*Phytopathol. Mediterr.* (2003) 42, 71–78

# Nematicidal and allelopathic responses of *Lantana camara* root extract

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**Summary.** The impact of root leachates of *Lantana camara* L., a tropical weed, against *Meloidogyne javanica*, the root-knot nematode, was tested under laboratory and pot conditions. Concentrated and diluted root leachate caused substantial mortality of *M. javanica* juveniles. Significant suppression of the nematode was achieved when soil was treated with a full-strength concentration of the leachate. Whilst this high concentration retarded plant height and shoot fresh weight, more diluted concentrations actually enhanced plant growth. To establish whether this inhibition of plant growth from the leachate was the result of depleted nitrogen levels in the soil due to the leachate, soil treated with such leachates was given urea as an additional nitrogen source. Urea not only enhanced nematode suppression activity of the root leachates but also increased seedling emergence and growth of mungbean. Application of the *L. camara* root leachates in combination with *Pseudomonas aeruginosa,* a plant growth-promoting rhizobacterium, significantly reduced nematode population densities in roots and subsequent root-knot infection, and enhanced plant growth. While a high concentration of root leachate slightly reduced *P. aeruginosa* colonization in the rhizosphere and inner root tissues, the nematicidal efficacy of the bacterium was unaffected. The root leachate of *L. camara* was found to contain phenolic compounds, including *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, ferulic acid and a quercetin glycoside, 7-glucoside. It also contained weak enzymic hydrogen cyanide.

**Key words:** *Lantana camara,* root extract*, Meloidogyne javanica*, allelopathy.

## Introduction

Many plant species are highly resistant to nematodes. The best documented of these are the marigolds (*Tagetes* spp.), rattlebox (*Crotalaria spectabilis*), chrysanthemums (*Chrysanthemum* spp.), castor bean (*Ricinus communis*), margosa (*Azardiracta indica*), and many members of the family Asteraceae (family Compositae) (D'Addabbo, 1995). The active principle(s) for nematicidal activity have not been identified in all these species and no plant-derived products are sold commercially for control of nematodes. In the case of the Asteraceae, the photodynamic compound alpha-terthienyl has been shown to account for the strong nematicidal activity of the roots (Duke, 1990).

*Lantana camara* L. (Verbinaceae) is a perennial weed commonly found in the semiarid regions of the Indo-Pakistan subcontinent. It is one of the 10 worst weeds of the world and is a serious problem with 14 crops in 47 countries (Holm *et al*., 1979). *L. camara* has an allelopathic potential because it contains a number of phenolic compounds (Narwal, 1994). In our previous study, leaf-extract

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and decomposed leaves of *L. camara* not only inhibited germination but also caused marked suppression of several root-infecting fungi (Shaukat *et al*., 2001) and of a plant-parasitic nematode (Ali *et al*. 2001). Later studies indicated that decomposed leaves of *L. camara* caused marked changes in the fungal community structure of the soil and the endorhiza, favouring fungal species that exhibited strong nematicidal and hatch-inhibiting activity (Shaukat and Siddiqui, 2001a). Rice (1984) and Inderjit and Dakshini (1994) earlier found that root leachates of several plant species influenced the growth and establishment of other plant species. The aim of the present study was to determine the impact of root leachates of *L. camara* on invasion by *Meloidogyne javanica*, the root-knot nematode in mungbean and on the subsequent development of root knot in that plant.

## Materials and methods

### Preparation of root extract

Roots of *L. camara* were collected in April, 2001 from shrubs grown at Karachi University. Roots were carefully washed to remove soil and dried for 48 h at room temperature. Root extract was prepared by soaking 15 g of root in 220 ml distilled water for 72 h, followed by filtration using coarse filter paper (Whatman No. 54, Clifton, NJ, USA). This filtrate was identified as full strength root extract (FSRE). FSRE was also diluted with distilled water to concentrations of 1:2 and 1:4 (v:v). The root extract was amended with appropriate amounts of penicillin and streptomycin sulphate to avoid bacterial contamination. The extract was then stored in a refrigerator at 6°C prior to use.

