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Nematicidal Potential of Tulsi (*Ocimum tenuiflorum* L.) Extracts against *Meloidogyne incognita* (Kofoid and White)

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ABSTRACT

Nematicidal efficacy of tulsi extracts were evaluated against root-knot nematode (*Meloidogyne incognita*) in the laboratory. Treatments comprised of aqueous and hot water extracts of tulsi at different concentration (10, 20 and 30 %) and control. The treatments were imposed on second stage juveniles of *M. incognita* and the mortality was recorded after 12, 24, 48 and 72 hours of incubation. Aqueous and hot water extracts of tulsi at different concentrations were found lethal to second stage juveniles of root-knot nematode. Aqueous extract of tulsi at 30 per cent caused the highest mortality of J_2s at all the periods of exposure and it was significantly higher than all other treatments. The per cent mortality increased with increase in the concentration of extracts and exposure time. As compared to hot water extracts, aqueous extract of tulsi gave better results. The present study prooved nematicidal activity of tulsi extracts and suggests that tulsi extracts at higher concentration can be used for the management of root knot-nematode.

Keywords: Aqueous extract, *Meloidogyne incognita*, *Ocimum tenuiflorum*, Root-knot nematode, Tulsi

PLANT parasitic nematodes cause significant damage to almost all crops (Elling, 2013). Among plant parasitic nematodes, root-knot nematode (*Meloidogyne incognita*) is the most frequently observed and key damaging genera (Mukhtar *et al.,* 2017). Use of chemical nematicides is the common and most effective practice in nematode management but it causes severe environmental pollution (Wachira *et al.,* 2009). Moreover, the use of synthetic pesticides has also been banned. Thus, alternative approaches involving the use of antagonistic plants for their pesticidal potential are now increasingly being implemented for the management of plant parasitic nematodes.

A wide variety of plant species are reported to have insecticidal and nematicidal properties and genus *Ocimum* is well known for its properties due to diverse group of compounds in it and which may be utilized as bio-pesticides (Patel *et al.,* 2016). Specialized compounds released from plants, called as secondary metabolites such as alkaloids, glucosinolates, isothiocyanates, diterpenes, fatty acid, phenols, polyacetylenes, sesquiterpenes, thienyls etc., serve as potential source of new nematicidal compounds and which are safer for man and the environment (Chitwood, 2002). Popovic *et al.* (2006) reported that tulsi contains bioactive constituents that have insecticidal and repellent action. Inhibitory effect of *Ocimum tenuiflorum* extracts on *M. incognita* was reported by Bharadwaj and Sharma (2007) and Priya and Pandyan (2019).

Application of plant extracts is an advantageous and environment-friendly strategy to manage the root-knot nematode. Hence, the present study was undertaken to evaluate the nematicidal potential of aqueous and hot water extracts of tulsi (*Ocimum tenuiflorum* L.) against root-knot nematodes in the laboratory.

MATERIAL AND METHODS

The experiment was conducted during September 2020 - October 2021 in the laboratory and glass house of the Division of Entomology-cum-Nematology, Banana Research Station, Kerala Agricultural University, Thrissur.

TABLE 1

Biochemical properties of aqueous and hot water extracts of tulsi

Tulsi extracts	pH	EC (dS/m)	Phenols (mg/100ml)	Tannins (mg/100ml)	Alkaloids (mg/100ml)	Flavonoids (mg/100ml)
Aqueous extract	6.16	0.220	4.79	0.45	166.67	41.76
Hot water extract	5.97	0.240	4.13	0.49	126.67	32.72

Preparation of tulsi extracts : To prepare aqueous extracts, hundred gram fresh crushed leaves of tulsi were added to 200 ml. of distilled water, shaken for one hour continuously in an electric shaker. It was kept at room temperature for 48 hours and filtered through No. 2 Whatman filter paper to obtain the concentration of 50 per cent w/v and is used as stock solution (El-Rokiek and El-Nagdi, 2011). For the preparation of the hot water extract, 200 ml of hot water (70 °C) was added to hundred gram of crushed tulsi samples and kept for 12 hours and filtered through No. 2 Whatman filter paper to make the stock solution (50 % w/v) (Asimiea *et al.,* 2015). From the stock, further dilutions of 10, 20 and 30 per cent concentrations were made.

Aqueous and hot water extracts used for the study were analyzed for the biochemical parameters, pH, EC, phenols, tannins, alkaloids and flavonoids and are presented in Table 1. The pH and EC were measured with a digital pH meter and conductivity meter, respectively. Total phenols, tannins, alkaloids and flavonoids were determined using Folin-Ciocalteu reagent, Folin-Denis reagent, 10 per cent *acetic acid* in *ethanol* and aluminium chloride, respectively (Harborne, 1973).

