EFFICACY OF FOUR PLANT EXTRACTS ON NEMATODES ASSOCIATED WITH PAPAYA IN SINDH, PAKISTAN

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Summary. This investigation attempts to evaluate the effect of ethanolic extracts of four plant species, *Azadirachta indica* (neem), *Withania somnifera* (ashwagandha), *Tagetes erecta* (marigold) and *Eucalyptus citriodora* (eucalyptus), against nematodes associated with papaya (*Carica papaya*) and to assess their influence on papaya growth and yield. While fresh shoot weight of papaya seedlings in pots was significantly increased by the plant extracts, in general, the fresh root weight remained unaltered compared to the controls. The number of *Meloidogyne incognita* juveniles, root-knot index and the number of egg masses per root were remarkably reduced by all treatments. The population densities of *Meloidogyne incognita*, *Helicotylenchus multicinctus* and *Hoplolaimus indicus*, the main nematode species associated with papaya, were significantly reduced in the field by all four plant extracts. *Meloidogyne incognita* and *H. multicinctus* were most affected by *Withania*, *H. indicus* by *Tagetes.* The papaya yield was increased by the treatments in the following order: *Withania somnifera* > *carbofuran* > *Azadirachta indica* > *Tagetes erecta* > *Eucalyptus citriodora*. The possible mechanisms of nematicidal effects of the plant extracts are discussed.

Key words: *Azadirachta indica*, control, *Carica papaya*, *Withania somnifera, Tagetes erecta, Eucalyptus citriodora*.

Plant-parasitic nematodes, particularly root-knot nematodes, are widely distributed and cause significant yield losses in a wide range of crops (Sasser and Freckman, 1987). It has been estimated that worldwide overall annual crop yield losses due to plant-parasitic nematodes are approximately 78 billion dollars (Barker *et al.*, 1994).

A preliminary survey of parasitic nematodes associated with papaya (*Carica papaya* L.) in Sindh province, Pakistan, disclosed that the three common species were: *Meloidogyne incognita* (Kofoid *et* White) Chitw., *Helicotylenchus multicinctus* (Cobb) Golden and *Hoplolaimus indicus* Sher (Khan *et al*., 2007). *Meloidogyne* spp. are the most widespread parasitic nematodes, causing root-knot disease of numerous crops and responsible for 70% of the total loss due to nematodes (Sasser and Freckman, 1987). *Meloidogyne incognita* parasitizes a number of crops in the tropics and semi-tropics (Williamson and Hussey, 1996). *Helicotylenchus* spp. are ectoparasites of roots but may also occur in cortical tissue (Siddiqui, 2000). Similarly, *Hoplolaimus indicus* is also an important and widely distributed ectoparasitic nematode, causing root damage and consequently limiting crop yield.

Nematode control is largely based on synthetic nematicides, which besides being costly present potential risk to non-target organisms. In the search for more benign acceptable alternatives to chemicals, the possibilities are being investigated of exploiting nematode-antagonistic plants for the management of plant parasitic nematodes (Chitwood, 2002; Akhtar, 2004). Certain cover crops as green manure have been shown to improve soil fertility as well as suppress plant parasitic nematodes (Barker and Koening, 1998; McSorley, 1999; Wang *et al.*, 2002, 2003). Soil amendment with leaves of many plants has been shown to reduce the rhizosphere population of *Meloidogyne* spp. (Wang *et al.*, 2004; Tsay *et al.*, 2004; Wani, 2006). Shaukat and Siddiqui (2001a) found that aqueous extracts of various weeds from Karachi, Pakistan, caused significant mortality of *M. javanica* (Treub) Chitw. juveniles *in vitro*. Shaukat *et al.* (2004) demonstrated that extracts of certain weeds are able to cause substantial mortality of plant parasitic nematodes thereby improving plant growth and yield. The objectives of this study were to test 1) the effects of ethanolic extracts of four plant species, containing known toxins, on *M. incognita* soil population density, root-knot disease intensity and growth parameters of papaya seedlings, and 2) to test the effects of these extracts on the parasitic nematodes associated with papaya and on yield of papaya.

MATERIALS AND METHODS

Pot experiment. Papaya cv. Bengali seeds were grown in 20-cm diam. plastic-pots filled with 2 kg of solarized sandy loam soil. Three weeks after germination, single seedlings were transferred to 9-cm diam. plastic pots containing 250 g of solarized sandy loam soil. Pots were randomized on a glasshouse bench at 28-30 °C and relative humidity of 55-60%. One week later, the soil in each pot

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was inoculated with 200 three-day-old second stage juveniles (J_2) of *M. incognita* obtained from a pure culture raised on tomato; and one hour later the pots were treated with the plant extracts of *Azadirachta indica* A. Juss., *Withania somnifera* Dun., *Tagetes erecta* L. and *Eucalyptus citriodora* Hook., at 25 ml of extract per pot.