### Biological assays

*Effect of root extract on egg hatching*

To determine the effects of root extract on egg hatching, two medium-size egg masses each of *M. javanica* were placed in glass cavity slides (3-cm-diam.) each containing 2-ml of one of the concentrations of the root extract. Egg masses hatched in 2 ml sterile distilled water served as controls. Each treatment was replicated four times and cavity glass slides were arranged in a randomized complete block design at 28°C. After 48 h, hatched juveniles were counted; the egg masses were then transferred from the cavity slides to sterile distilled water to ascertain whether the egg masses treated with root extract had been permanently or only temporarily inactivated. The emergence of any more juveniles was recorded after a further 48 h (Siddiqui, 2000).

*Effect of root extract on juveniles of* M. javanica

To determine the effect of root extract on the juveniles, 2 ml of the leachate was transferred to glass cavity slides to which 1 ml water containing 30–35 surface-sterilized juveniles $ml^{-1}$ was added. After 48 h, dead juveniles were counted and percent mortality was calculated. Juveniles were considered dead when they did not move on probing with a fine needle (Cayrol *et al.,* 1989).

*Effect of root extract on survival and infectivity of* M. javanica *and growth of mungbean*

A pot experiment was conducted to examine the effectiveness of root extract of *L. camara* against *M. javanica* root-knot. The soil used for this experiment was obtained from the experimental field of the Department of Botany, University of Karachi. The soil (sandy-loam, pH 8.1, moisture holding capacity 40%) was passed through a 2-mm sieve to discard stones and placed in 8-cm-diam plastic pots. The soil in each pot was drenched with 50 ml root extract at full-strength or at dilutions of 1:2 and 1:4, and was sown with eight mungbean (*Vigna radiata* [L.] Wilczek) seeds. Soil drenched with 50 ml sterile distilled water served as control. Treatments were replicated four times and arranged in a randomized complete block design in a glasshouse at 33±4°C. One week after sowing, percent seed germination was recorded. One week after seedling emergence, the roots in each pot were infected with 2000 freshly hatched juveniles of *M. javanica* by making three holes around the seedlings. The experiment was terminated 45 days after nematode addition and at that time growth-parameters such as plant height and fresh weight of the shoots were recorded. The galls produced by *M. javanica* on the root system of each seedling were counted under a low-power microscope (×6). To determine the extent of nematode invasion, the root system was washed with running tap water, blotted dry and boiled in 0.225% lactic acid fuchsin. The

stained roots were macerated in an electric grinder and the macerate was used to count the penetrated nematodes.

*Effect of* L. camara *root extract and urea on root-knot infection by* M. javanica *and growth of mungbean*

One of the concerns regarding allelopathy is that the addition of plant debris or leachates may lead to a temporary depletion of nitrogen (Harper, 1977) so that any growth response after the addition of plant debris or leachates will be due to the lowered nitrogen in the soil rather than the organic molecules added (Inderjit and Foy, 1999). An experiment was carried out to determine whether nitrogen added to the soil would affect the allelopathic potential of soil amended with different dilutions of *L. camara* root extract. This experiment was a 2×4 factorial. The factors included 2 levels of N (0 and 0.18 g urea $kg^{-1}$ soil) and 4 concentrations of root extract of *L. camara* (0, FSRE, 1:2 and 1:4 dilutions). The soil samples, 350 g each, were amended with 25 ml of root extract at full-strength or at a dilution of 1:2 or 1:4 and with 0.18 g of urea $kg^{-1}$ soil (prepared in 25 ml sterile distilled water) as N fertilization. All amendments were replicated four times. Plant growth and the number of galls induced by *M. javanica* on the root system were recorded as mentioned above.

*Effect of* L. camara *root extract and* Pseudomonas aeruginosa *on root-knot infection by* M. javanica *and growth of mungbean*

