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Nematicidal, Antifungal and Phytotoxic Responses of *Coryza canadensis*

S.S. Shaukat, I.A. Siddiqui and Nadia Munir
Soil Biology and Ecology Laboratory, Department of Botany, University of Karachi, Pakistan

Abstract: The study was carried out during June-September 2001 at the Department of Botany, University of Karachi. Present investigation concluded that aqueous extract of *Conyza canadensis* had no significant impact on egg hatch of *Meloidogyne javanica in vitro*. However, aqueous extract of *C. canadensis* caused considerable mortality of *M. javanica* juveniles at 24 h but not at 48 h. Ethanolic extract of powdered shoot of *C. canadensis* did not exert any inhibitory effect on radial growth of root-infecting fungi including *Macrophomina phaseolina, Fusarium solani* and *Rhizoctonia solani in vitro*. Soil amendment with powdered shoot of *C. canadensis* at 5% concentration significantly reduced galling due to *M. javanica* in mungbean roots grown in non-sterilized soil but not in sterilized soil. *C. canadensis* at 2.5% concentration did not affect root-knot development in either of the soil types tested. Soil amendment with 5% C. canadensis in non-sterilized soil markedly reduced plant height and fresh weight of mungbean shoots. Fresh root weights were markedly lowered, in both sterilized and non-sterilized soil, amended with 5% *C. canadensis*. *C. canadensis* suppresses root-knot nematode disease indirectly by enhancing soil microbial assemblages, particularly the microfungi antagonistic to root-knot nematodes.

Key words: Organic amendments, plant-parasitic nematodes, soil-borne fungi, diversity

INTRODUCTION

Soil-borne plant pathogens cause hundreds of millions, if not billions of dollars of economic losses to agricultural crops[1]. When cultivars with disease resistance are lacking, growers have only soil fumigants as guaranteed means for disease control. These products are highly effective, providing broad-spectrum disease control with rapid turnaround time from application to planting. Replacement technologies should posses these desirable traits. Fumigants, however, are expensive and their use can result in development of new disease problems requiring further fumigation. Replacement technologies can compete if they are less costly and if they provide long-term disease suppression.

Organic amendments, such as animal manures and composts, are commonly used in agricultural production for their fertility value prior to the availability of chemical fertilizers. It is likely that these amendments also provide other benefits such as improved plant health due to reduction of pathogens[1]. The efficacy of botanical toxicants against plant-parasitic nematodes and soil-borne fungi has been investigated under glasshouse conditions[2,3]. Of the various plants tested, *Tegetes* spp., produce α-terthienyl while *Crotalaria* spp. produce mono-crotaline, both these compounds possess nematicidal properties[4,5]. Likewise, Ali *et al.*[6] observed that methanolic extracts of *Lantana camara* induced significant mortality of *M. javanica juveniles in vitro*[6]. In another study, Shaukat and Siddiqui[2] found that *L. camara* also possesses antifungal agents, which inhibit radial growth of root-infecting fungi including *Macrophomina phaseolipa*, *Fusarium solani* and *Rhizoctonia solani in vitro*. Shaukat *et al.*[3] further demonstrated that aqueous extract of powdered shoot of *Argemone mexicana* contains a number of nematicidal compounds (phenolic acids including salicylic acid) which caused substantial mortality of *M. javanica juveniles in vitro*. The efficacy of the organic derivatives depends on their chemical composition and the type of microorganisms that develop during degradation[7]. Several antimicrobial compounds (e.g, organic acids, hydrogen sulfide, nitrogenous ammonia, phenols, tannins) are released during degradation of organic amendments, or synthesized by microorganism involved in such degradation[8].

The formal definition of allelopathy is any direct or indirect harmful or beneficial effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment[9]. Reduction in populations and infection of deleterious microorganisms following soil amendments

Corresponding Author: Dr. S. Shahid Shaukat, Soil Biology and Ecology Laboratory, Department of Botany, University of Karachi, Pakistan

with plant material is thought to be due to the release of toxic compounds, inhibitory to pathogens, in such environments. However whether suppressiveness occurs primarily due to the release of toxic coynpound(s) in the vicinity of the pathogen cannot be established until the active compound(s) from the rhizosphere and root is isolated and activity of the compound(s) proven. Alternatively, stimulation or suppression of soil microorganisms inhibitory to plant pathogens and enhancement of host defence mechanism are the factors that may hinder the build-up of populations of deleterious soil organisms.

