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Department of Botany, University of Karachi, Karachi-75270, Pakistan

NEMATICIDAL POTENTIAL OF *LANTANA CAMARA* AGAINST *MELOIDOGYNE JAVANICA* IN MUNGBEAN

by

N. I. Ali, A. Siddiqui, M. J. Zaki and S. S. Shaukat

Summary. Extracts of *Lantana camara* in organic solvents caused significant mortality of *Meloidogyne javanica* juveniles *in vitro*. Aqueous and methanolic extracts demonstrated greater inhibition compared to ethyl acetate or hexane extracts indicating that active principles were polar in nature. Decomposing leaves of *L. camara* used alone or in combination with *Pseudomonas aeruginosa* markedly suppressed population densities of *M. javanica* and subsequent root-knot development in mungbean. Taken together, the data suggest that soil amendment with *L. camara* extract was less effective compared with the decaying leaves indicating that the latter provides greater release of the toxic principles.

Lantana camara a native of tropical America, is widely naturalized in many tropical and subtropical regions. Various parts of the plant are used in folklore and indigenous systems of medicine for the treatment of cuts, ulcers, swellings, eczema, malaria and tumors (Sastri, 1962; Kirtikar and Basu, 1981). Although literature is available on the allelopathic potential of *L. camara* (Casado, 1995; Rajbansi and Inubushi, 1997), little attention has been given to the possibility that it may also be toxic to nematodes attacking crop plants (Sharma, 1996; Jomati *et al.*, 1998). Recently, Begum *et al.*, (2000) isolated four different compounds, lantanoside, linoroside, camarinic acid and lantanone, from the aerial parts of *L. camara*, of which the first three showed nematicidal acitivity against *Meloidogyne incognita*.

As the rhizosphere provides the first line of defense against attack by the pathogens, microorganisms that can grow in the rhizosphere are ideal for use as biological control agents in the control of soilborne plant pathogens (Weller, 1988). Of the various potential biological control agents, pseudomonads are aggressive colonizers of various crop plants and have shown promising results in the control of *Meloidogyne javanica*, the root-knot nematode (Siddiqui and Ehteshamul-Haque, 2000).

There are many reports that organic amendmets of the soil enhance the activity of biocontrol agents in the suppression of plant pathogens (Cook, 1977; Sitaramaiah, 1990). Therefore, experiments were carried out to evaluate the potential of *L. camara* alone or in combination with *Pseudomonas aeruginosa* in the control of *Meloidogyne javanica*, the root-knot nematode in mungbean.

Materials and methods

Leaves of *Lantana camara* L. were collected from shrubs grown as hedges in the Karachi University Campus. The leaves were air-dried

and powdered in an electric grinder. A 200 g aliquot of powdered *L. camara* leaves was extracted in 500 ml distilled water for 48 h. The extract was then filtered twice through Whatman No. 1 filter paper, lyophilized and stored at 6° C. Further, 200 g aliquots of powdered leaves were soaked in 500 ml methanol, ethyl acetate or hexane for 48 h. The extracts were filtered twice and the filtrates concentrated in a rotary vacuum evaporator. An appropriate amount of extract was dissolved in the respective solvents to make 10, 1 and 0.1 mg/ml concentrations.

To assess the nematicidal activity of different extracts of *L. camara*, 1 ml of an extract was placed in a glass-cavity-slide and left for 48 h to evaporate the organic solvent. Suspensions of juveniles of *Meloidogyne javanica* (Treub) Chitw. containing 30-35 nematodes in 1 ml water were added to the evaporated extracts. Treatments were replicated four times and kept at 28 °C. Glass-cavity-slides containing evaporated organic solvents without *L. camara* served as controls. The number of dead juveniles were counted after 48 h of incubation period. The nematodes were considered to be dead if they did not move when poked with a fine needle (Cayrol *et al.*, 1989).

Unsterilized sandy loam soil, pH 8.1, was mixed with chopped leaves of *L. camara* to make 0.5, 1, 3 or 5% concentrations and put into 8-cm-diam. plastic pots at 350 g/pot. The soil was watered daily to allow for decomposition of the plant material. After 21 days, a 25-ml aqueous cell suspension of *Pseudomonas aeruginosa* (Schroeter) Migula containing $3.1x10^8$ cfu/ml was drenched in each pot. Pots without *L. camara* and *P. aeruginosa* served as controls. Eight mungbean *Vigna radiata* (L.) Wilczek seeds were sown in each pot and after germination four seedlings were retained in each pot. Each treatment had four replicates and pots were kept in a randomized block design.

Concentrations of 3% and 5% were markedly injurious to plants, and were excluded from the experiments. One week after seedling emergence, soil in each pot was inoculated with 2000 juveniles (< 5-day-old) of *M. javanica*.

To establish whether decomposition was necessary for the release of toxic principles or the aqueous extract was responsible for the nematicidal activity, in another experiment soil was drenched with 12.5 or 25 ml extract (200 g/500 ml water = stock solution). The bacterium was added simultaneously. The rest of the procedure was the same as outlined above.