In previous reports, phenolic compounds were not only found to produce highly significant control of *M. javanica* (Calvet *et al.,* 2001; Shaukat and Siddiqui, 2001a) but they also affected the survival of the mycorrhizae *Glomus mosseae* and *Glomus intraradices* (Calvet *et al*., 2001). Another experiment was conducted to evaluate the impact of root leachates of *L. camara* on the survival of *Pseudomonas aeruginosa*, a plant growth-promoting bacterium (PGPB) in the rhizosphere and inner root tissues, and the bacterium's subsequent effectiveness against *M. javanica*. The bacterial culture was centrifuged at 2,800 *g* for 20 min., the supernatant discarded and the pallet resuspended in sterile distilled water prior to use. The experimental design was a 4×2 factorial which included four concentrations of root extract (0, FSRE, 1:2 and 1:4) and two levels of *P. aeruginosa* (0 and $2.3\times10^8$ cfu $ml^{-1}$ suspension). The top layer of soil was removed to a depth of 3 cm and the remaining soil drenched with 25 ml per pot of root extract at full strength or at dilutions of 1:2 or 1:4. Then eight mungbean seeds per pot were sown and the removed soil was replaced. For bacterial application, leachate-treated soil was drenched with a cell suspension ($2.3\times10^8$ cfu $ml^{-1}$) of *P. aeruginosa* strain IE-6S$^+$ (a streptomycin resistant derivative of strain IE-6 cultured on King's medium B (KB, King's *et al*., 1954) prepared in 25-ml sterile distilled water. Soil drenched with 25 ml sterile distilled water served as control. Each treatment was replicated four times and pots were arranged in a randomized complete block design. The seedlings were thinned to four per pot and the roots of seedlings inoculated with 2000 freshly hatched juveniles of *M. javanica*. The experiment was terminated 45 days after nematode addition, at which time plant growth parameters were recorded. To determine nematode penetration, roots were thoroughly washed with tap water to remove adhering soil, blotted dry, weighed and boiled in 0.1% lactic acid fuchsin. After homogenization in an electric blender, the juveniles that had penetrated the roots were counted under a low-power microscope (×6). The rhizosphere population of the bacterium was isolated by placing the fresh roots with adhering soil in a 100 ml Erlenmeyer flask containing 10 ml of 0.1 M $MgSO_4$ solution (pH 6.5) plus 0.02% Tween-20. Ten-fold serial dilutions of the suspension were prepared and 100 ml aliquots from the appropriate dilutions were plated onto KB supplemented with 100 mg $l^{-1}$ of streptomycin. For the assessment of the endophytic populations, fresh roots were surface-sterilized with 1% $Ca(OCl)_2$ for 1 min, rinsed twice with sterile distilled water, submerged for 30 s in 15% $H_2O_2$ and again rinsed twice in sterile distilled water. The tissue was then macerated in 10 ml of 0.1 M $MgSO_4$ solution (buffered to pH 6.5) with 0.02% Tween-20. Ten-fold serial dilutions of the suspension were prepared and 100 ml aliquots from the $10^{-3}$ and $10^{-4}$ dilution were plated onto KB supplemented with 100 mg $l^{-1}$ streptomycin. The plates were incubated at room temperature (25°C) for 48 h and the number of cfu was recorded.

**Detection of phenolic acids and hydrogen cyanide from *L. camara* root extract**

Plant roots exude a variety of phenolic acids into the surrounding medium (Inderjit *et al*., 1999). Phenolic compounds in the root extract of *L. camara* were therefore determined using a thin layer chromatography (TLC) technique and two-dimensional paper chromatography (PC). For TLC, 30-ml of root extract was mixed with 30 ml of ether and after shaking in a separating funnel, the ether fraction was concentrated to dryness. The residue was dissolved in 1 ml of 95% ethanol and was separated on analytical TLC plates (Merck Si 60 $F_{254}$, Merck, Darmstadt, Germany) with a mobile phase, acetic acid: chloroform (1:9 v:v, top layer). Phenolic compounds were detected by fuming the plates with ammonia and examining under short UV (Harborne, 1973). For PC, the ether extract of *L. camara* was evaporated to dryness, dissolved in 2 ml of 80% ethanol and used for loading Whatman No. 1 chromatographic paper. The chromatograms were developed in n-butanol-acetic acid-water (50:2:48 v:v:v). *Rf* values of the spots were compared with those of commercially available reference phenolic compounds. Phenolic principles were detected using ferric chloride-ferric cyanide reagents and UV light. For the detection of hydrogen cyanide from the root leachate of *L. camara*, a picrate paper disc method was used. Picrate papers were prepared by dipping 10×7 mm pieces of filter paper in saturated (0.05 M) aqueous picric acid previously neutralized with $NaHCO_3$ and filtered. Twenty ml of the FSRE was placed in a 100 ml conical flask. The flask was then firmly corked with a picrate paper suspended inside from the cork, and left to incubate at 40°C for 2 h. A colour change from yellow to reddish-brown indicated the enzymic release of HCN from the leachate (Harborne, 1973).