The aims of the present investigation were: I) to determine the influence of aqueous extract of *Coryza canadensis* on egg hatch and mortality of *M. Javanica in vitro*, ii) to determine the effect of methanolic extract of *C. canadensis* on radial growth of root infecting fungi including *Macrophomina phaseolina, Fusarium solani* and *Rhizoctonia solani in vitro*, iii) to determine the influence of soil amendment with *C. canadensis* on root infection caused by root-knot nematode and root-infecting fungi and growth of mungbean [*Vigna radiata* (L.) Wilczek] and iv) to determine the influence of the organic amendment on the diversity of culturable soil fungi in the rhizosphere of mungbean.

MATERIALS AND METHODS

Plant material and preparation of shoot extract: The study was conducted during July-September 2001 at the Department of Botany, University of Karachi. *C. canadensis* was collected from Karachi University Campus. The plant was air dried under shade for 2 weeks and finally chopped to small pieces in an electric grinder (Electra mini chopper, Japan). The chopped shoot material (50 g) was soaked in 500 mL sterile distilled water and left for 72 h at room temperature. The resulting aqueous extract was passed through two layers of Whatman No. 1 filter paper and kept in a refrigerator at 6°C prior to use. Appropriate quantities of the antibiotic were added to the extract to avoid microbial contamination.

Nematode and fungal inoculum: *Meloidogyne javanica* (Treub) Chitw. was obtained from pure cultures maintained on roots of eggplants (*Solanum melongena* L.). The heavily infested root pieces with large number of cream coloured egg masses were kept in 50 mL sterile distilled water for 4 to 5 days. The hatched juveniles were stored in 100 mL capacity beaker. Eggs were extracted by vigorous shaking of infested roots in a 1% sodium hypochlorite solution for three minutes. The resulting suspension was then passed through a range of different mesh sieves. The eggs collected on a fine sieve (38 μm) were washed in tap water to remove all traces of sodium hypochlorite before use. *Macrophomina phaseolina* was isolated from infected bean (*Phaseolus vulgaris* L.) roots grown at Ghulamullah Goth, 62 km east of Karachi while Rhizoctonia solani and Fusarium solani, respectively, were isolated from infected tomato and brinjal roots obtained from Mirpur Sakro, 69 km east of Karachi. The fangi were purified on Potato Dextrose Agar (PDA) plates supplemented with penicillin and streptomycin sulphate.

Effect of shoot extract on egg hatch of *M. javanica*: Two medium sized egg masses with 2 mL of the aqueous extract of *C. canadensis* were transferred into a 3 cm diam cavity glass slide. The egg masses placed in sterile distilled water served as controls. Each treatment was replicated four times and the cavity glass slides were randomized. The hatched juveniles were counted after 48 h. Subsequently, the egg masses were transferred into cavity glass slides containing 2 mL sterile distilled water to ascertain whether the egg masses kept in the culture filtrate had been temporarily or permanently inactivated. The juveniles were counted again after a further 48 h period.

Effect of shoot extract on mortality of *M. Javanica juveniles*: Two mililiter of each filtrate were poured in a glass cavity slide and about 45±6 second-stage juveniles (J_2) of *M. javanica* placed in each glass cavity slide. Juveniles kept in sterile distilled water served as controls. Treatments and controls were replicated three times and dead nematodes in each cavity slide were counted after 24 and 48 h. The nematodes were considered to be dead when they did not move on probing with a fine needle.

