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NEMATICIDAL EFFECT OF ROOT EXTRACT OF CERTAIN MEDICINAL PLANTS IN CONTROL OF *MELOIDOGYNE INCOGNITA IN VITRO* AND *IN VIVO* CONDITIONS

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Abstract

In the present study aqueous extract of medicinal plants viz., *Amaranthus spinosus*, *Chenopodium album*, *Catharanthus roseus*, *Solanum nigrum* and *Ocimum sanctum* were investigated for their nematicidal activity against second stage juvenile of *Meloidogyne incognita* by *in vitro* technique. Aqueous extract of all plant roots exhibited nematicidal activity. LC_{50} values calculated as 1024 ppm for *C. roseus*, 1867 ppm for *S. nigrum*, 1968 ppm for *A. spinosus*, 3428 ppm for *C. album* and 962 ppm for *O. sanctum*. LC_{50} values showed that out of these extracts *O. sanctum* found to be the most effective. *In vivo* experiment was conducted to study the effect of root extract of *O. sanctum* on *M. incognita* infecting French bean. Different concentrations viz., 10000 ppm (S), 5000 ppm (S/2), 2500 ppm (S/4), 1250 ppm(S/8), 625 ppm (S/16) and 312.5 ppm (S/32) of aqueous extract of *O. sanctum* roots was applied by seed soaking and foliar spray methods.

The plant parasitic nematodes are known to cause severe damage to crop plants. Nematodes are considered to be highly diversified and economically important organisms. The root-knot nematode, *Meloidogyne incognita* is one of the limiting factors in commercial production of vegetable and responsible for 15 to 60% yield loss (Krishnappa *et al.*, 1992). As the synthetic chemicals possess various bad effects to the environment and life, now - a -day, plant products have been given precedence in pest management than chemicals. Vegetable crop in India is very susceptible to root knot nematodes, *Meloidogyne* spp. (Singh *et al.*, 1979). Infested plants are stunted, and generally unthrifty and yellowish foliage suggest that may be deficient in nitrogen. Several workers have reported that root – knot nematode cause reduce nodulation in legume crop. The object of present study was to ascertain the extent to which the root knot nematode interferes with growth and yield of this crop. French bean is an important available cultivar. French bean (*Phaseolus vulgaris*), an economically important plant, is cultivated in hilly tract of Jammu and Kashmir, Himachal Pradesh (Kullu, Barot, Chamba and Shimla valley) and Uttar Pradesh. While its cultivation is mainly restricted to hilly region of North India, Himachal Pradesh produces about 2.1 thousand tons in the form of dry bean (Paul, 2002). About 43.4% yield loss in French bean due to *M. incognita* have been reported (Senthamizh & Subramanian, 2004). To

control this loss many nematicides as well as herbal products have been tried besides various other physical or biological control devices. Many indigenous plants, plant parts or by products are known to have phytonematotoxic properties (Miller *et al*., 1973; Egunjobi & Afolami, 1976; Khanna, 1997). Due to ill effects of chemicals on soil biosphere and environment, presently, attention is now a day given to the use of such plants under "Integrated Pest Management" (IPM) practices.

Therefore, the present investigation was carried out to evaluate the influence of certain medicinal plants (*Amaranthus spinosus*, *Chenopodium album*, *Catharanthus roseus*, *Solanum nigrum* and *Ocimum sanctum*) root extract for management of root-knot nematode, *Meloidogyne incognita* under *in vitro* as well as *in vivo* conditions.

Materials and Methods

Aqueous extracts: Aqueous extract of plants viz., *Amaranthus spinosus*, *Chenopodium album*, *Catharanthus roseus*, *Solanum nigrum* and *Ocimum sanctum* were tested under laboratory condition. Dried roots were powdered and strained through 20 mesh sieve. Twenty five g of each powder was soaked in equal amount of distilled water and filtered through Whatman`s filter paper No. 1. This was considered as stock (S) solution from which other concentrations S/2 (5000 ppm), S/4 (2500 ppm), S/8 (1250 ppm), S/16 (625 ppm) and S/32 (312.5 ppm) were prepared. These concentrations were used for comparison of the efficacy of materials used in the experiment (Vijayalakshmi *et al*., 1979). 1 ml of nematode suspension containing 30-35 J_2 of *M. incognita* was treated with 1 ml of each concentration of each extract. The mortality of $J_2$was recorded after 24, 48 and 72 h. Each experiment was replicated thrice. A control set was also run parallel with distilled water only.