**Statistical analysis**

The data sets were subjected to analysis of variance (ANOVA) or factorial analysis of variance (FANOVA) using STATISTICA software version 5.0 (StatSoft, Inc. 1995, Tulsa, OK, USA). The follow up of ANOVA or FANOVA included a least significant difference (LSD) test or Duncan's multiple range test. The bacterial populations were transformed to $\log_{10}$ (×+1) prior to analysis.

## Results and discussion

Under laboratory conditions, root extract of *L. camara* at full-strength and at 1:2 and 1:4 dilutions significantly ($P$<0.001) inhibited egg hatch and produced mortality of *M. javanica* juveniles *in vitro* (Table 1). Mortality of juveniles decreased gradually with decreasing concentrations of the extracts. FSRE caused 100% mortality of the juveniles. Tanda *et al.,* (1989) similarly reported that root exudates and extracts from sesame seedlings inhibited egg hatch and juvenile penetration of *M. incognita*. Root exudates of neem (*Azadirachta indica* A. Juss) and Persian lilac (*Syringa persica* L.) caused extensive nematode mortality and inhibited larval hatching of a number of plant nematodes (Siddiqui and Alam, 1989). Root exudates of *Lolium multiflorum* (Lam.) reduced populations of *Heterodera glycines* by increasing egg hatching of the nematode in the absence of the host (soybean), thus depleting the lipid reserves for the juveniles (Riga *et al.*, 2000). In contrast, the allelopathic compound glycinoeclepin A, found in kidney bean (*Phaseolus vulgaris* L.) roots, acted as a hatching stimulus of soybean cyst nematodes (Kraus and Vander Louw, 1996).

Soil treatment with root extract of *L. camara* significantly reduced ($P$<0.05) nematode population densities in roots and consequent root-knot infection in mungbean (Table 2). The greatest reduction in galling intensity was achieved with

Table 1. Effect of root extracts of *Lantana camara* at various concentrations on mortality of *Meloidogyne javanica* juveniles after 24 hours.

| Treatment | Number of eggs hatched in | | Mortality % |
|---|---|---|---|
| | Root extract | Distilled water | |
| Control | 193 a | 33 b | 1 c |
| FSRE[a] | 67 d | 18 c | 100 a |
| 1:2 dilution[b] | 103 c | 57 a | 84 b |
| 1:4 dilution[b] | 155 b | 39 b | 72 b |
| $LSD_{0.05}$ | 38 | 12 | 14 |

[a] FSRE, full-strength root extract.
[b] FSRE was diluted with distilled water to 1:2 and 1:4 (v:v) concentrations.
Data in column followed by the same letters are not significantly ($P$<0.05) different according to Duncan's multiple range test; n=4.

FSRE (>51%; $P<0.05$). Similarly, FSRE caused >39% reduction in nematode invasion over the controls. In general, nematode-suppressive activity also decreased with decreasing concentrations of the root leachates. FSRE also significantly ($P<0.05$) inhibited seedling emergence and growth of mungbean. However, when applied at the lower concentrations, root extract actually enhanced plant growth. Root extract at 1:2 dilution increased plant height and fresh weight of shoots by >14% and >19% respectively compared with the controls. Phytotoxic responses depend on both the quality and quantity of phenolic compounds. Some times lower concentrations will enhance growth, whereas higher concentrations will inhibit such growth (Inderjit *et al.,* 1999).

Urea with or without root extract significantly ($P<0.05$) reduced galling by *M. javanica* (Table 3). However, when urea and root extract were combined the decrease in galling was still greater. FSRE achieved a >45% reduction in root-knot development without urea; when it was used with urea, the reduction was >53%. FSRE without urea reduced seed germination over normal levels, but with urea it increased seed germination to above control levels. Compared with soils not treated with urea, the addition of 0.18 g $kg^{-1}$ of urea to the soil not only increased seedling emergence, but also

Table 2. Effect of root extract of *Lantana camara* at various concentrations on *Meloidogyne javanica* root-knot infection, nematode root populations and growth of mungbean.

| Soil with root extract[a] | Galls per root system | Juveniles per root system | Germination percentage | Plant height (cm) | Shoot weight (g) |
|---|---|---|---|---|---|
| Control | 94 | 174 | 91 | 16.4 | 0.5 |
| FSRE | 46 | 105 | 53 | 13.4 | 0.4 |
| 1:2 dilution | 68 | 127 | 75 | 16.7 | 0.5 |
| 1:4 dilution | 78 | 151 | 81 | 18.8 | 0.6 |
| $LSD_{0.05}$ | 18 | 26 | 17 | 1.3 | 0.1 |

[a] Roots of *L. camara* (15 g) were soaked in 220 ml of distilled water for 72 h followed by filtration. The resulting filtrate was identified as full–strength root extract (FSRE) and was further diluted with distilled water to dilutions of 1:2 (v:v) and 1:4 (v:v).

