Phytopathol. Mediterr. (2001) 40, 245–252

Lantana camara in the soil changes the fungal community structure and reduces impact of *Meloidogyne javanica* on mungbean

Syed Shahid Shaukat[1] and Imran Ali Siddiqui[2]

[1]National Nematological Research Centre, University of Karachi-75270, Pakistan
[2]P.A.F. Intermediate College, Korangi-Creek, Karachi-75190, Pakistan

Summary. Changes in the fungal community resulting from nematode suppression were studied by comparing the number of fungal species in the soil and mungbean roots between soils with and without amendment with *Lantana camara*. Addition of *L. camara* to the soil at 1% (w:w) significantly reduced the *Meloidogyne javanica* population density in the soil and in the roots of mungbean, as well as subsequent root-knot infestation of mungbean, confirming that nematode suppressiveness was induced by this organic amendment. *L. camara* amendment was associated with an increase in fungal populations, especially those with nematicidal activity. The soil was probably the major source of fungal endophytes in mungbean roots since nearly all the endophytic fungi were also found in the soil. *L. camara*-amended soils contained several fungi that were not found in unamended soil, including an *Acremonium* sp., *Aspergillus fumigatus*, *Drechslera australiensis*, *Fusarium culmorum*, *Penicillium notatum* and *Trichoderma viride*. The fungi in the inner root tissues of mungbean growing in *L. camara*-amended soil included an *Acremonium* sp., *F. solani*, *Macrophomina phaseolina*, *Penicillium* sp., and *Trichoderma viride*. Only *T. viride* was not encountered in the inner root tissues of mungbean in unamended soil. *M. phaseolina* and *F. solani* were isolated with relatively high frequency from the inner root tissues of mungbean growing in unamended soils while the remaining endorhizal fungi were more frequent in amended soils. *M. phaseolina,* though isolated extensively from the inner root tissues, either did not occur in the soil or was not isolated by the soil dilution technique used. When fungi from soil amended with *L. camara* were tested for their nematicidal and hatch-inhibiting activity, *Aspergillus niger* produced the greatest nematode mortality, while *T. viride* caused the greatest inhibition of egg hatching of *M. javanica*.

Key words: organic amendment, endophytic fungi, soil fungi, root-knot nematode.

Introduction

Naturally-occurring nematode suppressiveness has been reported for several agricultural systems (Stirling *et al*., 1979; Kerry, 1982; Kluepfel *et al*., 1993), but it is also induced by crop rotation with nematode-antagonistic plants such as switchgrass (*Panicum virgatum* L.) (Kokalis-Burelle *et al*., 1995) and velvetbean [*Mucuna deeringiana* (Bort.) Merr.] (Vargas *et al*., 1994), and by organic amendments with pine bark (Kokalis-Burelle *et al*., 1994), hemicellulose (Culbreath *et al*., 1985) and chitin (Mankau and Das, 1969; Spiegel *et al*., 1986; Rodríguez-Kábana and Morgan-Jones, 1987). A major component of the suppressiveness caused by chitin amendments is believed to be biotic and several studies report increased numbers of nematode-antagonistic micro-organisms in soils with chitin-induced nematode suppressiveness (Rodríguez-Kábana *et al*., 1984; Hallmann *et al*., 1999).

Corresponding author: I.A. Siddiqui
E-mail: imran_75850@yahoo.com

In a previous study, an aqueous extract of *Lantana camara* L. Linn. caused high mortality of *Meloidogyne javanica* juveniles *in vitro*, but not in a pot test under greenhouse conditions. However, decomposed *L. camara* material in the soil significantly reduced the *M. javanica* population density and subsequent root-knot infection of mungbean growing in that soil (Ali *et al*., 2001). These results suggested that some other factors were involved in suppressing the root-knot nematode. We postulated that a shift in the microbial community structure due to *L. camara* was responsible for the observed suppression of *M. javanica*. The purpose of the present investigation was to determine if *L. camara*-mediated suppression of plant parasitic nematodes was related to changes in fungal communities in soils.