***In vivo*:** Seeds of French bean variety contender were immersed for 4 h in aqueous extract of *O. sanctum*. Each treatment was replicated thrice. After soaking the seeds were allowed to dry before sowing in 13 x 7.5 ft^2 plots separately.

***In vitro*:** Aqueous root extract of all of these plants showed nematicidal effects against the sized plot. One week old seedlings were inoculated with 1000 J_2 of *M. incognita*. Uninoculated plants were treated as normal control. Foliar application of extract was given at crop age of 40 days. After 60 days of inoculation, plants were uprooted and various observations recorded.

Result and Discussion

Juveniles (J_2) of *M. incognita* have shown mortality ranging from 11.11 to 100% (Table 1).The highest concentration, 10000 (stock solution 'S') and 5000 ppm concentration of *C. roseus* exhibited 100% mortality in 72 h of exposure. Other concentrations 2500-625 ppm showed 91.92-58.83% mortality after 72 h exposure, while its lowest dose (312.5 ppm) showed 45.13% mortality after 72 h exposure.The present study is in agreement with Desai *et al*., (1973); Rao *et al*., (1986); Saxena & Lalita (2006) as they also recorded reduced population of *M. incognita* with roots and leaves of same plant. Extract of *A. spinosus* and *O. sanctum* also showed the maximum mortality (100 %) after 48 h and the extract of *S. nigrum* and *C. album* exhibited 85.56-100% mortality with 10000 ppm concentration within 72 h, respectively.

In general, the efficacy of all root extracts of *C. roseus*, *A. spinosus*, *C. album*, *S. nigrum* and *O. sanctum* increased with time and concentration of dose. Aqueous root extract of *C. album* revealed that at its lowest dose (312.5 ppm) the % mortality of J_2 was nil after 24-48h. But as the concentration and the exposure time increased % mortality also increased in proportional manner. Similarly 625 ppm was little more effective after 24 h than its 312.5 ppm dose as 10% mortality had been observed in 24 h with non-significant increase after 72 h (12%) with 625 ppm. Higher reduction in number of J_2 of *M. incognita* by *C. album* extract has been documented by many other workers (Nandal & Bhatti, 1983; Goswami & Vijayalakshmi, 1986; Tabil & Walia, 1996, 1997).

The root extract of *S. nigrum* showed 2500 ppm (37.78%), 1250 ppm (25.0 %), 625 ppm(11.11%) and 312.5 ppm (5.67%) after 24 h. Like other root extract similar increase in mortality was observed with *A. spinosus* as the exposure time increase gradually (53.33 to 77.78% with 2500 ppm, 46.67 to 62.22% with1250 ppm, 34.44 to 43.11 % with 625 ppm and 28.89 to 42.22 % with 312.5 ppm in 24-72 h exposure). Increase larval kill with *A. spinosus* was also observed by other workers (Nandal & Bhatti, 1983; Goswami & Vijayalakshmi, 1986).

The highest dose of *O. sanctum* (10,000 ppm) showed 88.78% mortality after 24 h exposure and 100% mortality after 48-72h exposure. The lowest dose (312.5 ppm) showed 34.67% within a short exposure of 24 h while the same dose showed 40.0 and 41.11% mortality after 48 and 72 h, respectively. Desai *etal*., (1973) observed 57.62, 50.00 and 73.81% kill with root, stem and leaf extracts of *O. sanctum*, respectively. The present study is in agreement with Vijayalakshmi *et al*., (1979) as they also observed 100% mortality after 48 h exposure.

The major essential oil of *O. sanctum* and *O. basilicum* are reported to be eugenol (Dutt, 1940) and linalool (Nigam *et al*., 1970), respectively. Chaterjee *et al*., (1982) also showed that eugenol and linalool as the major constituents of *O. sanctum* and *O. basilicum*, respectively and were prime candidate for the nematicidal principle.

Table 1. Nematicidal effect of various root extracts on J_2 of *M. incognita*.

