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Effect of medicinal plant extracts on inoculated *Meloidogyne javanica* in tomato

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Abstract

In this study, the inhibitory effect of medicinal plant extracts viz., marigold (*Tagetes* spp.), rosemary (*Rosmarinus officinalis* L.) and nigella (*Nigella sativa* L.) on root-knot nematode, *Meloidogyne javanica* @ 1500, 2500 and 5000 juveniles were studied on susceptible tomato cv. Karoon under greenhouse condition. Although all the treatments reduced root infections rate and significantly effect on root weight ($P \leq 0.05$). It was observed that when the nematode inoculum increased, reproduction factor also increased while extract treatments effect decreased. In between treatments, rosemary extract has the greatest impact on the reduction of nematode populations @ 40% concentration as compared to control.

Keywords: *Meloidogyne javanica*, tomato, medicinal plant extracts, gall index, root weight.

Tomato (*Lycopersicon esculentum* Mill.) is one of the important agricultural crops in Iran with a production of 58, 000 metric tons per annum. Nematodes of the genus *Meloidogyne*, commonly known as root-knot nematodes (RKNs), belonging to a group of plant-parasitic nematodes and widely dispersed around the world. Root-knot nematodes parasitize most crops, affecting production and quality (Maleita *et al.*, 2012). Root-knot nematodes are important group of plant parasites that cause heavy damage to the tomato fields. *Meloidogyne javanica* reduced about 50% of the tomato crops in Iran (Hosseini-Negad, 2004).

In many crops, the control of plant-parasitic nematodes, including, *M. javanica* is mainly based on chemical nematicides, though safe, environmentally appropriate and nonchemical methods are desirable (Moosavi & Zare, 2012). Numerous plant extracts have been reported to suppress plant-parasitic nematode population (Ferris & Zheng, 1999; Zasada *et al*., 2002; Kokalis-Burelle & Rodriguez-Kabana, 2006). Several plants in Iran have been reported as anthelminthics and have been traditionally used to treat human and animal diseases caused by nematodes, but there are few reports of their effects on plant-parasitic nematodes (Sholevarfard & Moosavi, 2015). Analysis showed that capability of non-toxic metabolites is the best alternative strategy for controlling nematodes but use of compounds and secondary metabolites of plants was limited (Khayyat *et al.*, 2014). In recent decades, numerous studies have been reported on plant compounds in order to achieve safe and effective methods to control the root-knot nematodes (Khayyat *et al.*, 2014).

Use of plant and their products is one of the safe methods to control root-knot nematodes. These methods are low cost, easy to apply and also have the ability to improve soil texture and fertility (Feizi *et al.*, 2014). Various herbal products to manage plant parasitic nematodes have been introduced, such as; marigold (*Tagetes* spp.), wormwood (*Artemisia*

absinthium L.), thyme (*Thymus vulgaris* L.), cloves hindi (*Syzygium aromaticum* (L.) Merrill & Perry) and members of Apiaceae family such as cumin (*Cuminum cyminum* L.) and fennel (*Foeniculum vulgare* Mill.) (Feizi *et al*., 2014). In this study, the effect of three inoculum levels (1500, 2500 and 5000 juveniles) with three medicinal plant extracts (tagetes, rosemary and nigella) with three concentration levels (10, 20 & 40%) on susceptible species of tomato were examined.

Materials and Methods

Preparation of plant extracts: Plants were collected from field, washed and dried in laboratory conditions. After drying, plants were macerated and kept in separate bags. For the extraction whole of the plant, including stems, leaves and seeds were used. To prepare the concentrations of 10, 20 and 40%, plant powder 100, 200 and 400 g, respectively were mixed in 1000 ml of distilled water in aluminum foil covered flasks. These flasks were then placed into shaker with the idle speed for 24 hours at room temperature. After 24 hours, the contents of the flasks were passed from thin gauze muslin to isolate extracts. Extracts were kept in dark and sealed.

