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TOXICITY OF METHANOLIC LEAF EXTRACTS AND ESSENTIAL OILS FROM VARIOUS PLANTS TO THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*

by
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Summary. The methanolic leaf extracts and essential oils of eight plants viz., *Callistemon lanceolatus, Cymbopogon winterianus, Eucalyptus* sp., *Lantana camara, Nerium oleander, Ocimum basilicum, Ocimum sanctum* and *Vitex negundo* were tested *in vitro* against second stage juveniles of *Meloidogyne incognita* at 0.5, 0.1 and 0.02 per cent concentrations. In plant extracts of *O. sanctum, C. winterianus, Eucalyptus* sp., *O. basilicum*, and *V. negundo*, hundred per cent mortality was observed at 0.5 per cent concentration, whereas at 0.1 per cent concentration only *O. sanctum* gave similar results. Essential oils of *O. sanctum* resulted in 100 per cent mortality even up to 0.02 per cent concentration while *O. basilicum* also was very effective at the same concentrations. It is speculated that nematicidal activity in these plants may be due to compounds like terpinen-4-ol in methanolic extracts and linalool in essential oils, respectively.

The development of nematicides of plant origin as an alternative to synthetic nematicides may have an advantage due to their low pollution level and capacity to improve soil fertility (Chatterjee *et al.*, 1982; Malik *et al.*, 1985, 1987a, 1987b; Sangwan *et al.*, 1985). In continuation of the search for environmentally non-toxic nematicides (Malik *et al.*, 1989 and 1995) studies were undertaken on eight plant species for their nematicidal activity against the root-knot nematode, *Meloidogyne incognita.*

Materials and methods

Callistemon lanceolatus Sm., *Cymbopogon winterianus* Jowitt., *Eucalyptus* sp., *Lantana camara* L., *Nerium oleander* L., *Ocimum basilicum* L., *Ocimum sanctum* L. and *Vitex negundo* L. were selected for this investigation.

Young and mature leaves (1000 g) of each plant were collected from the Landscape section, CCS Haryana Agricultural University, Hisar. The plant material was washed with water and allowed to dry at room temperature. It was then chopped and immersed in methanol for 48 h to extract the maximum of chemical (mainly organic) constituents. After filtration the extractives were obtained by dissolving in methanol. This methanol extract of the leaves was concentrated under reduced pressure and the viscous material so obtained was allowed to solidify at room temperature in large diameter Petri-plates.

The extraction of essential oils was done by steam distillation (Bhardwaj, 1974). Each extract

was obtained from 500 g leaves to which 500 ml water was added and the contents were distilled for about 2 h. Some water always accompanied the essential oils. The extracted material was kept in a refrigerator for a few hours, allowing the contents to get separated from the water, which was then pipetted out.

A pure culture of *Meloidogyne incongita* (Kofoid *et* White) Chitw. (race 4) was obtained from the Department of Nematology, CCS Haryana Agricultural University, Hisar. The egg masses of *M. incognita* were isolated from infected roots and put on a wire gauge covered with a double layer of facial tissue paper placed on a Petri-dish filled with water. They were allowed to hatch in a biological oxygen demand (B.O.D.) incubator for 2-3 days at 28±2 °C. Second stage juveniles (J2s) were hatched out, collected, counted under a stereozoom binocular microscope and tested against plant extracts and essential oils.

One g crude material of each plant species was dissolved in 100 ml of 5000 ppm methanol and designated as standard 'S'. It was further steam sterilized at 15 psi for 20 min and diluted as required by adding sterile distilled water.

One bundred J2s of *M. incognita* in 3 ml of water were poured into 5 cm diameter Petriplates and 3 ml of the respective plant extract (double strength) was added to make 0.5, 0.1 and 0.02 per cent concentrations. The Petri plates were kept in a B.O.D. incubator at 25±1 °C for 24 h. Dilution of methanol was made with distilled water and it was kept as control along with sterile distilled water. Each treatment was replicated three times and juvenile mortality was observed after a 24 h exposure period.

One ml of each essential oil was dissolved in 9 ml of sterile distilled water along with 1-2 drops of Triton-X-100 to make a clear emulsion and it was designated as 'S'. This emulsion was further diluted with distilled water to 0.5, 0.1 and 0.02 per cent concentrations and tested for nematode mortality as described above.

Results

Among the methanolic extracts of the eight plant species tested, maximum juvenile mortality was obtained with *C. winterianus, O. basilicum* and *O. sanctum*, irrespective of concentration, and with no statistical differences among them (Table I). The nematode mortality decreased significantly with the lowering of concentration, irrespective of plant species. There was a significant interaction between plant species and extract concentration. Leaf extracts of *C. winterianus, Eucalyptus* sp., *O. basilicum, O. sanctum* and *V. negundo* at 0.5% concentration and those of *C. winterianus, O. basilicum* and *O. sanctum* at 0.1% concentration gave almost 100% juvenile mortality. Among these, *O. sanctum* is considered best since it gave 100% juvenile mortality both at 0.5 and 0.1% concentrations (Table I).