Extracts	Exposure time(h)	Doses (ppm)					
		10000	5000	2500	1250	625	312.5
C. roseus	24	66.68	64.45	63.33	60.00	32.24	18.98
	48	85.88	83.71	82.12	68.04	45.45	21.12
	72	100	100	91.92	78.86	58.83	45.13
	Mean	84.18	82.19	79.65	68.96	45.50	28.41
C.album	24	95.55	86.67	15.56	13.33	10.00	00.0
	48	97.78	88.89	28.89	17.78	10.00	00.0
	72	100	98.89	35.56	22.22	12.22	4.45
	Mean	97.77	91.55	26.67	17.77	10.74	1.48
S. nigrum	24	53.34	49.00	37.78	25.00	11.11	5.67
	48	70.00	58.89	46.23	28.89	15.56	7.78
	72	85.56	60.00	51.11	32.22	30.00	13.34
	Mean	69.63	55.96	45.04	28.70	18.88	8.93
A. spinosus	24	78.89	67.78	53.33	46.67	34.44	28.89
	48	100	94.44	64.44	51.11	36.67	31.11
	72	100	100	77.78	62.22	43.11	42.22
	Mean	92.96	87.40	65.18	53.33	38.07	34.07
O. sanctum	24	88.89	78.89	74.45	53.33	45.00	34.67
	48	100	84.44	80.00	75.78	53.33	40.00
	72	100	100	94.44	91.00	68.89	41.11
	Mean	96.66	87.71	82.96	73.37	55.74	38.59

LC_{50} values were recorded as 962 ppm of *O. sanctum*, 1024 ppm for *C. roseus*, 1867 ppm for *S. nigrum*, 1968.2 ppm for *A. spinosus* and 3428.7 ppm for *C. album* after 24 h against *M. incognita*. Thus, LC_{50} values shows that among all examine extracts *O. sanctum* was found to be the best, however, other plant extract also showed nematicidal properties to reduce the population of *M. incognita* under *in vitro* condition.

***In vivo*:** *In vivo* experiment reveal that pre-soaking seed and foliar spray together improve plant growth and proved to be effective against *M. incognita* but 312.5 ppm concentration was found to be the best. In infected control 110 galls were recorded whereas only 62.3 galls were noticed in 312.5 ppm (Fig. 1-3). Many workers have also followed the method of seed soaking and foliar spray for nematode control. Hong & Sethi (1988) showed that seed soaking in trizophos followed by phenomiphos and carbofuran hampered the larval penetration. Reddy (1984) used three known nematicides, aldicarbsulfone, carbofuran and

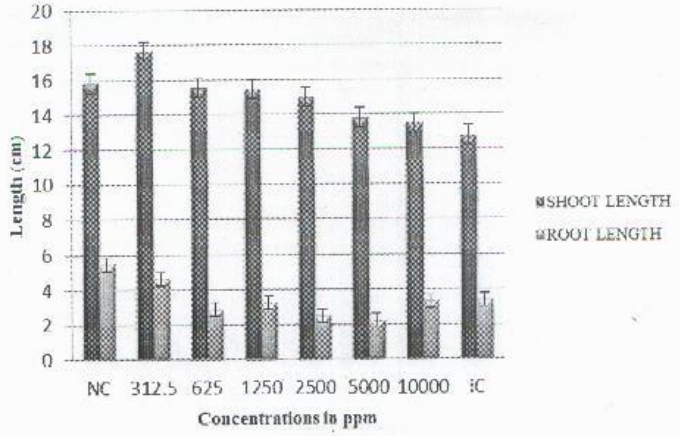

Fig. 1. Effect of different concentrations of *Ocimum sanctum* by seed soaking and foliar spray and *M. incognita* on shoot and root length; NC– Normal Control, IC= Infected Control.