Separation, multiplication and detection of nematodes: Root-knot nematodes were collected from infected tomato fields from Mashhad city belonging to Razavi Khorasan province of Iran. Population of *M. javanica* was reared with single egg-mass inoculation on young tomato seedling for pure culture. Sub-culturing was done subsequently by inoculation new tomato seedlings with at least 15 egg-masses obtained from pure culture in order to maintain sufficient inoculum for the experiment (Nasr-Esfahani & Ansari, 2006). The pure isolate was identified by means of nematode morphology as described by Jepson (1987). Two week old tomato seedlings cv. Karoon were planted in 1 kg clay pots filled with steam sterilized sandy loam soils (1:1) with four replication and three inoculation level of nematode.

Pot experiments and statistical analyses: Holes were made near the root of each plant and juveniles were inoculated @ 1500, 2500 and 5000 when the tomatoes were in 3-4 leaf period. After a week from nematode inoculation 100 ml of each plant extracts (Marigold, Rosemary and Nigella) were added to the pots. Plants were maintained under controlled condition at 85% humidity and temperatures between 25-30 °C. Pots were irrigated after two days interval so that the water was not drained out under the pots.

After 60 days of inoculation, 100 g soil samples were collected from around each rhizosphere and assessed for *M. javanica* juvenile population levels as described by De Grisse method (1969). Nematode egg-masses were extracted from infested roots using a 2% NaOCl solution, the released eggs from the roots were collected using the modified technique described by McClure *et al*., (1973).

Total number of nematode galls were counted as galls/root system, irrespective of their size, 60 days after inoculation for root infestation grading (Heald *et al.*, 1989). The number of galls/root system was assessed and assigned a severity scale from 0 to 5 (0 = no galls, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = 100 galls per root system) as given by Taylor & Sasser (1978). Susceptibility of the plant was assessed on the basis of root gall index (GI) and the reproduction factor (Rf = number of eggs and J_2 in roots and J_2 in soil/Pi) (Sasser *et al.*, 1984)

The experiment was multi-factorial, based on randomized complete block design. There were nine treatments and one control with four replications. Data analysis was done by JMP software and mean comparison based on the least significant difference test.

Results

In this study, nematodes inoculum @ 1500 showed that there is a significant difference between plants extracts, concentrations, and their interactions ($P \leq 0.05$). Data (Table 1) showed that the least number of galls (22) was observed at highest concentration (40%) of rosemary extract while highest number of galls (54) in root system was seen at lowest concentration (10%) in marigold extract that was far less than control (109).

Same trend was found in number of juveniles in 100 g soil and their reproduction factors i.e., at highest concentration of rosemary extracts (40%), the lowest number of juveniles (45) with least Rf (0.33) was observed while in marigold extract highest number of juveniles (225) with highest Rf value (1.56) was observed at lowest concentration (10%) as compared to control (J_2= 290; Rf= 2.02). Egg-masses were found in lowest number (38) at highest concentration (40%) of rosemary extract while number of egg-masses were highest (105) at lowest concentration (10%) of rosemary extract as compared to control (131). Similarly, at the highest concentration (40%) of rosemary extract highest fresh weight of root was obtained (35 g) while it was lowest (30.2) at lowest concentration (10%) of marigold extract. Data in Table 2 & 3 show that similar effect was produced by rosemary and marigold extracts on gall formation in roots, and juveniles in soil and their Rf values at 2500 and 5000 nematode inoculum levels. However, egg-masses and root weight varied at different concentrations in 2500 and 5000 nematode inoculum levels. At 5000 nematode inoculum the lowest (153) and highest egg-masses (427) were formed at highest (40%) and lowest (10%) concentration of nigella plant extract (Table 3).

Results showed that highest percentage of all three plant extracts proved most effective against root-knot nematodes as they reduced number of galls, egg-masses and nematode population in soil. However, their efficacy varied at different inoculum levels of root-knot nematodes on roots of tomato plant.

