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MEDICINAL PLANT EXTRACTS AS POTENT SOURCE OF NEMATOCIDAL ACTIVITIES

by

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Summary. Extracts of two medicinal plants, *Curcuma aromatica* and *Swertia chirata*, showed a marked nematocidal and nematode-hatching inhibitory activity against root-knot nematode, *Meloidogyne incognita*. The nematocidal activity differed between extracts and plants. Maximum toxic activity was recorded in the hexane extract of *S. chirata* while the butanol extract of *C. aromatica* exhibited maximum inhibition to hatching of *M. incognita* eggs after 120 hours exposure period at 1000-ppm concentration.

Extracts of the rhizome of turmeric (*Curcuma aromatica*) have been reported for their pharmaceutical properties such as anthelmintic action against *Ascaris lumbricoides* (Raj, 1975), wound healing activity (Santhanam and Nagarajan, 1990) and anti-inflammatory properties (Jangde *et al.*, 1998). Although recent papers report on their effect on spore germination (Karade and Sawant, 1999) and inhibition of mycelial growth of plant pathogenic fungi (Raja and Kurucheve, 1998, 1999), their effect on plant parasitic nematodes is unknown. It is known, however, that another species of *Curcuma* i.e. *C. longa* exhibits nematocidal activity against the root-knot nematode (Pillai and Desai, 1978). In contrast to *C. aromatica*, aqueous extracts of *Swertia chirata* inhibit egg hatch in *Meloidogyne incognita* (Goswami and Vijailakshmi, 1987). Furthermore, extracts of *S. chirata* are reported to exhibit several other biological activities viz. anthelmintic (Shilaskar and Parashar, 1989), antileishmanial (Singha *et al.*, 1992; Sutapa *et al.*, 1996) and insecticidal activity (Das and Singh, 1998). Although plant extracts in their crude form have been shown to have nematocidal activity (Scramin *et al.*, 1987; Jatala *et*

al., 1995), there is need to isolate, characterise and identify the compounds involved which are nematocidal. Therefore, an investigation was carried out to determine the efficacy of different fractions of *Curcuma aromatica* Salisb and *Swertia chirata* Buch-Ham ex. Clarke on hatching and mortality of the root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw.

Materials and methods

The plant material of *C. aromatica* was collected from Assam, India. Air dried rhizome powder (4.5 kg) was extracted with n-hexane (2.5 l x 6) followed with the aqueous methanol (methanol: water = 80:20). The aqueous extract was further fractionated with n-hexane, chloroform, ethyl acetate, methanol and saturated butanol using a Soxhlet apparatus. The different extracts obtained after removal of solvents under reduced pressure were: hexane (CAH-1, 93.33 g), chloroform (CAC-2, 117.96 g), ethyl acetate (CAE-3, 5.71 g), methanol (CAM-4, 7.11 g) and butanol (CAB-5, 17.04 g), respectively. The plant material of *S. chi-*

rata was collected from Darjeeling (West Bengal), India. The air-dried aerial parts of *S. chirata* (4.12 kg) were extracted with n-hexane followed by 20% aqueous methanolic solvent. The aqueous methanol extract was concentrated and then subjected to further fractionation by the Soxhlet apparatus to yield a series of extracts i.e. hexane (SCH-1, 55.58 g), chloroform (SCC-2, 9.19 g), ethyl acetate (SCE-3, 8.07 g), methanol (SCM-4, 13.36 g) and butanol (SCB-5, 83.26 g).

For *in vitro* studies egg masses of *M. incognita* were collected from a stock culture maintained on tomato in a glasshouse. The egg masses were placed on wire gauge supported with tissue paper in a funnel containing water at 25 °C. Stock solutions 2000 ppm of the test extract/fraction were prepared by dissolving different extracts in 0.5 ml dimethyl sulphoxide (DMSO), made up to 5 ml with distilled water. These solutions were further diluted to give a concentration of 1000 ppm. Five ml of nematode suspension containing around 300 juveniles (J2) were put in the vial with 5 ml double concentration of 1000 ppm solution. The loosely capped vials (so that gaseous exchange could easily take place) were stored at 25 °C. Vials containing 0.5 ml DMSO and 9.5 ml distilled water with nematodes served as a control. After 24 hours incubation the nematodes were transferred in tap water and kept overnight at 25 °C; J2 which did not resume

motility were assumed to be dead. There were three replicates for each treatment. The number of dead and surviving juveniles were counted after 24 hours and the mean percent mortality was calculated. The efficacy of the extracts on hatching of *M. incognita* egg masses was also determined by adding five fresh egg masses of average size into each vial. The vials were incubated at 25 °C and the total numbers of hatched juveniles in each vial were counted after 120 hours.

Results and discussion

The results presented in Tables I and II indicate that *C. aromatica* and *S. chirata* contain compound(s) that exhibit strong to mild nematocidal activity. The various extracts of these plants inhibited hatching of eggs and killed the juveniles of *M. incognita* at a concentration of 1000 ppm after 24 hours of exposure. The hatching of eggs and mortality of the juveniles, however, varied depending upon the type of extract and the plant. The highest mortality was recorded in the hexane extract (80%) of *S. chirata* (Table II) while the methanol extract of *C. aromatica* was least effective (25%). Significantly higher levels of mortality were also recorded in almost all the extracts of both the test plants (Tables I and II). Butanol extract of *C. aromatica* and hexane extract

TABLE I - Effect of different extracts of *Curcuma aromatica* on hatching and mortality of *Meloidogyne incognita*.

Name of / Fraction	Type of fractions	Percent mortality (after 24 hours)	Hatched juveniles (after 120 hours)	% inhibition in hatching over control
Control	(DMSO + Water)	1	321	—
CAH-1	Hexane	54	210	34.6
CAC-2	Chloroform	60	143	55.5
CAE-3	Ethyl acetate	64	245	23.7
CAM-4	Methanol	25	256	20.3
CAB-5	Butanol	60	89	72.3
C.D. = (P = 0.05)	—	5.32	6.38	—
C.D. = (P = 0.01)	—	8.45	9.32	—

TABLE II - Effect of different extracts of *Swertia chirata* on hatching and mortality of *M. incognita*.

Name of / Fraction	Type of fractions	Percent mortality (after 24 hours)	Hatched juveniles (after 120 hours)	% inhibition in hatching over control
Control	(DMSO + Water)	2	348	–
SCH-1	Hexane	80	123	54.7
SCC-2	Chloroform	50	189	45.7
SCE-3	Ethyl acetate	63	167	52.0
SCM-4	Methanol	55	269	22.7
SCB-5	Butanol	51	270	22.4
C.D. (P = 0.05)	–	6.64	7.90	–
C.D. (P = 0.01)	–	10.63	10.30	–

of *S. chirata* were quite effective in reducing hatching of eggs. Goswami and Vijayalakshmi (1987) reported that even an aqueous extract of *S. chirata* had an inhibitory effect on juvenile hatch from egg masses of *M. incognita*.

These results clearly indicate that both *C. aromatica* and *S. chirata* possess strong nematode inhibitory activity which may be exploited by the extraction of specific nematicidal compound(s).

Acknowledgement. Authors are grateful to Prof. Dr. Sushil Kumar, Director, Central Institute of Medicinal and Aromatic Plants, Lucknow, for providing facility and encouragement during course of present investigation.

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