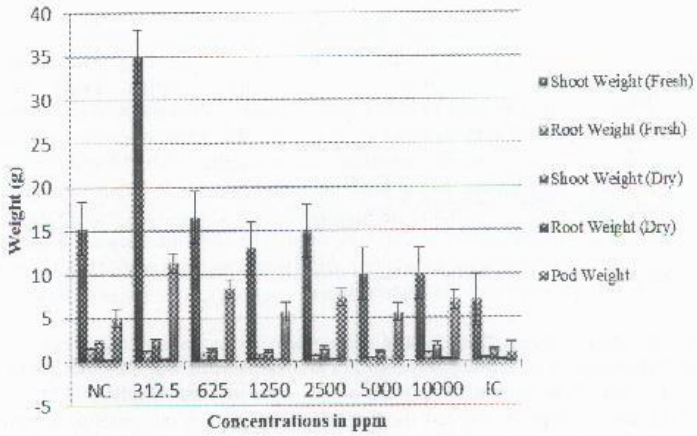

Fig. 2. Effect of different concentrations of *Ocimum sanctum* by seed soaking and foliar spray and *M. incognita* on shoot and root weight (Fresh & Dry) and pod weight; NC= Normal Control, IC– Infected Control.

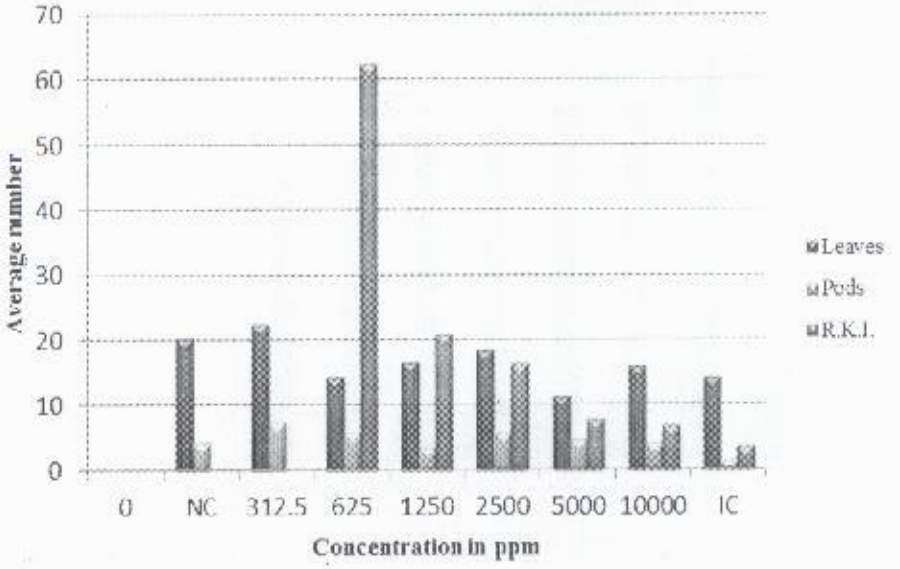

Fig. 3. Effect of different concentrations of *Ocimum sanctum* by seed soaking and foliar spray and *M. incognita* on leaves and pods number and RKI; NC= Normal Control, IC= Infected Control; RKI= Root-knot index.

fenamiphos for seed soaking. Seed soaking with triazophos 40 EC, monocrotophos 35 EC and carbosulfan 25 EC improved plant growth as well as effective in reducing the number of galls and egg-mass/plant (Pareek *et al.*, 1998). Chakraborti & Mishra (2001) used bio-pesticide (neem product) alone as well as incombination with carbofuran to control this nematode. Their findings were in agreement with the present observations as they also showed improved plant growth with treatment. In the same year Maheshwari & Sunderbabu (2001) and Senthamizh & Subramanian (2004) also showed efficacy of seed soaking for nematode management under field condition. On the other hand, Saxena & Kaushik (2005) showed that application of effluent as seed soaking also play a promising role in mean gall index reduction.

Foliar application with 15% and 10% neem extract showed increase shoot length in comparison to untreated control (Maheshwari & Sunderbabu 2001). In present work foliar spray of extract of *O. sanctum* was applied in different concentrations which increased shoot length (17.6 cm) in comparison to normal control (15.8 cm). Di. Sanzo (1981) performed foliar spray of carbofuran resulted in 70-80 % of development of nematode galls in root system of control plants but foliar application of carbofuran reduced the number of galls on tomato roots (Prasad *et al.*, 1997). Trizophos at 500 ppm concentration, when applied as

spray on 10th day, the growing seedlings of groundnut, found to be effective in reducing root-knot and reniform nematode. In present work foliar spray of *O. sanctum* extract was given at 40th day of crop age which reduced penetration of J_2 of *M. incognita* and improved plant growth, thus confirming its nematicidal effect.

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