Materials and methods

Soil (sandy loam; pH 8.1; moisture holding capacity 38%) obtained from a field near the Department of Botany, University of Karachi, was mixed with powdered leaves of *L. camara* to give concentrations of 0.5 and 1.0% w:w. Concentrations greater than 1% were very injurious to the mungbean seedlings and were therefore not considered here. Unamended soil and the two amended soils were placed in 8-cm-diam plastic pots at 350 g/pot. The soil was watered daily to facilitate decomposition of *L. camara*. Three weeks after the amendments, eight mungbean seeds were sown in each pot, and following germination four seedlings were retained in each pot. One week after seedling emergence, the soil in each pot was inoculated with 2000 juveniles of *M. javanica*. Juveniles less than one-week-old were obtained from infected brinjal roots using the Baermann funnel technique (Whitehead and Hemming, 1965). The juveniles were counted and the requisite amount of inoculum was introduced in 10 ml water by pipetting the juveniles into three holes around the roots of the seedlings. Each treatment had eight replicates and the pots were arranged in a randomized complete block design. The soil pH (1:5 w:v soil-water suspension) and total fungal count were measured at the start of the experiment (day 0), at the time of seed sowing (21 days) and at the time of harvest (73 days). Four replications were sufficient to demonstrate significant differences between soil pH and total fungal populations, so only the first four replicates of each treatment were used.

The experiment was terminated 45 days after nematode addition, at which time plant growth parameters (plant height and fresh weight of shoots and roots) were determined and the galls produced in the entire root system were counted under a stereomicroscope (x6). To determine nematode penetration, 1-g root samples were thoroughly washed with tap water, stained in 0.25% acid fuchsin with lactic acid, and macerated in an electric grinder for 45 seconds. The macerate was suspended in 100-ml water, and *M. javanica* females and juveniles in 5 samples of 5 ml each were counted with the aid of a low-power microscope (x6). To assess nematode populations, each type of soil (from all replicates) was mixed thoroughly in a plastic container and the nematodes were extracted from five 50-g samples using a modified Baermann funnel technique. To determine the entire fungal population, 10 g of moist soil was taken at each sampling time, added to 250-ml Erlenmeyer flasks containing 100 ml sterile distilled water and stirred for 2–4 min. A serial dilution of the soil suspensions was prepared and 0.1 ml aliquots of 10^{-1}, 10^{-2} and 10^{-3} dilutions were plated on potato dextrose agar (PDA) and Czapek's Dox agar (CDA) medium, supplemented with penicillin and streptomycin sulphate to avoid bacterial contamination. After incubation at 28°C for 1 wk, the plates were examined for total fungal counts. The fungi were then purified on PDA at 28°C and were tested for nematicidal activity against *M. javanica*. The fungi were grown in a 250-ml Erlenmeyer flask containing 100 ml Czapek's Dox liquid medium. After incubation at 28°C for 1 wk, the extract was filtered through Whatman No. 1 filter paper and the filtrate was collected in a beaker prior to use. One ml of fungal filtrate and one ml of surface-sterilized *M. javanica* juveniles (35–50 juveniles ml^{-1}) were placed in a glass cavity slide and kept at 28°C. There were three replicates for each fungal filtrate. After incubating for 48 h, the dead and surviving juveniles were counted and mean percent mortality was calculated. The effect of the filtrates on the hatching of *M. javanica* egg masses was also determined by placing two equal masses of medium-size eggs in the glass-cavity-slides. Three slides for each fungal filtrate were kept at 28°C and the hatched juveniles were counted after 120 h. The nematicidal

and hatch-reducing activity of the filtrate was compared with egg masses on Czapek's Dox broth grown without the fungal filtrate. Some of the fungi were not tested for their effectiveness against nematodes because the large number of other fungi in the medium meant that they could not be purified on PDA.