Multiplication and maintenance of root-knot nematode culture : Pure mother cultures of root- knot nematode (*Meloidogyne incognita*) were procured from National Research Centre for Banana (NRCB), Tiruchirappalli, Tamil Nadu. Rooted cuttings of *Plectranthus amboinicus* (Lour.) commonly known as Indian borage or Mexican mint (Family: Lamiaceae) was used as host plant for multiplication of root-knot nematode cultures. The potted plants were inoculated with infective juveniles of *M. incognita* and watered regularly for the multiplication of nematodes on the host plant. Repotting and subculturing were carried out from the

Fig. 1 : Multiplication of root-knot nematode cultures on roots of *Plectranthus amboinicus*

mother culture to ensure sufficient population of nematodes (Fig. 1).

Extraction of second stage juveniles of M. incognita : The second stage juveniles (J_2s) of *M. incognita* were extracted using Modified Baermann Funnel Method (Schindler, 1961). From the pure culture pots, heavily infested plants were uprooted and roots were washed gently with water to remove adhering soil particles. Egg masses in the galled roots were picked up using forceps and kept over tissue paper supported on a wire mesh (Fig. 2). Then the wire mesh was placed over a petri dish filled with water enough to moisten the egg masses on tissue paper and was kept at room temperature (27 $\pm$ 2 ^{0}C). In order to obtain required number of second stage juveniles, sufficient numbers of petri dishes were kept for extraction. The active juveniles settled at the bottom of petri dishes, they were collected in a beaker and these nematode suspensions were used for further study.

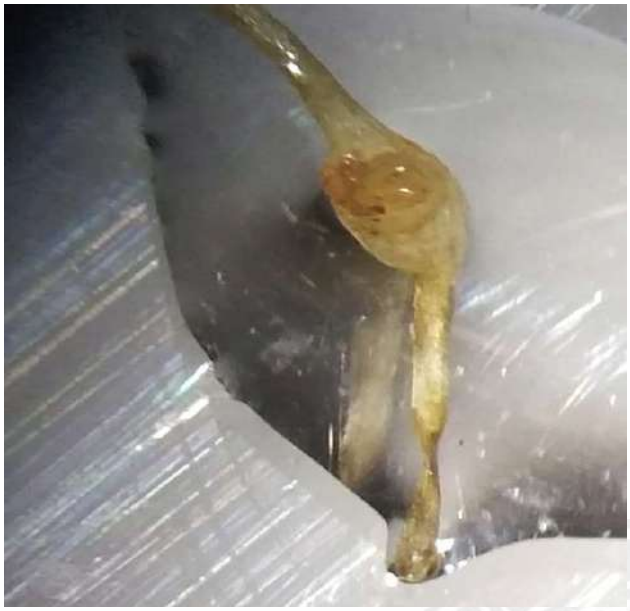

Fig. 2 : Egg mass of root-knot nematode

Experimental details : The experiment was set up in completely randomized design (CRD) with seven treatments and five replications. Treatments comp rised of aqueous and hot water extracts of tulsi at 10, 20 and 30 per cent and control (distilled water). The treatments were imposed on second stage juveniles (J_2s) of *M. incognita* extracted from pure culture maintained in the glasshouse on host plant.

Nine millilitres of extract was added to one ml of nematode suspension containing 100 J_2s of *M. incognita* in petri dishes (Fig. 3). Double distilled water was used as the control. The mortality of second stage juveniles (J_2s) was recorded after 12, 24, 48 and 72 hours of incubation using stereo scopic microscope of make Motic-SMZ -168 (50 X). The juveniles were considered as dead when they attained the shape of straight line and not responding to touch with a fine needle (Siddiqui and Shaukat, 2004). The mortality of J_2s for each treatment was calculated as the ratio of dead J_2s / number of total J_2s and expressed as percentage.

Fig. 3 : Experimental set up of exposure of root-knot nematode J_2s to tulsi extracts

Results and Discussion

Aqueous and hot water extracts of tulsi at different concentrations had significant effect on the mortality of second stage juveniles (J_2s) of *M. incognita*. Aqueous extract of tulsi at 30 per cent caused the highest mortality of J_2s after 12, 24, 48 and 72 hours of exposure (14.14, 23.81, 44.13 and 48.46 %, respectively) and it was significantly higher than all other treatments (Table 2). The control (distilled water) recorded the lowest juvenile mortality (0.00 %) for all the periods of exposure and it was significantly lower than all other treatments. The second best treatment was 20 per cent aqueous extract of tulsi followed by hot water extract at 30 per cent concentration. Hot water extract of tulsi at 20 per cent and 10 per cent showed less effect on mortality of juveniles. However, all the extracts of tulsi exhibited some level of toxicity toward second stage juveniles (J_2s) of the root-knot nematode. Inhibition of egg hatching and high juvenile mortality of the root-knot nematode by the application of leaf extracts of tulsi was reported by Claudius-cole *et al.* (2010). As per Pandey *et al.* (2000), essential oil of *O. basilicum* was highly toxic to *M. incognita* even at the lower concentrations. According to Asimiea *et al.* (2015), aqueous extract of *O. gratissimum* leaves at 20 ml / kg of soil was comparable to carbofuran in the management of *M. incognita* in okra. Neeraj *et al.* (2017) also observed mortality in juveniles of root-knot nematode due to the presence of aqueous extracts of tulsi.