Preparation of ethanolic extract and its antifungal activity: Fifty gram fresh leaves of *C. canadensis* were soaked in 100 mL ethanol and disintegrated in a homogenizer. After 2 weeks, ethanoilic extract was filtered through 2 layers of Whatman No. I filter paper. The extract was dried in a rotary vacuum evaporator (EYELA) under reduced pressure at 30°C. The resulting gummy substance was weighed an dissolved in ethanol. To determine the antifungal activity, the extract (10 mg mL^{-1}) was impregnated on a 5 mm diam disc of Whatman No. 1 filter paper at 10 μL $disc^{-1}$ and placed 5 ram inside of the 9 cm diam petri plates containing Czapek Dox agar medium, pH 7.2. Disc inoculated with ethanol served as control,, was placed apart from the disc containing ethanolic extract of *C. canadensis*. A 5 mm diam disc of the test fungus was placed at the center of the petri plate.

There were four replicates for each test fungus and plates were incubated at room temperature (30°C). Zone of inhibition (if any) was measured after a one week incubation period.

Nematicidal, antifungal and allelopathic responses of *Conyza canadensis* in mungbean: The sandy loam soil (pH 7.8 and moisture holding capacity 38%) was obtained from Crop Disease Research Institute, Karachi University campus. The experiment was set up as a randomized complete block design with four replications. The soil was thoroughly mixed with *C. canadensis* at 3% w/w and filled in 8 cm diam plastic pots at 400 g pot^{-1}. The pots with soil were placed in a glasshouse (20-29°C) and sprinkled daily with 40 mL sterile distilled water. Three week after amendment, eight surface sterilized mungbean seeds were sown in each pot and after germination only four seedlings were kept pot^{-1}. One week after germination, 2000 freshly hatched juveniles of *M. javanica* were introduced in the soil by making four holes around the seedlings in the pots. The plants were fertilized at alternate weeks with 0.8 g kg^{-1} of urea. The experiment was terminated 45 days after nematode inoculation and plant height, fresh weight of shoot and root, number of galls induced by *M. Javanica* and nematode populations in the soil were estimated. For nematode counts, 100 cm^3 soil aliquots were incubated for 72 h using a modified Baerman funnel technique[10]. An identical experiment was performed with the exception that steam-sterilized soil was used. The soil was tested for the presence or absence of the microbial population before filling the pots; soil with no microbial population was used for the experiment.

Isolation and identification of fungi from rhizosphere: At harvest, one mungbean plant was randomly chosen from each replicate pot to study the rhizosphere culturable fungi. The roots were excised and weighed after the excess soil had been shaken-off The roots were then shaken vigorously in a test tube containing sterile distilled water, blotted dry and reweighed. A serial dilution of the soil suspensions was prepared and was tested for the enumeration of fungi. A 0.5-mL aliquot from 10^2 and 10^3 dilutions was plated on Czapek's Dox Agar (CDA) medium, supplemented with penicillin (100,000 units L^{-1}) and streptomycin sulphate (0.2 g L^{-1}) to avoid bacterial contamination. After incubation at 28°C, the plates were examined for total fungal counts. Most isolates were obtained after a few days of incubation, but plates-were checked over several weeks to allow isolation of slow-growing fungi. Developing fungal colonies were sub-cultured into pure isolates and identified by their microscopic morphology using mycological literature[11-13].

Isolation of fungi from roots: The roots of all plants (including the one which was tested for the isolation of rhizosphere fungi) were cut into small segments (5-mm) and after surface sterilization in 1% $Ca(OCl)_2$ for 3 min, 5 such segments were plated onto Potato Dextrose Agar (PDA) plates supplemented with penicillin (100,000 units L^{-1}) and streptomycin sulphate (0.2 g L^{-1}). The plates were incubated at 28°C for one week and emerging fungi from each root segment were identified. Colonization percentage was determined by using the following formula:

Colonization (%) = (No. of root pieces colonized by a fungus/total No. of root pieces) x 100

Statistical analyses: Data were subjected to one-way analysis of variance (ANOVA) followed by the Least Significant Differences test (LSD) or Duncan's multiple range test using STATISTICA software (ver. 5.0, StatSoft Inc., Tulsa, Oklahoma, U.S.A.). Fungal rhizosphere populations were transformed to logio (x+l) before the analyses.