The extracts were prepared as follows. One hundred g of air-dried plant material was plunged into 400 ml of 80% ethanol at 50 °C, stirred well and soaked for 24 h. This material was then reduced to a gummy mass in a Rotary evaporator (Buchi Rotavapor R-200) and dissolved in 100 ml distilled water. The extract was used for the treatments.

In addition, there were positive and negative controls for different amendments. The nematicide carbofuran (commercial product, Furadan a.i. 3 percent) was used at 2 g (dissolved in 25 ml water) per pot.

Each treatment and control were replicated four times. Sixty days after nematode inoculation, the plant in each pot was uprooted and the soil particles around the roots gently shaken off. The nematode juveniles in the soil were extracted by a sieving and centrifugation technique (Barker, 1985). The eggs/egg masses of *M. incognita* from the roots were extracted by shaking the roots in a 0.5% sodium hypochlorite solution for 3 minutes (Hussey and Barker, 1973). Egg-masses were kept in a Petri dish containing distilled water and placed in an incubator at 28 °C in the dark. J_2 were collected on the third day. Root-knot and egg mass indices were rated according to the scale of Hadisoeganda and Sasser (1982). Fresh and dry weights of shoots and roots were recorded.

Table I. Effects of different treatments on plant growth components of papaya seedlings and reproduction of *Meloidogyne incognita* in pots inoculated or not with 200 second stage juveniles (J_2) of the nematode.

Treatment	Fresh weight (g)		Dry weight (g)		Nematode J_2/ 250 g soil	Root-knot index (0-5)	Nematode egg masses/ root	Eggs/ egg mass
	Shoot	Root	Shoot	Root				
Control positive	1.19	0.27	0.11	0.052	286.5	3	8.5	20
Control negative	1.62	0.38 n.s.	0.14 n.s.	0.067 n.s.	0	0	0	0
Carbofuran + 200 J_2	1.58	0.35 n.s.	0.10 n.s.	0.06 n.s.	0	0	0	0
Tagetes erecta + 200 J_2	1.46	0.33 n.s.	0.13 n.s.	0.067 n.s.	2	0.25	0.25	0.50
Azadirachta indica + 200 J_2	1.58	0.28 n.s.	0.12 n.s.	0.04 n.s.	4	0.25	1	0.50
Withania somnifera + 200 J_2	1.92	0.36 n.s.	0.13 n.s.	0.035 n.s.	0	0	0	0
Eucalyptus citriodora + 200 J_2	1.43 n.s.	0.27 n.s.	0.10 n.s.	0.04 n.s.	4.25	0.25	0.50	1
Carbofuran	1.49	0.38 n.s.	0.16 n.s.	0.080 n.s.	0	0	0	0
Tagetes erecta	1.66	0.37 n.s.	0.16 n.s.	0.075 n.s.	0	0	0	0
Azadirachta indica	1.62	0.45	0.13 n.s.	0.062	0	0	0	0
Withania somnifera	2.10	0.47	0.17 n.s.	0.09 n.s.	0	0	0	0
Eucalyptus citriodora	1.50	0.35 n.s.	0.12 n.s.	0.065 n.s.	0	0	0	0
L.S.D. $_{0.05}$	0.27	1.18	0.07	0.03	28	1.7	4	1.6

Values are averages of four replicates.
Significance is given compared to positive controls.
Control positive = control in conjunction with inoculation of *M. incognita* juveniles.
Control negative = control without inoculation of *M. incognita* juveniles.

Field trial. A farmer's field in Malir, Sindh, was chosen for the trial. Papaya trees (var. Bengali) were growing in rows and were apparently free from diseases. Summer temperature in the area varies from 30 to 35 °C while the winter average is 18 °C. The average annual rainfall is 200 mm but in 2006 it was 116 mm. Soil samples were collected in Oct. 2006 (fifteen days prior to treatment) from near the tree trunks at a depth of 5-25cm, taking 6-8 cores per tree that were pooled to form about 1.5 kg of composite sample. Soils from six rows of papaya were sampled with three samples in each row. Population density of all the stylet-bearing nematodes associated with papaya were determined by a sieving and decantation and modified Baermann funnel technique (Southey, 1986). Five ml aliquots (fifteen replicates) of nematode suspension were used for nematode counts and value converted to number of nematodes per 200 ml of soil sample. The three stylet-bearing nematodes (see below) comprised 88% of the total nematode population and the rest were free-living (non-pathogenic). The initial populations were 45 ± 3.9 *Meloidogyne incognita*, 55 ± 15.3 *Helicotylenchus multicinctus* and 30 ± 7.2 *Hoplolaimus indicus*.