Overall gall index and reproduction factor in all samples have shown (Fig.1) that the pathogen levels were reduced by applying different concentrations of medicinal plants extracts. Control treatments at all levels, accounted the most of the disease and this results re-affirm the positive impact of medicinal plants in reducing nematode populations and disease.

However, when the population was 5000 nematodes per kg of soil, the efficacy of extract treatments was seemingly broken as the extracts on nematode control had the very little effect.

Table 1. Effect of plant extracts to control nematodes at 1500 inoculum level.

Plant extracts	Conc. (%)	Galls in roots	Larvae in 100 g soil	Eggs-masses in root system	Rf	Root fresh weight (g)	GI
Control	0	109^{a}	290^{a}	131^{a}	2.02^{a}	29.03^{i}	5
Rosemary	10	48^{b}	178^{c}	105^{b}	1.26^{c}	31.09^{g}	4
	20	29^{ef}	99^{fg}	50^{ef}	0.75^{fg}	32.83^{e}	3
	40	22^{g}	45^{h}	38^{f}	0.32^{h}	35.00^{a}	3
Nigella	10	37^{cd}	147^{d}	68^{cd}	1.02^{d}	32.07^{f}	4
	20	35^{cde}	119^{ef}	58^{de}	0.83^{ef}	34.00^{b}	4
	40	27^{fg}	83^{g}	43^{f}	0.58^{g}	33.13^{d}	3
Marigold	10	54^{b}	225^{b}	97^{b}	1.56^{b}	30.20^{h}	4
	20	41^{c}	129^{de}	79^{c}	0.91^{de}	32.16^{f}	4
	40	33^{def}	113^{ef}	60^{de}	0.79^{ef}	33.52^{c}	4

Different letters in the same column indicate significant difference (LSD test, $P \leq 0.05$).

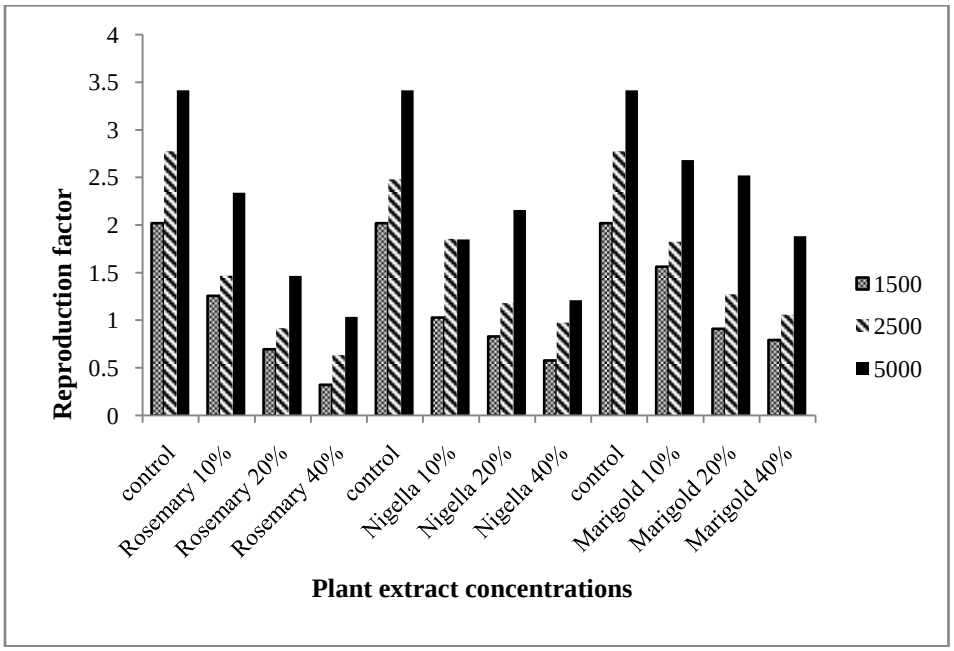

Fig. 1. Comparison of population growth at different concentrations and inoculums levels.

