patula* L., eucalyptus-*Eucalyptus globulus* Labill, rosemary-*Rosmarinus officinalis* L., thyme-*Thymus vulgaris* L., mint-*Mentha piperita* L. and bay-*Laurus nobilis* L. were selected for the present study. The selected plants were obtained from the Antalya region public market of Turkey.

2.5 Preparation of aromatic leaf extracts

Five concentrations (w/v) i.e., 5.0, 7.5, 10.0, 12.5 and 15.0% of hot water aromatic leaf extracts were prepared by boiling 45, 65, 85, 105 and 125 g chopped fresh aromatic leaves of each plant for 15 min. in 650 ml of water. The leaf extracts were filtered through cotton plug and 5 ml of extract from each concentration was placed separately in a Petri dish (90 x 17 mm). Water was used for the control. Each treatment was replicated three times.

2.6 Extract-nematod applications

Root-knot infected cucumber plants from the culture pots were uprooted and washed gently under running tap water. Fresh and uniform egg-masses of *M. javanica* were collected using needles and forceps under a stereomicroscope. One egg-mass of *M. javanica* was added to each Petri dish containing the extracts. The effect of different concentration levels on the hatching of nematodes was determined at six observation time specified as 1, 3, 6, 12, 24 and 48 h. Data were recorded and comparisons of hatching percentage were made on the basis of average of 260 eggs per egg-mass. The data was analysed statistically by z-test using SigmaStat software programme, version 3.1.

3. Results and Discussion

The aromatic leaf extract of all selected plants delayed the hatching of *M. javanica* eggs (Table 1). The effect of different concentration percentages and exposure times on hatching of eggs varied with leaf extract type and exposure time. In the control, hatching started just after 1 h, whereas for the other treatments there was no hatching until 1 h. The percentage hatch increased from 0.22 % at 1 h to 44.04 % until 48 h in the control.

No hatching was observed at any dose with *T. patula* and *E. globulus* leaf extracts. In *R. officinalis*, with 5.0 and 7.5 % concentration percentages, there was continuous increase in percentage hatching until 48 h while with 10.0 % concentration percentage the number of live larvae was found the decrease after 24 h. The decrease in the number of live larvae may be due to the larvicidal effect value of these leaf extracts. No hatching occurred with 12.5 and 15.0 concentration percentages. In *T. vulgaris* there was 0.56 and 0.22 % hatching with 5.0, 7.5, 10.0 and 12.5 % concentration, respectively, after 48 h. but higher concentration percentage (15.0 %) of *T. vulgaris* inhibited hatching completely. In the case of *L. nobilis*, hatching started after 6 h with all the concentrations. The hatching percentage with 5.0 % concentration was increased from 0.44 to 1.67 % at 24 h but decreased to 0.56 % at 48 h. A similar trend was observed in other concentration percentages. This decrease in hatching, as also found in case of *R. officinalis*, could possibly be due to mortality of some larvae. In *M. piperita*, there was 0.33 and 0.44 % hatching with 5.0, 7.5 and 10.0 % concentration, respectively, after 48 h. Higher concentration percentages (12.5 and 15.0 %) of *M. piperita* inhibited hatching completely.

Tagetes patula and *E. globulus* leaf extracts were found to be the most effective herbals among all the selected herbals in delaying and controlling the hatching of *M. javanica*, followed by *M. piperita*, *T. vulgaris*, *R. officinalis* and *L. nobilis*.

Table 1: Effect of hot water aromatic leaf extracts on hatching of eggs on root-knot nematode, *Meloidogyne javanica*

Plant species	Concentration (%)	Egg-hatching (%)					
		1 h	3 h	6 h	12 h	24 h	48 h
T. patula (French marigold)	5.0	-	-	-	-	-	-
	7.5	-	-	-	-	-	-
	10.0	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
	15.0	-	-	-	-	-	-
E. globulus (Eucalyptus)	5.0	-	-	-	-	-	-
	7.5	-	-	-	-	-	-
	10.0	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
	15.0	-	-	-	-	-	-
R. officinalis (Rosemary)	5.0	-	-	0.33	1.33	1.33	1.67
	7.5	-	-	0.33	0.77	0.66	0.44
	10.0	-	-	0.22	0.33	0.44	0.33