The leaves of neem (*A. indica*), ashwagandha (*W. somnifera*), eucalyptus (*E. citriodora*) and marigold (*T. erecta*) were air-dried in the shade and ground to coarse powder from which the plant extracts were prepared as described earlier (see pot experiment). Four hundred ml of each extract was applied to 2 m of row of the papaya trees at 20 cm depth by mixing the extract in the soil using a spade. The extract was uniformly broadcast in the furrow and the application rate corresponded to approximately 1,300 kg plant material in the form of extract per hectare of papaya field. Each treatment and controls were replicated three times. The plant spacing was 1.5 m between rows and 1.2 m along the row. Weeding was done manually and cow manure was used as fertilizer. No fungal disease was observed and insect populations were at a low level and not problematic. For comparison of the potential phytonematicides, the chemical nematicide carbofuran (commercial product, Furadan) was applied at a rate of 15 kg/ha. Untreated trees were kept as controls. Twelve months after the application, soil samples were collected from a depth of 5-25 cm with a soil auger to determine nematode populations. Each final sample was a composite of 6-8 cores. For yield determination, the first picking started after 11 months, and a total of four pickings were taken into account over a period of three weeks. There were four replicates for each observation.

Data were subjected to either one-way analysis of variance (ANOVA) (pot experiment) or the factorial analysis of variance (FANOVA) (field experiment). Each variable was treated separately in the case of ANOVA. The post-hoc tests performed included Fisher's least significant difference (LSD) at P = 0.05 and Dunett's test for comparing control with treatment means (Zar, 1999).

RESULTS

Pot experiment. Growth components were slightly elevated in the negative control over the positive control. Shoot fresh weight of papaya was significantly increased in all treatments except *Eucalyptus citriodora* + 200 nematode J_2 as compared to the positive control (Table I).

Differences in root fresh weight from the positive control were not significant in any treatments except for *A. indica* and *W. somnifera* without the nematode. Shoot dry weight also was not significantly different in all treatments compared to the positive control. Moreover, root dry weight was not significantly different in any treatments except for *A. indica* (without the nematode). Compared to negative controls, the growth parameters of treatments (either plant extract alone or in conjunction with the nematode), in general, did not exhibit any signif-

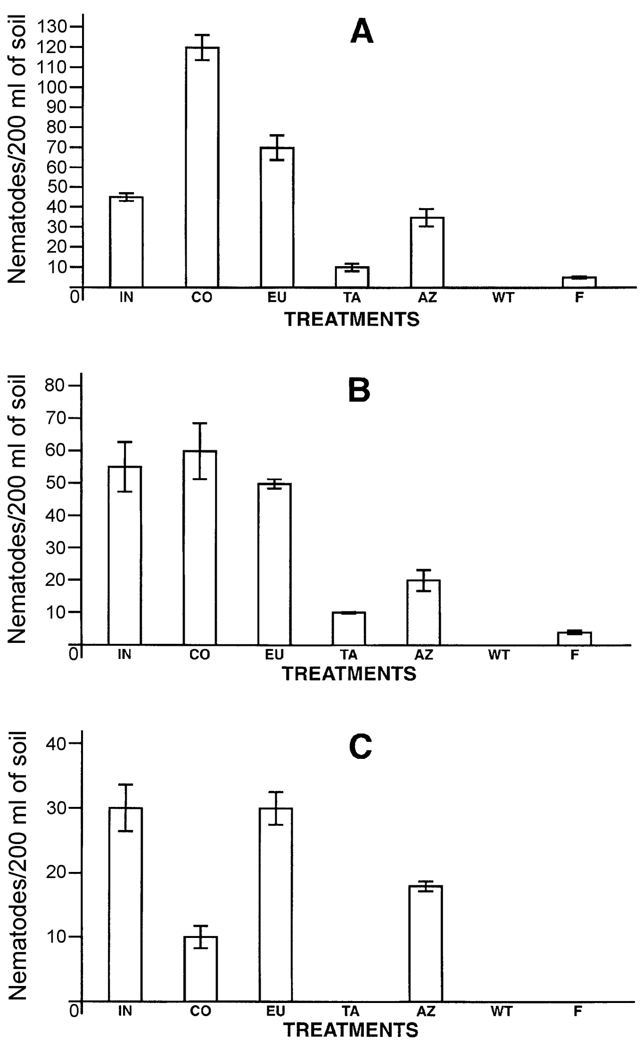

Fig. 1. Effect of different treatments, under field conditions, on nematode soil population densities of: A, *Meloidogyne incognita*; B, *Helicotylenchus multicinctus*; C, *Hoplolaimus indicus*. IN = Initial population density; CO = Control; F = Furadan; EU = *Eucalyptus citriodora*; TA = *Tagetes erecta*; AZ = *Azadirachta indica*, and WT = *Withania somnifera*. LSDs: nematodes = 5.05; treatments = 7.15.