Table 3. Effect of root extracts of *Lantana camara* at various concentrations with and without urea as N fertilization, on the development of *Meloidogyne javanica* root-knot infection and growth of mungbean.

| Soil with root extract[a] and with or without urea | No. galls per root system | Germination (%) | Plant height (cm) | Shoot weight (g) |
|---|---|---|---|---|
| No urea added | | | | |
| Control | 102 | 91 | 17.4 | 0.55 |
| Full strength | 56 | 53 | 13.7 | 0.41 |
| 1:2 dilution | 73 | 75 | 16.2 | 0.52 |
| 1:4 dilution | 84 | 91 | 18.7 | 0.72 |
| 0.18 g urea $kg^{-1}$ soil added | | | | |
| Control | 84 | 100 | 18.4 | 0.63 |
| Full strength | 39 | 75 | 15.3 | 0.58 |
| 1:2 dilution | 61 | 81 | 15.9 | 0.62 |
| 1:4 dilution | 68 | 91 | 20.1 | 0.79 |
| $LSD_{0.05}$ Root extract (RE) | 14 | 13 | 2.1 | 0.19 |
| Urea (U) | 10 | 10 | 1.5 | 0.14 |
| RE × U | 23 | 21 | 3.4 | 0.31 |

[a] See Table 2.

enhanced growth of mungbean seedlings. The addition of urea may have stimulated microbial activity, which would lower the level of phenolics in the soil amended with root leachate (Inderjit and Foy, 1999).

Soil application of root extract, with or without *P. aeruginosa,* significantly ($P<0.05$) reduced nematode penetration rates compared with the untreated controls (Table 4) but with the addition of *P. aeruginosa,* the reduction was greater ($P<0.05$) than with root extract alone at all dilutions. Similarly, when compared with application of *P. aeruginosa* alone, root extract at 1:4 dilution mixed with the bacterium caused a >18% reduction in nematode population densities in the root. *P. aeruginosa* alone also significantly (>36%; $P<0.05$) reduced nematode root populations compared with the untreated controls.

FSRE significantly reduced plant height and fresh weight of shoots compared to *P. aeruginosa* treatment alone or to the untreated controls (Table 4). However, the root extract at the 1:2 or 1:4 dilutions and *P. aeruginosa* used together gave greater plant height ($P<0.05$) while the 1:4 dilution with the bacterium produced a greater fresh weight of shoots than did root extract alone. The allelopathic potential of decomposed leaves and of leaf extract has been reported in a previous study (Ali *et al.,* 2001). The results of the present study suggest that the allelopathic effects of root extract can to some extent be alleviated by the application of specific rhizobacteria.

Whereas FSRE significantly ($P<0.05$) reduced bacterial rhizosphere colonization, the ability of *P. aeruginosa* to colonize the inner root tissues remained unaffected (Table 4). However, root extract at 1:2 or 1:4 dilutions enhanced bacterial colonization in both the rhizosphere and the roots. It is therefore recommended that the compatibility between organic amendments, biocontrol bacterium and the host plant should be ascertained in the laboratory by pot trials before the benefits (control of plant pathogens, enhanced plant growth) of these agents are exploited in practical agriculture. In a previous study, AlSaadawi and Rice (1982) found that four phenolic phytotoxins isolated from *Polygonum aviculare* inhibited the growth of selected strains of the nitrogen-fixing bacteria *Azotobacter* and *Rhizobium*. Since streptomycin-resistant bacteria rarely occur in a natural environment, reisolation of the streptomycin-resistant *P. aeruginosa* from the rhizosphere was undertaken, which con-

Table 4. Effect of root leachates of *Lantana camara* at various concentrations with or without *Pseudomonas aeruginosa* on nematode penetration, growth of mungbean and population of *P. aeruginosa* in the rhizosphere and root.