Diversity measurement: Species diversity is an important parameter of natural or organized community and several diversity indices have been proposed[14]. Diversity indices represent a useful means to quantify community diversity and have been instrumental in revealing the impact of biocontrol inoculants on resident population assemblages[15]. Several diversity indices were employed to compare treatment effects. Various diversity measures estimate different aspect of community structure. The general species diversity of the fungal communities was measured by the generally accepted Shannon-Wiener information theorv function:

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

where, H' is the general species diversity and pi the proportion of total number of cfu for fungi or counts for nematodes, N belonging to the ith species[16]. The variance of general diversity var (H') was calculated in accordance with Magurran[14], as follows:

$$\text{Var } (H') = \Sigma P_i (\log P_i)^2 - (\Sigma P_i \log P_i)^2/N + 2 (S-I)/2N^2$$

Dominance concentration (complement of diversity) was measured by using Simpson's index[17] as: $D = \Sigma\{[n_i(n_i-1]/[N(N-1]\}$ in which n_i = number of CFU for fungi or counts for nematodes. 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 that measures the evenness of allotment of individuals among the species[14]. The equitability component of diversity and its variance were measured in accordance with Pielou[18]: $J' = H'/H'_{max}$. The equitability index Y is the ratio between observed diversity (H') and maximal diversity (H'_{max}). Variance of equitability was estimated as: $Var(J') = Var(H')/(\log S)^2$. Species richness was calculated in accordance with Menhinick[19] as $d = S/\sqrt{N}$, where, S equals the number of species and N the total number of individuals (colony counts).

RESULTS

Effects of *C. canadensis* on egg hatch and mortality of *C. canadensis* juveniles and radial growth of root-infecting fungi *in vitro*: When compared with the control, egg hatch activity of *M. javanica* did not affect markedly when exposed to aqueous extract of *C. canadensis* (Table 1). However, when compared to the controls, egg hatch activity was significantly ($p<0.05$) reduced when egg masses were transferred from *C. canadensis* extract to sterile distilled water. Aqueous extract of *C. canadensis* caused significantly ($p<0.05$) mortality of *M. javanica* at 24 h exposure period, ompared to the controls (Table 2). However, at 48 h no significant difference on mortality of *C. canadensis* juveniles was detected over the controls. Ethanolic extract of *C. Canadensis* had no inhibitory effect on radial growth of any of the root-infecting fungi; colonies of fungi grew over disc impregnated with *C. canadensis* extract.

Effect of *C. canadensis* on the development of root-knot infection and the growth of mungbean: When compared with the controls, soil amendment with *C. canadensis* at 5% significantly ($p<0.05$) reduced galling on mungbean roots grown in non-sterilized soil (Table 3). However, in steam-sterilized soil, *C. canadensis* at any concentration did not reduce root-knot infection. When two soil types (sterilized and non-sterilized) were compared, root-knot infection was significantly ($p<0.05$) higher in sterilized soil amended with 5% *C. candensis*. Soil amendment with 5% *C. canadensis* in non-sterilized soil reduced plant height, compared to the controls while shoot material of *C. canadensis* had no, significant m pact on mungbean plant height in steam-sterilized soil. In both sterilized and non-sterilized soils, amendment with 5% *C. canadensis* significantly ($p<0.05$) reduced fresh weight of shoots. However, with respect to fresh shoot weight of mungbean plants, steam sterilized and non-sterilized soils did not differ markedly. In both sterilized and non-sterilized soils, *C. canadensis* at 5% concentration markedly ($p<0.05$) reduced fresh weight of root while in steam- sterilized soil, *C. canadensis* at 2.5% concentration enhanced root weights.