Table II. Effect of plant extract treatments on the yield (kg/plant) of papaya, at different harvests, under field conditions.

Treatment	Harvest				Cumulative
	1	2	3	4	
Carbofuran	1.388	1.510	1.482	1.507	5.887
Azadirachta	1.291	1.366	1.430	1.652	5.739
Withania	1.304	1.328	1.646	1.774	6.052
Tagetes	1.121	1.245	1.368	1.504	5.238
Eucalyptus	1.132	1.386	1.260	1.391	5.169
Control	1.040	1.230	1.114	1.258	4.642

$LSD_{0.05}$ (treatments) = 0.086
$LSD_{0.05}$ (harvests) = 0.115

icant change. Total number of J_2/250 g soil, root-knot index, number of egg masses/root and mean number of eggs/egg masses were significantly reduced in all treatments compared to the positive control.

Field experiment. The effects of the treatments were significant ($F = 143.12$, $P < 0.001$). The treatment with *W. somnifera* was the most effective, followed by carbofuran and *T. erecta*. Significant differences were found for nematode species ($F = 15.47$, $P < 0.001$). While the root-knot nematode and *Helicotylenchus multicinctus* were most affected by ashwagandha (*W. somnifera*), the population of *Hoplolaimus indicus* was affected by *T. erecta*, carbofuran and *W. somnifera* extract (Fig. 1A-C). Interaction of treatments × nematode species was found to be significant ($F = 19.15$, $P < 0.001$).

The yield of papaya was significantly increased ($P < 0.001$) by carbofuran and the four plant extracts tested (Table II). The yield was increased over the controls in the order *W. somnifera* > carbofuran > *A. indica* > *T. erecta* > *E. citriodora*. Harvests also differed significantly ($P < 0.05$) and the yield tended to increase from first to last harvest. The interaction of treatments × harvest was also found to be significant ($P < 0.01$).

DISCUSSION

The pot and field experiments demonstrated the nematicidal potential of the four plant extracts tested, especially ashwagandha and neem, that also resulted in increased plant growth and yield of papaya.

Several mechanisms may be involved in the suppression of nematode soil populations and enhancement of plant growth and yield. Plant extracts often contain phenolic compounds, organic acids, terpenes and terpenoids, coumarin-like compounds and other secondary metabolites (Muller and Gooch, 1982; Insunza *et al*., 2001; Shaukat *et al*., 2004). Many of these compounds, such as phenolics, have been demonstrated to have nematicidal activity (Shaukat *et al*., 2004). The nematicidal activity of neem can be attributed to one or more of the biologically active principles it contains, including azadirachtine, nimbine, nimbidine, thionemone, kemferol, etc., that are known to possess nematicidal properties (Rossner and Zebitz, 1987; Abbasi *et al*., 2004). *Eucalyptus* spp. possess complex mixtures of plant secondary metabolites, including terpenoids, cyanogenic glycosides, tannins, flavonoids, long-chain ketones, phloroglucinols and leaf-oil 1-8-cineole eucalyptol (Boland *et al*., 1991; Brophy and Southwell, 2002). *Withania somnifera* contains inhibitory withaniloids and glyco-withaniloids that are known to be highly effective against *M. javanica* (Goel *et al*., 2005). Finally, leaves and flowers of *Tagetes erecta* contain some inhibitory essential oils (Oguvawande and Olawore, 2006; Tomava *et al*., 2005) that possess strong nematicidal activity (Wang *et al*., 2002; Evenhuis *et al*., 2004; Tsay *et al*., 2004). In addition, the application of plant extracts or other control agents can also alter the indigenous bacterial or fungal communities of the rhizosphere or endorhiza in a manner that leads to enhanced soil suppressiveness to nematodes or other soilborne plant pathogens (Vargas-Ayala *et al*., 2000; Shaukat and Siddiqui, 2001b).

The use of plant material for the management of nematode populations is apparently effective and environmentally friendly compared to synthetic nematicides. The pot experiment was a preliminary test and the rates of plant extracts used could be considered rather large, although the quantity of secondary metabolites present in the extracts, was probably more or less equivalent to the rates used for chemical nematicides. The amount of plant material used in the field experiment are comparable to those used as organic soil amendments by other workers, and provide effective nematode control. However, whether a smaller amount would still be effective needs further investigation.

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