| Soil with root extract and with or without *P. aeruginosa* | Juveniles per root system | Plant height (cm) | Shoot weight (g) | Bacterial population log cfu (×+1) | |
|---|---|---|---|---|---|
| | | | | Rhizosphere | Root |
| Without *P. aeruginosa* | | | | | |
| Control | 244 | 16.6 | 0.6 | 0 | 0 |
| Full strength | 168 | 14.5 | 0.4 | 0 | 0 |
| 1:2 dilution | 196 | 17.0 | 0.6 | 0 | 0 |
| 1:4 dilution | 228 | 18.2 | 0.6 | 0 | 0 |
| With *P. aeruginosa* | | | | | |
| Control | 157 | 18.9 | 0.7 | 4.31 | 3.28 |
| Full strength | 144 | 16.9 | 0.5 | 4.14 | 3.23 |
| 1:2 dilution | 128 | 18.8 | 0.7 | 4.42 | 3.44 |
| 1:4 dilution | 144 | 21.5 | 0.8 | 4.52 | 3.48 |
| $LSD_{0.05}$ Root extract (RE) | 18 | 1.8 | 0.1 | 0.09[a] | 0.06[a] |
| *P. aeruginosa* (PA) | 13 | 1.3 | 0.1 | - | - |
| RE × PA | 29 | 2.9 | 0.2 | - | - |

[a] One-way analysis of variance performed.

Table 5. *Rf*-values of the phenolic principle(s) in the ether fraction of aqueous root extract of *Lantana camara* and their reaction to a developing reagent and UV light.

| *Rf* | Reagent | UV-light | Compound |
|---|---|---|---|
| 0.57 | Ammonia fuming | Blue | *p*-hydroxybenzoic acid[a] |
| 0.84 | Ammonia fuming | Blue | Vanillic acid[a] |
| 0.76 | Ferric chloride-ferric cyanide | Light blue | Caffeic acid[b] |
| 0.90 | Ferric chloride-ferric cyanide | Bright blue | Ferulic acid[b] |
| 0.35 | Ammonia fuming | Bright yellow | 7-Glucoside[b] |

[a] Compound detected using TLC.
[b] Compound detected using PC.

firmed that endophytic bacteria form part of the rhizosphere microbial community (Hallmann *et al*., 1999). Variations in the extent to which *P. aeruginosa* colonizes the rhizosphere and inner root tissue when combined with various dilutions of root leachate also suggest that the application of *L. camara* root extract causes a change in the microbial community structure, particularly influencing those organisms that possess a nematode-suppressive capacity. It was noted in an earlier study that decomposed leaves of *L. camara* caused marked changes in the fungal community structure in the rhizosphere and roots of mungbean, and enhanced the hatch-inhibiting and nematicidal microbiota (Shaukat and Siddiqui, 2001b).

Root leachate of *L. camara* yielded various phenolic compounds and a quercetin glycocide on TLC and PC. The spots that appeared on TLC were similar to those of *p*-hydroxybenzoic acid and vanillic acid, whereas those on PC resembled caffeic acid, ferulic acid and 7-glucoside (Table 5). Various phenolic compounds including quercetin glycosides have been identified from the root leachate of *Verbesina enceloides*, an agent causing reduced plant growth in radish seedlings (Inderjit *et al*., 1999). The allelopathic and nematicidal activity of *p*-hydroxybenzoic acid and caffeic acid has been previously reported (Shaukat and Siddiqui, 2001a). It is however not certain that compounds like vanillic acid, ferulic acid and 7-glucoside also posses nematicidal characteristics. In the present study, root leachate of *L. camara* converted picrate paper from yellow to light brown, indicating the presence of weak enzymatic HCN. It has been known for many years that the release of HCN from intact plants is a sign that those plants contain cyanogenic glucosides, which produce HCN on either enzymic or non-enzymic hydrolysis (Harborne, 1973). HCN is known to play a significant role in the suppression of root-infecting fungi (Keel *et al*., 1989). Recently, Gallagher and Manoil (2001) found that hydrogen cyanide is the primary toxic factor produced by *P. aeruginosa,* and is the agent killing the nematodes. The role of HCN in plant growth remains to be ascertained.

## Acknowledgements

This investigation was supported by a grant from the office of the Dean, Faculty of Science, University of Karachi, which is gratefully acknowledged. Thanks are also due to M. Zafar of the National Nematological Research Centre, Karachi University, for providing technical assistance during the study.

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*Accepted for publication: March 11, 2003*