Data were subjected to one-way analysis of variance (ANOVA) followed by the least significant differences (LSD) test. Treatment means were also compared using Duncan's multiple range test in accordance with Sokal and Rohlf (1995). Fungal populations were transformed to $\log_{10}$ (x +1) before the analyses. The general species diversity of the fungal communities was measured by the generally accepted Shannon-Wiener information theory function:

$$H' = -\sum_{i=1}^{s} p_i \log p_i$$

where H' is the general species diversity and p_i the proportion of the total number of colonies N belonging to the ith species (Shannon and Weaver, 1963). The variance of general diversity var (H') was calculated in accordance with Magurran (1988), as follows:

$$\text{Var } (H') = \frac{\sum pi \, (\log p_i)^2 - (\sum p_i \log p_i)^2}{N} + \frac{S-1}{2N^2}$$

The general diversity incorporates two components of diversity: species richness, which expresses the number of species (S) as a function (ratio) of the total number of individuals (N), and equitability, which measures the evenness of allotment of individuals among the species. Species richness was determined simply by the number of species $\hat{S}$ (Margurran, 1988).

The equitability component of diversity and its variance were measured in accordance with Pielou (1975):

$$J' = H'/H'_{max}$$

The equitability index J' is the ratio between observed (H') and maximal diversity (H' max). Variance of equitability was estimated as:

$$\text{Var } (J') = \text{Var } (H')/(\log S)^2$$

Results

Effect of *L. camara* on the population density and infectivity of *M. javanica* and on the growth of mungbean

L. camara soil-amendment caused significant (P<0.001) suppression of *M. javanica* in mungbean (Table 1). Soil amendment with 1% *L. camara* gave the maximum reduction of nematode populations (42.3% in the soil and 24% in the roots compared with the controls) and the greatest subsequent root-knot development (36.6% less than the control). At 0.5% concentration *L. camara* produced better mungbean growth than at 1%. The greatest plant height (11.8% less than controls) and fresh weight of the shoots (+18.1%) were obtained in soil with 0.5% *L. camara*. However, root weight was significantly reduced in the amended soils.

Composition of fungi in amended and unamended soils

Amendment of soil with *L. camara* generally resulted in a significantly greater number of soil fungal species culturable on PDA and CDA, compared with the number of species in unamended

Table 1. Effect of soil amendment with powdered leaves of *Lanatana camara* on the development of *Meloidogyne javanica* populations in the soil and in the roots of mungbean, and its effect on the growth of mungbean.

Treatment	Galls/ root system	*M. javanica* population in		Plant height (cm)	Shoot weight (g)	Root weight (g)
		250 g soil	1 g root			
L. camara 0%	71	2753	104	16.1	2.2	1.4
L. camara 0.5%	58	2131	79	18.0	2.6	0.9
L. camara 1.0%	45	1587	66	17.5	2.4	0.7
$LSD_{0.05}$	9.6	408	19	1.2	0.3	0.4

soil. The full range of fungi isolated from both *L. camara*-amended and unamended soil is listed in Table 2. Thirty-three percent of these fungi occurred only in amended soil: an *Acremonium* sp., *Aspergillus fumigatus*, *Drechslera australiensis*, *Fusarium culmorum*, *Penicillium notatum* and *Trichoderma viride*. By contrast, all fungi in unamended soil also occurred in the amended soils. The Aspergilli were the most common, comprising 22% of all fungi in both amended and unamended soils.

Effect of *L. camara* on the diversity, equitability and species richness of the fungal community

In general, fungal species diversity and equitability decreased with time, while species richness increased slightly (Table 3). The greatest fungal

Table 2. Effect of soil-amendments with *Lantana camara* at concentrations of 0, 0.5 and 1%. Fungi in the soil fungal community were surveyed after 0, 15 and 73 days and expressed as $\log_{10}$ (x+1).