Table 1: Effects of *C. canadensis* on egg hatching of *Meloidogyne javanica*

	Number of eggs hatched		
Treatment	Extract	Distilled water[a]	Total No. of egg hatched
Control	221	73	273
C. canadensis	208	52	281
$LSD_{0.05}$	36	19	-

[a]After a 48-h hatching period in culture filtrate, the egg masses were transferred to sterile distilled water

Table 2: Effects of aqueous extract of *Conyza canadensis* shoot extract on mortality of *Meloidogyne javanica*

	Mortality %	
	Exposure time (h)	
Treatment	24	48
Control	9	14
C. canadensis extract	21	24
$LSD_{0.05}$	9	11

Effects of *C. canadensis* on culturable fungi in the mungbean rhizosphere: Regardless of amendments, a total of 15 fungal species belonging to 10 genera were isolated from the rhizosphere of mungbean (Table 4). When various treatments were compared, total fungal species and their colony counts were significantly ($p<0.01$) higher in soil amended with *C. canadensis* especially at higher concentration (i.e., 5%). Some fungal populations were either specifically enhanced or restrained following soil amendment with *C. canadensis*. For instance, *Curvularia lunata*, *Penicillium brefeldianum* and *P. notatum* were totally absent from

Table 3: Effects of *C. canadensis* on root-knot development due to *Meloidogyne javanica* and growth of mungbean plants in sterilized and non-sterilized soils

	Galls/g root		Plant height (cm)		Shoot weight (g)		Root weight (g)	
Treatment	NSS	SS	NSS	SS	NSS	SS	NSS	SS
Control	70	78	23.7	22.2	2.6	2.9	1.3	1.5
C. canadensis (2.5%)	61	69	24.1	23.7	2.2	3.1	1.4	1.9
C. canadensis	49	71	18.5	19.1	1.5	1.6	0.8	1.0
$LSD_{0.05}$ Treatment	12		4.1		1.1		0.4	
Soil type	10		2.9		1.7		0.3	

SS = Sterilized Soil; NSS = Non-sterilized Soil

Table 4: Effect of soil amendment with or without shoot powder of *C. canadensis* on soil fungal community structure expressed. as log, 0 (x+1) in mungbean

	[(log cfu g^{-1} dry soil)+I]		
Fungus	Control	*C. canadensis* (2.5%)	*C. canadensis* (5%)
Alternaria alternata	0.90	0.75	0.82
Aspergillus flavus	0.75	0.75	1.57
A. niger	0.90	1.50	2.25
A. quadrifinatus	0.00	0.00	0.82
Cladospotium herbarum	1.50	1.61	1.57
Curvularia lunata	0.00	0.75	0.75
Fusarium culmorum	0.00	0.00	0.90
F. kysponim	0.75	0.82	0.75
E. solani	0.90	1.61	0.75
Macrophomina phaseolna	0.86	0.75	1.50
Mycelia sterilia	0.75	1.57	2.25
Penicillum brefeldianum	0.00	0.82	0.75
P. notatum	0.00	0.75	0.90
Rhizoctonia solani	1.57	1.61	1.57
Rhizopus stolonifer	2.32	2.25	3.15
Total species	10.00	13.00	15.00
Total genera	8.00	10.00	10.00

Table 5: General diversity H', equitability (J'), species richness (d) and dominance (D) of the fungal communities affected by soil amendment with or without *Conyza canadensis* in mungbean rhizosphere. Var (H')= variance of H'; Var (J') = variance of J'.

Diversity	[Log cfu g^{-1} fresh root wt.)+ I]		
indices	Control	*C. canadensis* (2.5%)	*C. canadensis* (5%)
H,	1.325	1.8920	1.1130
Var (IT)	0.005	0.0030	0.0010
J'	0.575	0.7370	0.4110
Var (*J'*)	0.001	0.0004	0.0001
d	0.554	0.6420	0.3380
D	0.435	0.2310	20.0000

Table 6: Percent colonization of the fungi isolated from the mungbean roots growing in soil amended with *C. canadensis*

	Colonization (%)		
Fungus	Control	*C. canadensis* (2.5%)	*C. canadensis* (5%)
Alternaria alternata	0	5	3
Fusarium saloni	48	55	33
Marcrophomina phaseolina	23	37	25
Penicillium notatum	7	18	24
Rhizoctonia solani	13	25	27
$LSD_{0.05}$		16	
C. canadensis concentration		10	

unmended soil. On the other hand, all the fungal species, which were isolated from the rhizosphere of unamended soils were also isolated from *C. canadensis* amended soils but population level of *Alternaria alternata* was higher in unamended soils. Noticeably, colony counts for other species were markedly higher in amended soils. A few fungal species including *Aspergillus flavus*, *A. niger, Fusarium solani* and *Alyrothecium* sp. were isolated in negligible colony counts from steam sterilized soils (data not presented).