Table 2. Effect of plant extracts to control nematodes at 2500 inoculum level.

Plant extracts	Conc. (%)	Galls in root system	Larvae in 100 g soil	Egg-masses in root system	Rf	Root fresh weight (g)	GI
Control	0	119^{a}	676^{a}	178^{a}	2.77^{a}	27.71^{j}	5
Rosemary	10	63^{c}	357^{bcd}	103^{bc}	1.47^{bc}	30.29^{f}	4
	20	48^{ef}	223de	61^{d}	0.92^{de}	30.62^{e}	4
	40	41^{f}	156^{e}	31^{e}	0.64^{e}	31.88^{b}	4
Nigella	10	62^{c}	455^{cd}	89^{c}	1.85^{b}	29.30^{i}	4
	20	54c^{de}	288de	69^{d}	1.18c^{de}	31.69^{c}	4
	40	45^{ef}	238de	57^{d}	0.97^{cde}	32.58^{a}	4
Marigold	10	73^{b}	445^{bC}	117^{b}	1.82^{b}	29.90^{g}	4
	20	59^{cd}	309^{cd}	89^{c}	1.27^{cd}	31.54^{d}	4
	40	49^{def}	259de	64^{d}	1.06^{cde}	29.65^{h}	4

Different letters in the same vertical column indicate significant difference (LSD test, P≤0.05)

Table 3. Effect of plant extracts to control nematodes at inoculum level 5000.

Plant extracts	Conc. (%)	Galls in root system	Larvae in 100 g soil	Egg-masses in root system	Rf	Root fresh weight (g)	GI
Control	0	156^{a}	1658^{a}	494^{a}	3.414^{a}	24.51^{j}	5
Rosemary	10	112^{cd}	1139^{cd}	305^{de}	2.34^{cd}	25.12^{i}	5
	20	102^{def}	707^{g}	248^{fg}	1.46^{f}	28.59^{c}	5
	40	77^{g}	496^{h}	212^{gh}	1.03^{g}	30.40^{b}	4
Nigella	10	137^{ab}	882^{f}	427^{b}	1.85^{e}	27.38^{e}	5
	20	105^{cde}	1051^{de}	272^{ef}	2.16^{dc}	27.22^{f}	5
	40	91^{efg}	589^{gh}	153^{i}	1.21^{fg}	28.31^{d}	4
Marigold	10	132^{b}	1304^{b}	368^{c}	2.68^{b}	26.78^{g}	5
	20	125^{bc}	1228^{bc}	332^{cd}	2.52^{bc}	25.65^{h}	5
	40	85^{fg}	923^{ef}	186^{hi}	1.88^{e}	30.82^{a}	4

Different letters in the same vertical column indicate significant difference (LSD test, $P \leq 0.05$)

Discussion

The number of galls and egg-masses were inversely proportional to root fresh weight. In treatments where less egg-masses and juveniles were inoculated fresh root weight was increased; whereas at high nematode population, the fresh root weight decreased. Similarly, in control treatments where 5000 juveniles were inoculated, the least fresh root weight was observed, and in the rosemary and marigold plant extracts at a concentration of 40%, highest fresh root weight was observed. Nasr-Esfahani & Ansari (2006), Maleita *et al*., (2012) and Volvas *et al*., (2005) observed that when the rate of nematode population increased, fresh root weight decreased. Results obtained in this study are in conformity with the results of earlier researchers. Ramezani *et al*., (2013) stated that, increased root weight in sensitive varieties was due to hypertrophy and hyperplasia while in this research, when the infection rate was higher; the roots were smaller with lower fresh weight. Some species of tomato lost resistance and became susceptible with the increase in nematode population. Further research in this area particularly the chemical components of rosemary, nigella and marigold extracts need to be made.

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