In the study, when second stage juveniles were exposed to tulsi extracts for a longer period (72 h) higher mortality compared to shorter periods (48, 24 and 12 h) were observed. Hasabo and Noweer (2005) and Ranjitsingh and Sucheta (2009) reported that botanicals

TABLE 2
Effect of Tulsi extracts on mortality (%) of second juveniles of *M. incognita*

Treatments	Mortality (%) of J_2s of *M. incognita*			
	12 h	24 h	48 h	72 h
T_1 : Aqueous extract of tulsi @ 10 %	13.50 ^{d} (5.47)	19.67 ^{b} (11.36)	29.51 ^{c} (24.28)	36.58 ^{c} (35.55)
T_2 : Aqueous extract of tulsi @ 20 %	16.39 ^{b} (7.97)	20.89 ^{b} (12.74)	37.56 ^{b} (37.18)	40.01 ^{b} (41.35)
T_3 : Aqueous extract of tulsi @ 30 %	22.08 ^{a} (14.14)	29.20 ^{a} (23.81)	41.63 ^{a} (44.13)	44.11 ^{a} (48.46)
T_4 : Hot water extract of tulsi @ 10 %	11.87 ^{e} (4.25)	14.87 ^{d} (6.62)	17.04 ^{e} (8.69)	20.60 ^{e} (12.42)
T_5 : Hot water extract of tulsi @ 20 %	13.15 ^{d} (5.21)	17.51 ^{c} (9.08)	23.96 ^{d} (16.55)	24.97 ^{d} (18.05)
T_6 : Hot water extract of tulsi @ 30 %	14.57 ^{c} (6.34)	20.89 ^{b} (12.73)	31.54 ^{c} (27.42)	37.28 ^{bc} (36.71)
T_7 : Control (Distilled water)	0.29 ^{f} (0.00)	0.29 ^{e} (0.00)	0.29 ^{f} (0.00)	0.29 ^{f} (0.00)
SE m (±)	2.485	3.336	5.269	5.745
CD (0.05)	1.018	1.314	2.149	3.00

**Arc sin transformed values, original values are in parentheses

at longer exposure time were more effective in increasing egg-hatch inhibition and juvenile morality of *M. incognita* compared to the control. According to Pavaraj *et al.* (2012), plant extracts exhibited highly promising mortality against root-knot nematode after 72 h exposure.

Mortality of J_2s was influenced by concentration of extracts also. In general, juvenile mortality increased with increase in concentration. Both aqueous and hot water extracts of tulsi at higher concentration (30 %) showed higher mortality of J_2s, followed by its lower concentration (20 and 10 %). Research studies indicated that increase in concentration of aqueous extracts of *Moringa oleifera*, *Jatropha curcas* and *Ricinus communis* and increase in exposure time led to decrease in egg hatching ability and increase in mortality rate of *M. incognita* and vice versa.

The inhibitory activity of plant extracts might be due to the presence of chemicals that possessed ovicidal and larvicidal properties. *Ocimum* spp. contains phenolic constituents such as eugenol, methyl eugenol, iso eugenol, methyl chavicol and traces of terpenoids and traces of acids (Vasudevan *et al.,* 1999). Some of the bio-active compounds such as eugenol and methyl eugenol in *O. sanctum* exhibited insecticidal and antimicrobial properties (Bhavya *et al.,* 2018). Secondary metabolites such as phenols, tannins, alkaloids and flavonoids that were identified in the extracts (Table 1) could be responsible for the nematicidal effect in the present study. Direct contact of plant extracts might have effectively delivered the active ingredients to the juveniles of the root-knot nematodes. As per Knoblock *et al.* (1989), nematicidal effect of *Ocimum* extract is attributed to certain oxygenated compounds that dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure. Compared to hot water extracts, aqueous extract of tulsi gave better results at all the periods of exposure. The higher mortality found in aqueous extract might be due to the higher concentration of phenols, alkaloids and flavonoids compared to hot water extracts (Table 1).

The present study proved the nematicidal property of tulsi extracts on second stage juveniles of root-knot nematode (*M. incognita*). Among the treatments, 30 per cent aqueous extract of tulsi exhibited highest inhibitory effect and could be utilized for nematode control. However, further studies on identification of the exact active compound and its mode of action and field level evaluation of extracts have to be conducted.

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