The experiments were terminated 45 days after the nematode addition and plant height and fresh weight of shoot were recorded. Galls developed on the entire root system were counted with the aid of a low power microscope. Nematode populations in the soil were estimated using a modified Baerman funnel technique (Schindler, 1961). Populations of *P. aeruginosa* in the rhizosphere were estimated following modified method of Pillay and Nowak (1997). One g root samples with adhering soil were placed in 250 ml flasks containing 10 ml of 0.1M $MgSO_4$ solution (pH 6.5) plus 0.02% Tween-20 and shaken vigorously for 15 min. Ten-fold serial dilutions of the suspension prepared and 50 µl aliquots from the approrpiate dilutions were plated onto KB medium. The plates were incubated at room temperature (28 °C) for 48 h and the number of cfu recorded. The plants grown in soil not treated with *P. aeruginosa* were also checked for the presence of contaminants. No growth of *P. aeruginosa* was detected in non-bacterized soil.

The data were subjected to analysis of variance (ANOVA) followed by the least significant differences according to Gomez and Gomez (1984). The data were transformed to $\log_{10}x+1$ where necessary.

Results and discussion

Aqueous, methanolic, ethyl acetate and hexane extracts of *L. camara* caused significant mortality ($p<0.001$) of *M. javanica in vitro* (Table I). Aqueous extracts at 10 mg/ml

showed the greatest (93%) juvenile mortality followed by methanolic extracts (10 mg/ml) which caused 78% juveniles deaths. Ethyl acetate and hexane extracts showed least inhibition indicating that the active principles were polar in nature.

TABLE I - *Percentage death of* Meloidogyne javanica *at different concentrations of aqueous, methanol, ethyl acetate and hexane extracts of* Lantana camara.

Treatments	Concentrations	Mortality %
Aqueous extract	10 mg/ml	93
	1 mg/ml	76
	0.1 mg/ml	15
	0 mg/ml	0
Methanol extract	10 mg/ml	78
	1 mg/ml	43
	0.1 mg/ml	10
	0 mg/ml	0
Ethyl acetate extract	10 mg/ml	29
	1 mg/ml	13
	0.1 mg/ml	2
	0 mg/ml	1
Hexane extract	10 mg/ml	10
	1 mg/ml	2
	0.1 mg/ml	3
	0 mg/ml	1
$LSD_{0.05}$		12.33

Decomposing leaves of *L. camara* at 0.5% resulted in significant ($p<0.05$) reduction in gall formation due to *M. javanica* but did not influence plant height (Table II). However, at 1% *L. camara* significantly reduced galling rates and nematode density in the soil ($p<0.05$) although plant height was also significantly reduced. *P. aeruginosa* used alone reduced galling ($p<0.05$) and increased the shoot weight ($p<0.05$). *L. camara* (at 0.5 and 1.0%) in conjuction with *P. aeruginosa* dramatically reduced galling as well as nematode density ($p<0.001$) in the soil but only at 0.5% significantly enhanced plant height and shoot weight. Population density of *P. aeruginosa* was significantly greater in the combination of *L. camara* (0.5%) and *P. aeruginosa* (Table II). *L. camara* extract at both dosages did not significantly influence galling and nematode density (Table III). However, *P. aeruginosa* either used alone or in combination with *L. camara* extract gave significant reduction in galling and elevated shoot weight. Populations of *P. aeruginosa* in the rhizosphere declined ($p<0.05$) when 25 ml extract of *L. camara* was added.

Decomposing *L. camara* in the soil was more effective in the control of *M. javanica* than its aqueous extract, presumably because of more ef-

TABLE II - *Effects of chopped leaves of* L. camara *and of* Pseudomonas aeruginosa *on the development of root-knot infection, nematode population in the soil, growth of mungbean and population of* P. aeruginosa *(log cfu x+1) in the rhizosphere.*

Treatments	Galls per root system	Nematodes in 250 g soil	Plant height (cm)	Shoot weight (g)	Bacterial rhizosphere population
Control	58	2026	16.6	2.3	–
L. camara 0.5%	49	1822	17.4	2.2	–
L. camara 1.0%	42	1504	16.2	2.1	–
P. aeruginosa	44	1724	17.6	2.8	5.23
L. camara 0.5% + *P. aeruginosa*	35	1130	18.4	2.7	5.38
L. camara 1.0% + *P. aeruginosa*	37	1246	17.5	2.5	5.15
$LSD_{0.05}$	7.8	320	1.5	0.3	0.12

TABLE III - *Effects of aqueous extract of* L. camara *(Ss = stock solution) and of* P. aeruginosa *on the development of root-knot infection, nematode population in the soil, growth of mungbean and population of* P. aeruginosa *(log cfu x+1) in the rhizosphere.*

Treatments	Galls per root system	Nematodes in 250 g soil	Plant height (cm)	Shoot weight (g)	Bacterial rhizosphere population
Control	69	2338	16.8	2.4	–
L. camara 12.5 ml Ss	62	2490	16.4	2.3	–
L. camara 25 ml Ss	60	2230	16.3	2.1	–
P. aeruginosa	47	1980	17.7	2.6	5.17
L. camara 12.5 ml Ss + *P. aeruginosa*	51	2010	17.4	2.8	5.12
L. camara 25 ml Ss + *P. aeruginosa*	43	2190	17.3	2.6	4.95
$LSD_{0.05}$	16	419	1.7	0.2	0.15

fective and continuous release of the toxic principles. Furthermore, addition of growth-promoting bacteria such as *P. aeruginosa* with decaying *L. camara* provided better control of the nematode and enhanced shoot growth.

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