Fungus	*L. camara*								
	0			0.5			1.0		
	0	15	73	0	15	73	0	15	73
Acremonium sp.	0	0	0	0	1.39	0.99	0	0.34	1.06
Alternaria alternata	0.34	0.49	0	0	0.56	0	0.34	0	0
Aspergillus flavus	0	0.34	0	0	0.34	0.49	0	0.84	0.34
Aspergillus fumigatus	0	0	0	0	0	1.06	0	0.56	0
Aspergillus niger	0.49	1.06	1.84	0.56	1.59	2.45	0.56	1.06	2.76
Aspergillus sp.	0	0.34	0.49	0.34	0.84	0.34	0	0.34	0.99
Chaetomium globosum	0	0	0.34	0	0.34	0	0	0.49	1.43
Cladosporium sp.	0.34	0	0	0	0	0	0	0	0.44
Drechslera australiensis	0	0	0	0	0	0	0	0.34	0
Drechslera hawaiiensis	0	0	0.34	0	0.49	0	0	0	0.44
Fusarium culmorum	0	0	0	0	0.34	0.34	0	0.49	0
Fusarium oxysporum	0.34	0	1.38	0.56	1.59	1.53	0.34	1.59	0.99
Fusarium solani	0.44	0.34	1.41	0.44	2.25	2.76	0.56	2.45	2.15
Mycelia sterilia	0	0	1.26	0.34	0	0.49	0	0.34	1.41
Penicillium notatum	0	0	0	0	0.49	0	0	0	0.34
Penicillium sp.	0.34	0.34	1.38	0	0.34	1.26	0	0.53	0.99
Rhizopus stolonifer	0.84	0.84	1.26	0.49	1.38	2.26	0.34	0.99	2.15
Trichoderma viride	0	0	0	0.34	0.84	0	0	0	1.43

Table 3. General diversity (H'), equitability (J') and species richness ($\hat{S}$) of the fungal communities in *Lantana camara* amended soils (0, 0.5 and 1%) at various sampling times (0, 15, 73 days). Var (H') = variance of H'; Var (J') = variance of J'.

Diversity	*L. camara*								
	0			0.5			1.0		
	0	15	73	0	15	73	0	15	73
H'	1.856	1.703	1.797	1.952	1.641	1.301	1.612	1.001	1.410
Var (H')	0.014	0.019	0.003	0.003	0.004	0.008	0.005	0.005	0.001
J'	0.954	0.875	0.817	1.004	0.622	0.542	1.001	0.394	0.534
Var (J')	0.003	0.005	0.0004	0.001	0.0006	0.0001	0.002	0.0008	0.0002
$\hat{S}$	7	7	9	7	14	11	5	13	14

diversity and equitability were exhibited by the controls. In *Lantana*-amended soils fungal diversity and equitability were slightly lower, but species richness was markedly increased. The greater dominance of *A. niger, F. solani* and some other species in unamended soil explained why there was greater diversity and equitability in the amended soils.

Effect of *L. camara* on endophytic colonization by fungi

The fungi isolated from the roots of mungbean were an *Acremonium* sp., *Macrophomina phaseolina*, *F. solani*, *Penicillium notatum* and *T. viride* (Table 4). Of these root-colonizing fungi, *M. phaseolina* was not found in any of the soils, and *T. viride* was found only in amended, not unamended soil. With these exceptions, fungi that colonized the roots also occurred in the soils, whether amended or unamended. *M. phaseolina* and *F. solani* were very frequent in roots from amended soils but still more frequent in roots from unamended soils. *Acremonium* sp. and *P. notatum*, on the other hand, were more common in roots from amended soils.

Effect of fungi isolated from soil and the roots of mungbean on egg hatching and juvenile mortality of *M. javanica*

Of the 14 fungi tested for nematicidal activity, only three (*A. niger, F. solani* and *T. viride*) caused more than 50% mortality of *M. javanica* juveniles. *T. viride* caused the greatest inhibition (65% more than controls) in egg-hatching, followed by *A. niger* with 59% (Table 5).

Table 4. Percent colonization of fungi isolated from the roots of mungbean growing in soils with different *Lantana camara* amendments (0, 0.5 and 1%).

Fungus	Colonization %		
	0	0.5	1.0
Acremonium sp.	1.56	4.68	3.90
Fusarium solani	30.46	21.09	24.90
Macrophomina phaseolina	42.96	30.46	21.09
Penicillium sp.	0.78	1.56	1.56
Trichoderma viride	0	3.12	4.68
$LSD_{0.05}$	11.1	7.8	5.2

Table 5. Juveniles mortality (% after 48 h) and inhibition of egg haching (No. after 120 h) in *Meloidogyne javanica* by fungal species isolated from soil amended with *Lantana camara*.