In the second test investigating the effect of essential oils of different plants on the mortality of *M. incognita* J2, *O. sanctum* followed by *O. basilicum* were found to be best, and were statistically not different. The effect of concentration, irrespective of the plant species, was not discernible. The interaction of oils and concentrations was significant. As in the case of methanolic extracts, the essential oils of *O. sanctum* were the most effective since they resulted in 100 per cent juvenile mortality even up to 0.02% concentration. *O. basilicum* also gave similar results at all the three concentrations. Among the remaining plant species, the essential oils had a toxic effect only at 0.5% concentration (Table II).

In both tests, revival of immobilised nematodes was examined by randomly transferring ten J2s to water for 24 h. None of those immobilised J2s revived.

Discussion

A perusal of the literature for the compounds isolated from leaves and essential oils of the

TABLE I - *Effect of different crude methanolic leaf extracts on the mortality of second stage juveniles of* Meloidogyne incognita.

Plant leaf extract	Concentration (%)			
	0.5	0.1	0.02	Mean (Plants)
Callistemon lanceolatus	66.7 (55.7)	30.0 (33.6)	6.7 (14.8)	34.5 (34.7)
Cymbopogon winterianus	100.0 (90.0)	96.7 (88.0)	20.0 (26.8)	72.2 (68.3)
Eucalyptus sp.	100.0 (90.0)	16.7 (24.6)	3.3 (10.3)	40.0 (41.6)
Lantana camara	–	66.7 (55.4)	6.7 (14.8)	36.7 (35.1)
Nerium oleander	23.3 (29.5)	26.7 (31.7)	6.7 (14.8)	18.9 (25.3)
Ocimum basilicum	100.0 (90.0)	96.7 (88.0)	6.7 (14.8)	67.8 (64.2)
O. sanctum	100.0 (90.0)	100.0 (90.0)	3.3 (10.3)	67.8 (63.4)
Vitex negundo	100.0 (90.0)	33.3 (35.8)	3.3 (10.3)	45.5 (45.4)
Distilled water (Control)	5.0 (13.1)	1.7 (8.6)	3.3 (10.3)	3.3 (10.7)
Mean (Conc.)	(68.5)	(50.6)	(14.1)	

C.D. at 5%; plants = 6.08; concentration = 3.51; plants x concentration = 10.53; figures in parentheses are arcin $\sqrt{\text{percentage}}$ transformations.

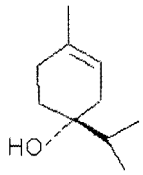
TABLE II - *Effect of different essential oils on the mortality of second stage juveniles of* M. incognita.

Plant essential oils	Concentration (%)			
	0.5	0.1	0.02	Mean (Oils)
Callistemon lanceolatus	85.3 (71.1)	33.3 (35.8)	18.3 (26.1)	45.6 (44.3)
Cymbopogon winterianus	96.7 (84.2)	10.0 (19.4)	13.3 (22.0)	40.0 (41.9)
Eucalyptus sp.	95.0 (80.3)	61.7 (53.5)	25.0 (30.6)	60.6 (54.8)
Lantana camara	76.6 (62.1)	56.6 (49.4)	10.0 (19.4)	47.7 (43.6)
Ocimum basilicum	83.3 (75.3)	94.3 (84.2)	96.7 (84.2)	91.4 (81.2)
O. sanctum	96.7 (84.2)	100.0 (90.0)	100.0 (90.0)	98.9 (88.1)
Vitex negundo	86.6 (69.8)	36.6 (37.8)	10.0 (19.8)	44.4 (42.3)
Triton-X-100 (Control)	5.0 (13.0)	5.0 (13.1)	5.0 (13.1)	5.0 (13.1)
Mean (Conc.)	(67.5)	(47.9)	(38.1)	

C.D. at 5%; oils = 19.6 concentration = N.S.; oils x concentration = 33.9; figures in parentheses are arcin $\sqrt{\text{percentage}}$ transformations.

plant species under investigation revealed that terpinen-4-ol (Fig. 1) is the common compound present in *C. winterianus, O. basilicum, O. sanctum* and *V. negundo* (Kries and Mosandl, 1994; Mallavarapu *et al.*, 1994; Grayer *et al.*, 1996). Several structurally similar monoterpenes are reported to show nematicidal activity against nematodes (Malik *et al.*, 1987). The toxicity observed against *M. incognita* in the methanolic leaf extracts of these plants may, therefore, be attributed to terpinen-4-ol having unsaturated and hydroxy group.

Similarly, linalool (Fig. 1) appeared to be a common compound isolated and characterized



Terpinen-4-ol **Linalool**

Fig. 1 - Chemical structure of the two compounds to which major nematicidal activity of the plant extracts is attributed.

from the four most active essential oils in the present studies i.e., *O. sanctum* (Anonymous, 1966), *O. basilicum* (Johanson *et al.*, 1999), *Eucalyptus* sp. (Brooker and Lassak, 1981; Boland *et al.*, 1982; Weston, 1984; Franich, 1986) and *C. winterianus* (Kries and Mosandl, 1994). Linalool also has both unsaturated and hydroxy groups.

Thus, the extracts and essential oils which are active against nematodes may be due to the presence of hydroxyl group and/or unsaturation in the constituents.

The nematoxicity traits of *O. sanctum* is further corroborated by *in vivo* studies conducted by Chattopadhyay (1991), who reported that the number of primary galls due to *M. incognita* was reduced to about 20 per cent and fruit weight increased by about 240 per cent by the application of leaf dust of *O. sanctum*.

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