	12.5	-	-	-	-	-	-
	15.0	-	-	-	-	-	-
T. vulgaris (Thyme)	5.0	-	-	0.22	0.44	0.44	0.56
	7.5	-	-	0.22	0.44	0.44	0.56
	10.0	-	-	-	-	0.33	0.22
	12.5	-	-	-	-	0.33	0.22
	15.0	-	-	-	-	-	-
M. piperita (Mint)	5.0	-	-	-	-	0.33	0.44
	7.5	-	-	-	-	0.11	0.33
	10.0	-	-	-	-	0.11	0.33
	12.5	-	-	-	-	-	-
	15.0	-	-	-	-	-	-
L. nobilis (Bay)	5.0	-	-	0.44	1.44	1.67	0.56
	7.5	-	-	0.33	0.56	0.44	0.67
	10.0	-	-	0.22	0.56	0.44	0.33
	12.5	-	-	0.22	0.33	0.33	0.11
	15.0	-	-	0.11	0.11	0.11	0.11
Control		0.22	1.22	2.56	4.01	4.56	44.04

Use of plant and their products is one of the safe methods to control RKNs. These methods are low cost, easy to apply and also have the ability to improve soil texture and fertility (Feizi *et al*., 2014) [12]. Different plant products to manage plant PPNs have been introduced, such as; marigold (*Tagetes* spp.), wormwood (*Artemisia absinthium*), thyme (*Thymus vulgaris*), cloves hindi (*Syzygium aromaticum*) and members of Apiaceae family such as cumin (*Cuminum cyminum*) and fennel (*Foeniculum vulgare*) (Feizi *et al*., 2014) [12].

According to previous studies, Hussain and Masood (1976) [13] reported complete inhibition of hatching of *M. incognita* eggs in certain leaf extracts like neem (*Azadirachta indica*) and Jerusalem oak (*Chenopodium anthelminticum*). Among plant oil extracts, karanj (*Millettia pinnata*) and neem oil extracts inhibited egg hatch to the extent of 34·00 and 23'53%, respectively. Similarly Khan *et al*., (1975) [14] reported that egg hatch of *M. incognita* was suppressed by aqueous extracts of mahua (*Madhuca longifolia*) and neem extracts. But in the subsequent study it was observed that in extracts of castor (*Ricinus communis*) and gingelly (*Sesanum indicum*), egg hatch was almost normal. This is in variance with the findings of Khan *et al*., (1975) [14] which could be due to the difference in the method of preparation of the extracts as well as the concentration used.

Mishra and Prasad (1975) [15] reported that 1% solution of neem plant extract was highly toxic to larvae of *M. incognita*. The adverse effect of plant products in suppressing nematode activities like egg hatch and larval mobility could be due to the presence of toxic principles like alkaloids, cyanogenic glycosides, glycosides, phenols, terpenoids etc.

Oostenbrink *et al*., (1961) [16] reported similar effect of root exudate of *Tagetes* spp. on *Pratylenchus penetrans* and *Tylenchorhynchus* spp. The toxic effect could be due to the release of certain toxic metabolites from roots. Gommers and Bakker (1988) [17] reported that the root exudate of *Tagetes* spp. contained compounds like cc-terthienyl which could have affected nematode activities.

Today, the use of herbal-based nematicides has gained importance. In many crops, the control of PPNs, including, *M. javanica* is mainly based on nematicides called synthetic chemicals, though safe, environmentally appropriate and nonchemical methods are desirable (Moosavi and Zare, 2012) [18]. Numerous plant extracts have been reported to suppress plant-parasitic nematode population (Ferris and Zheng, 1999; Zasada *et al*., 2002; Kokalis-Burelle and Rodríguez-Kábana, 2006) [19, 20, 21].

Makkar and Becker (1996) [22] showed that, extract of some plants such as moringa (*Moringa oleifera*), oregano (*Origanum vulgare*), ginger (*Zingiber officinale*) and miswak (*Salvadora persica*) contained the cytokinine group which effective in reducing nematode population in plants with a subsequent increase in plant growth and number of leaves. Also, obtained results confirmed with Guzman (1984) [23] who reported that aqueous extract of moringa is as toxic to *Meloidogyne* spp. as standard pesticides. The nematicidal effect of the moringa could be attributed to its high content of certain oxygenated compounds which are characterised 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) [24]. This property may also be responsible for the extract's efficacy in nematode management (Nchore *et al*., 2011, Maina *et al*., 2012, Pavaraj *et al*., 2012) [25, 26, 27].The mechanisms of plant extract action may include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation (Konstantopoulou *et al*., 1994) [28]. According to Claudius-Cole *et al*., (2010) [29], *M. oleifera* is a good inhibitor of nematode egg hatching and juvenile survival.

4. Conclusion

This study showed that water extracts of test plants could be useful for nematode control, which would be an economical and environmentally safe alternative for the control of nematodes. However, more studies are needed to determine the active ingredients of these extracts. Using botanicals as bio-pesticides will reduce dependence on chemical pesticides.

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