Fungus	Juveniles mortality %	Egg hatching No.
Czapek's dox broth	5	228
Acremonium sp.	37	175
Alternaria alternata	15	202
Aspergillus flavus	9	235
Aspergillus fumigatus	15	158
Aspergillus niger	60	93
Chaetomium globosum	18	168
Cladosporium sp.	4	213
Drechslera hawaiiensis	7	247
Fusarium oxysporum	23	136
Fusarium solani	51	112
Macrophomina phaseolina	21	193
Penicillium notatum	39	123
Penicillium sp.	18	184
Trichoderma viride	55	79
$LSD_{0.05}$	13.2	21.4

Discussion

Amendment of soil with *L. camara* caused soil suppressiveness to *M. javanica* and produced marked changes to the fungal communities in the soil and endorhiza. It is likely that those fungal species, especially the endophytes, that were reduced or specifically promoted by *L. camara* had a role in suppressing *M. javanica*. However, a role of toxic compounds produced by *L. camara* itself cannot be ruled out. *L. camara* is known to produce unknown compounds of a polar nature that caused juvenile mortality of *M. javanica* (Ali *et al*., 2001). In that same study, *L. camara* added to the soil caused a strong reduction in nematode population density and root-knot of mungbean (Ali *et al*., 2001). In an earlier study we found that phenolic compounds including caffeic acid, *p*-hydroxybenzoic acid and *p*-coumaric acid inhibited *M. javanica* in the soil (Shaukat and Siddiqui, 2001). These and other phenolic compounds of varying nature are also reported from *L. camara* (Narawal, 1994).

L. camara in the soil changed the frequency with which some species were isolated. Six fungi occurred only in *L. camara*-amended soil: an *Acremonium* sp., *Aspergillus fumigatus*, *D. australien-*

sis, *F. culmorum*, *P. notatum* and *T. viride*. Species in the genus *Acremonium*, *Aspergillus* and *Fusarium* are known to be antagonists of nematodes; the role of *Drechslera* and *Penicillium* to inhibit nematodes is only speculative. In this study, *M. phaseolina* was isolated from the roots of mungbean but was not found in any of the soils. Several reasons may explain this absence of the fungus. The wet sieving and dilution technique normally used to isolate sclerotia of *M. phaseolina* was not employed (Sheikh and Ghaffar, 1975). Instead of a selective medium that maximizes the chance of isolating *M. phaseolina*, only PDA or CDA were used. The fungus could also be lacking in the soil as a result of competition between soil organisms in the rhizosphere. Another possibility is that the fungus survived inside the mungbean seeds as a seedborne fungus. The release of phytoalexins from the plant roots may also have had a role in eliminating *M. phaseolina* from the rhizosphere.

With the sole exception of *M. phaseolina*, all endophytic species were also found in the soil, supporting the hypothesis that root-colonizing bacteria are mostly soil-organisms (Hallmann *et al*., 1997). However, not all soil micro-organisms became endophytes. Hallmann *et al*. (1998) reported that two dominant bacterial genera in the soil and rhizosphere, *Bacillus* and *Arthrobacter*, did not occur in the roots. The roots of plant are different from a soil environment and may not meet the requirements of non-colonizing bacteria. The plant itself also seems to be able to prevent the invasion of potential endophytic-colonizers under certain conditions.

Adult female nematodes require considerable amounts of nutrients for egg production, and compete with the host for the nutrients in the roots (Hussey, 1985). The increased metabolic activity of the giant cells on which the nematode feeds stimulates the mobilization of photosynthates from the shoots to the roots and in particular to the giant cells, where they are removed and fed on by the nematode (Bird and Loveys, 1975; McClure, 1977). The mobilization and accumulation of substances in the giant cells peaks when the adult females commence laying their eggs and declines thereafter (Meon *et al*., 1978).

M. phaseolina and *F. solani* were more common in roots from unamended soil than in roots from *L. camara*-amended soils. Since roots growing in unamended soil supported larger nematode populations causing severe wounds to the root, it is likely that these two fungi proliferated more extensively on nematode-damaged roots because there was greater leakage of root exudates from galled roots, or because photosynthates were excreted directly by the developing nematodes. However, pronounced root-rot was not evident in the present study. *M. phaseolina* and *F. solani* are well-known plant pathogens and their lower colonization in amended soils suggests that, apart from inhibiting *M. javanica*, *L. camara* also to some extent controlled these soilborne root-infecting fungi.