Effects of *C. canadensis* on the diversity of rhizosphere fungi: Highest general diversity (H') of culturable soil fungi was recorded in 2.5% *C. canadensis* amendment (Table 5). However, doubling of the application rate reduced the general diversity compared to controls. Likewise, equitability was also highest in 2.5% *C. canadensis* amendment and lowest in 5% dosage. Species richness exhibited the same trend. On the other hand, Simpson's index of dominance concentration showed an opposite trend to diversity and the maximum value was recorded for 5% *C. canadensis* amendment.

Effects of *C. canadensis* on root colonization by fungi: Regardless of treatments, a total of five fungal species were isolated from the inner tissue of mungbean roots (Table 6). The percentage colonization of roots by *Penicillium notatum* and *Rhizoctonia solani* significantly ($p<0.05$) increased following soil amendment with *C. canadensis*, over the untreated controls, at both the concentrations. Root colonization by *Fusarium solani* was reduced at 5% *C. canadensis* while that of *M. phaselina* markedly increased at 2.5% *C. canadensis*. Interestingly, *Alternaria alternata* was exclusively isolated form the roots growing in the amended soils.

DISCUSSION

Present results indicate that aqueous extract of powdered shoot of *C. canadensis* inhibited egg hatch and caused appreciable mortality of *C. canadensis* juveniles *C. canadensis*. These results are in large part due to the fact that *C. canadensis* possesses compounds toxic to root-knot nematode. However, the nematicidal activity of the extract remained effective only during early incubation period (24 h) most likely due to fact that nematode might have overcome extract toxicity at a later incubation period (48 h). In the present study, whereas *C. canadensis* extract exhibited nematicidal activity to some extent, it did not inhibit radial growth of root-infecting fungi including *M. phaseolina*, *F. solani* and *R. Solani in vitro*. It is possible that either the compounds were not toxic to fungi and if present, might not have been soluble in ethanol. In our previous investigation[2], methanol extract of the leaf material of Argemone mexicana, a tropical weed, caused greater mortality of *M. javanica* juveniles than did ethyl acetate or hexane extracts. We suggested that active nematicidal compounds from *A. mexicana* were soluble in ethyl acetate and hence polar in nature.

In the current study, soil amendment with *C. canadensis* shoot material at 5% markedly suppressed nematode galling in mungbean roots. On the other hand, organic amendment below 5% failed to reduce nematode infection. Soil sterilization also plays a vital role in the disease suppression following organic amendments in the

soil. For example, *C. canadensis* inhibited root-knot nematode galling efficiently in non sterilized soils compared to the steam-sterilized soils. These results clearly suggest that *C. canadensis* inhibits root-knot infection indirectly by enhancing antagonistic microflora in the amended soils. This notion was proved from the isolation of culturable mycobiota from *C. canadensis* amended soils. Soil amendment with *C. canadensis* harboured larger number of Fungal species with high colony forming units. Although the application of organic amendments in the field for control of nematode pests is generally for large-scale vegetable production, it is feasible to use these amendments in transplant mixes. Organic amendments that have demonstrated efficacy in reducing damage caused by root-knot and other nematodes include chitin, pine bark and hemicellulose[7,20-23]. Hemicellulosic waste, a product of the paper pulp industry generated by alkaline and bisulfate wood treatments that release cellulose, has been investigated as a soil amendment for nematode control[24]. Decomposing tissues of *A. mexicana* in soil at 30 or 50 g kg^{-1} of soil significantly reduced population densities of *M. javanica* in soil and root and inhibited root-knot development in tomato[2]. Nematicidal activity of organic amendments in soil can be attributed to chemical mineralization with the ultimate release of ammonia, increased nitrogen and carbon dioxide levels, lowered oxygen concentration, release of toxic compounds from plant tissues, or growth of fungi and bacteria antagonistic to nematodes. Shaukat and Siddiqui[25] demonstrated the nematicidal potential of a number of phenolic compounds.