Of the fungi tested for nematicidal activity, *A. niger, F. solani* and *T. viride* caused heavy mortality of *M. javanica* juveniles and reduced egg hatching activity. It has been reported in earlier studies that all three fungi have great potential in suppressing *M. javanica* (Amer-Zareen *et al*., 2001; Siddiqui *et al*., 2001a, 2001b). Nematode suppression found in this study could be related to high nematode mortality and lesser egg hatching activity, which may have reduced the nematode population density in the soil and roots and thus lowered root-knot severity.

Although mungbean growth was greater in *L. camara*-amended soils than in unamended soil, at higher concentrations the reduction in plant growth was only slight. *L. camara* is a well-known allelopathic plant (Casado, 1995) containing a variety of phenolic acids (Narawal, 1994). Allelopathic plants at higher concentrations may well produce phytotoxic symptoms. It is therefore advisable that before *L. camara* is applied under field conditions to suppress plant-parasitic nematodes, optimal concentrations are determined which are toxic to the nematodes but not to the plants to be protected, nor to any associated beneficial micro-organisms, such as those possessing biocontrol and growth-promoting properties.

This study focused on changes in the fungal community structure; the effect of *L. camara* on other micro-organisms (especially bacteria) was not examined. Furthermore, observations were continued for 45 days after nematode inoculation, during which time only one or two generations of the nematode could display their effect. An organic amendment-mediated shift in the microbial community, with subsequent suppression of plant diseases in the second or third harvest, is not unusual. It may therefore be more appropriate to com-

pare the degree of nematode inhibition achieved by the addition of an organic amendment at later harvests with that achieved after nematicide treatment. Further study should be directed towards agro-systems in which crops are exposed to amendments for longer periods to confirm that *L. camara* effectively suppresses nematode over the longer term.

Acknowledgements

The present work was carried out under a research grant provided by the University of Karachi, which is sincerely acknowledged.

Literature cited

Ali N.I., I.A. Siddiqui, M.J. Zaki and S.S. Shaukat, 2001. Nematicidal potential of *Lantana camara* against *Meloidogyne javanica* in mungbean. *Nematologia Mediterranea* 29, 99–102.

Amer-Zareen, I.A. Siddiqui, F. Aleem, M.J. Zaki, and S.S. Shaukat, 2001. Observation on the nematicidal effects of *Fusarium solani* against *Meloidogyne javanica*, the root-knot nematode. *Journal of Plant Pathology* 83(3) (in press).

Bird A.F. and B.R. Loveys, 1975. The incorporation of photosynthates by *Meloidogyne javanica*. *Journal of Nematology* 7, 111–113.

Casado C., 1995. Allelopathic effects of *Lantana camara* (Verbenaceae) on morning glory (*Ipomea tricolor*). *Rhodora* 97, 264–274.

Culbreath A.K., R. Rodríguez-Kábana, and G. Morgan-Jones, 1985. The use of hemicellulosic waste matter for reduction of the phytotoxic effects of chitin and control of root-knot nematodes. *Nematropica* 15, 49–75.

Hallmann J., A. Quadt-Hallmann, W.F. Mahaffee and J.W. Kloepper, 1997. Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology* 43, 859–914.

Hallmann, J., A. Quadt-Hallmann, R. Rodríguez-Kábana, J.W. Kloepper, 1998. Interactions between *Meloidogyne incognita* and endophytic bacteria in cotton and cucumber. *Soil Biology and Biochemistry* 30, 925–937.

Hallmann J., R. Rodríguez-Kábana and J.W. Kloepper, 1999. Chitin-mediated changes in bacterial communities of the soil, rhizosphere and within roots of cotton in relation to nematode control. *Soil Biology and Biochemistry* 31, 551–560.

Hussey R.S., 1985. Host-parasite relationships and associated physiological changes. In: *An Advance treatise on Meloidogyne. Vol. I: Biology and Control*. (J.N. Sasser, C. Carter ed.). North Carolina State University, SC, USA, 143–153.

Kerry B.R., 1982. The decline of *Heterodera avenae* populations. *European Plant Protection Organization Bulletin* 12, 491–496.

Kluepfel D.A., T.M. McInnis E.I. Zehr, 1993. Involvement of root-colonizing bacteria in peach orchard soils suppressive of the nematode *Criconemella xenoplax*. *Phytopathology* 83, 1240–1245.

Kokalis-Burelle N., R. Rodríguez-Kábana, C.F. Weaver, P.S. King, 1994. Evaluation of powdered pine bark for control of *Meloidogyne arenaria* and *Heterodera glycines* on soybean. *Plant and Soil* 162, 163–168.

Kokalis-Burelle N., R. Rodríguez-Kábana, D.G. Robertson, W.F. Mahaffee, J.W. Kloepper and K.L. Bowen, 1995. Effects of forage grass rotations on soil microbial ecology and nematode populations. *Phytopathology* 85, 1124.

Magurran A.E. 1988. *Ecological Diversity and its Measurement*. Croom Helm, London, UK, 179.

Mankau R., and S. Das, 1969. The influence of chitin amendments on *Meloidogyne incognita*. *Journal of Nematology* 1, 15–16.

McClure M.A., 1981. Electron microscope histochemistry. In: *Plant parasitic nematodes. Vol. III*. (B.M. Zuckerman, R.M. Rohde ed.). Academic Press, New York, NY, USA, 89–124.

Meon S., J.M. Fisher and H.R. Wallace, 1978. Changes in free proline following infection of plants with either *Meloidogyne javanica* or *Agrobacterium tumefaciens*. *Physiological Plant Pathology* 12, 251–256.

Narwal S. 1994. *Allelopathy in Crop Production*. Department of Agronomy, CCS, Haryana Agricultural University, Hisar, India.

Pielou E.C., 1975. *Ecological Diversity*. Willey, New York, NY, USA,165.

Rodríguez-Kábana R. and G. Morgan-Jones, 1987. Biological control of nematodes: soil amendments and microbial antagonists. *Plant and Soil* 100, 237–247.

Rodríguez-Kábana R., G. Morgan-Jones, and B.O. Gintis, 1984. Effects of chitin amendments to soil on *Heterodera glycines,* microbial populations and colonization of cysts by fungi. *Nematropica* 14, l0–25.

Shannon C.E. and W. Weaver, 1963. *The Mathematical Theory of Communications*. University of Illinois Press, Urbana, IL, USA 367 pp.

Shaukat S.S. and I.A. Siddiqui, 2001. Effect of some phenolic compounds on survival, infectivity and population density of *Meloidogyne javanica* in mungbean. *Nematologia Mediterranea* 29(2), 23–26.

Sheikh A.H. and A. Ghaffar, 1975. Population study of sclerotia of *Macrophomina phaseolina* in cotton fields. *Pakistan Journal of Botany* 7, 13–17.

Siddiqui I.A., Amer-Zareen, M.J. Zaki, and S.S. Shaukat, 2001a. Use of *Trichoderma* species in the control of *Meloidogyne javanica*, the root-knot nematode in okra and mungbean. *Pakistan Journal of Biological Sciences* 4, 846–848.

Siddiqui I.A., N.I. Ali, M.J. Zaki, and S.S. Shaukat, 2001b. Evaluation of *Aspergillus* species for the biocontrol of *Meloidogyne javanica* in mungbean. *Nematologia Mediterranea* 29(2), 15–22.

Sokal R.R. and F.J. Rohlf, 1995. *Biometry.* 3d ed. Free-

man, San Francisco, CA, USA, 887 pp.
Spiegel Y., E. Cohn, and I. Chet, 1986. Use of chitin for controlling plant-parasitic nematodes. I. Direct effects on nematode reproduction and plant performance. *Plant and Soil* 95, 87–95.
Stirling G.R., M.V. McKenry and R. Mankau, 1979. Biological control of root-knot nematodes *(Meloidogyne* spp.) on peach. *Phytopathology* 69, 806–809.
Vargas R., R. Rodríguez-Kábana, and J.W. Kloepper, 1994. Study of microbial ecology in a crop rotation program with soybean, velvet-bean and winter crops. *Phytopathology* 84, 1084.
Whitehead A.G. and J.R. Hemming, 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55, 25–38.

Accepted for publication: October 3, 2001