In the present study, soil treatment with *C. canadensis* increased general diversity and equitability of the culturable soil fungi compared to the untreated controls. In general populations of soil fungi was higher in amended soils. Furthermore, a few fungal species were either enhanced or suppressed following *C. canadensis* applications in the soil. For example, *Curvularia lunata*, *Penicillium brefeldianum* and *P. notatum* were isolated exclusively from *C. canadensis* amended soils. On the other hand, population levels of *Alternaria alternata* were lowered following soil amendment. The exploitation of antagonistic activities by microorganisms decomposing organic amendments may be one of the important practical biocontrol tools that could be developed to manage plantparasitic nematodes[26]. The addition of organic matter to soil stimulates microbial populations of bacteria and fungi, some of which might be antagonistic to nematodes[27]. For instance, Shaukat and Siddiqui[2] observed that soil amendment with powdered shoot of *Lantana camara* caused soil suppressivness to *M. javanica* which was not related with the release of nematicidal compounds but the addition of plant material to soil altered the fungal community structure and composition that could indirectly influence the nematode population. Shaukat *et al.*[28] demonstrated that fungal species diversity and equitability declined with the passage of time following soil amendment with *Avicennia marina*, but species richness slightly increased in the amended soils. Chavarria-Carvajal *et al.*[29] studying the effects of combinations of organic amendments and an aromatic compound benzaldehyde for the suppression of plant parasitic nematodes found that most amendments reduced damage from nematodes. Also, it was observed that amendments exerted a selective action on the activity and composition of microbial populations in the soybean rhizosphere. Caffeic acid, a phenolic compound, when used in conjunction with aromatic aldehyde (benzaldehyde) exhibited considerable activity against *M. javanica* populations, also the combination of two compounds markedly reduced population density of *Pseudomonas aeruginosa*, a plant disease suppressive bacterium[30] However, manipulation of desirable microbial community, a phenomenon referred to as induced suppressiveness, for the control of plant-parasitic nematodes in response to *C. canadensis* may have practical limitations. One limitation in this regard is that the development of antagonistic microbial community requires multiple years of crop monoculture[31,32]. Stirling[33] stated that with efforts to induce nematode-suppressive soils, the long cropping cycle allowed for considerable nematode damage before suppressiveness developed. In addition, Stirling[33] indicated that all documented examples of effective natural suppression of plant parasitic nematodes appear to be due mainly to the action of one or two specific biological control, agents and that these are highly host specific.

Phytotoxicity levels for the amendments tested are known for a number of crops and this information provides a framework for initial studies with amendments for tomato[20,22,24]. In the present study, soil amendment with 5% *C. canadensis* substantially reduced plant height and fresh weight of shoots of mungbean while 2.5% *C. canadensis* did not alter plant growth. Rates of amendments are also important, as levels above 1% with chitin[21,34] and 5% with pine bark[22] are phytotoxic to certain crops including summer squash and soybean, respectively. Similarly, soil amendment with 50 g kg^{-1} of soil of *A. mexicana* was highly deleterious causing 80% mortality of tomato plants[2]. Aqueous extract of *C. canadensis* inhibited the germination, root and shoot growth of six test plants including tomato, radish, wheat, corn, millet and mungbean. In the same study, decaying shoot of *C. canadensis* in sandy-loam soil at 5, 10 or 20

g/400 soil, substantially inhibited germination and seedling growth of bulrush millet[9]. Chromatography of the shoot extract of *C. canadensis* disclosed the presence of four phenolic compounds including gallic acid, vanillic acid, catechol and syringic acid[9]. Our previous and current investigations clearly suggest that *C. canadensis* contains a variety of phenolic allelochernicals that may interfere with the normal growth of crop plants as well as their associated microorganisms.

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