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INVESTIGATIONS ON SOME ASPECTS OF CHEMICAL ECOLOGY OF COGONGRASS, *Imperata cylindrica* (L.) BEAUV.

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Abstract—To understand the interference mechanism of the weed, cogongrass, *Imperata cylindrica* (L.) Beauv., its effect on nutrient availability and mycoflora of its soil rhizosphere as well as nodule characteristics, root length, and root/shoot ratio of *Melilotus parviflora* Desf. were investigated. Additionally, the effect of the leachates of leaves and root/rhizome of cogongrass on seed germination and seedling characteristics of radish, mustard, fenugreek, and tomato were examined. Furthermore, to assess the qualitative and quantitative differences in phytochemical components, the leachates and the soils from three sampling sites (with cogongrass and 1.5 m and 3 m away from cogongrass) were analyzed with high-performance liquid chromatography (HPLC) on a C18 column. No significant difference in nutrient availability was found, but qualitative and quantitative differences in phenolic fractions were recorded in the three sampling sites. Furthermore, of the 19 fungi recorded in the soils, decreases in the number of colonies (per gram of soil) of *Aspergillus fumigatus, A. niger, A. candidus*, and an increase of *A. flavus* was recorded in the soils with cogongrass. The inhibition in nodule number, weight, nitrogen fixation (acetylene reduction activity), root length, and root/shoot ratio of *Melilotus parviflora* were noted. Percent seed germination, root and shoot length, fresh and dry weight of seedlings of different seeds were affected by the leachates of leaves and root/rhizome. It was found that root/rhizome leachate was more inhibitory than leaf leachate. However, the inhibition was higher in soil + leaves leachate than soil + root/rhizome leachate. HPLC analysis established that four compounds were contributed by the weed to the soil system even though their relative concentration varies in various leachates. It is surmised that these compounds cause allelopathic inhibition of growth characteristics of seeds tested. Significance of the data vis-à-vis the interference potential of the cogongrass is discussed.

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Key Words—Allelopathy, cogongrass, competition, *Imperata cylindrica*, HPLC, interference, weed.

INTRODUCTION

Cogongrass, *Imperata cylindrica* (L.) Beauv. is among the 10 worst weeds of the warmer regions of the world (Holm, 1969). However, how this weed achieves success has not been thoroughly explained. Abdul-Wahab and Al-Naib (1972) identified scopoletin, scopolin, chlorogenic acid, and isochlorogenic acid in water extract of leaves and culm of this weed. These phenolic acids have been reported to effect growth and physiological characteristics of plant species (Rice, 1984; Einhellig and Kuan, 1971; Lodhi and Rice, 1971), but whether these compounds were present in the rhizosphere of this weed, thus giving allelopathic success to the weed, has not been established. In view of such a gap in our understanding, the present investigations on this weed have been undertaken.

METHODS AND MATERIALS

These investigations were conducted at the Botanical Garden, University of Delhi, where cogongrass is distributed in various densities. Data to study the effect of the weed on nutrient availability, soil mycoflora, nodule forming characteristics of *Melilotus parviflora* Desf., seed germination, seedling characteristics, and phenolic fractions of soils and test solutions were collected. The methods for each of these investigations are described below.

Analysis of Phenolic Fractions. Different test solutions [leaf leachate (TS1), soil + leaves leachate (TS2), root/rhizome leachate (TS3), soil + root/rhizome leachate (TS4), garden soil leachate, as well as soils from the three different sampling sites (with cogongrass and 1.5 and 3 m away from cogongrass)] were analyzed for phenolic fractions by high-performance liquid chromatography (HPLC). Soils (5 g) and plant materials (0.1 g) were shaken with 10 ml of methanol for 1 hr at room temperature. The ratio of extractant to soil was 2 : 1 (v/w) and that for plant material was 100 : 1 (v/w). The extracts were carefully filtered and subjected to HPLC (Shimadzu LC-4A) using a variable wavelength UV detector set at 275 nm. Reversed-phase chromatography was carried out using a steel column (15 cm × 4.6 mm ID) containing Zorbax C18 with the flow rate of 1 ml/min. The volume injected each time was 10 μl and 30 μl for leaf and soil leachates, respectively. The phenolic fractions, differentiated on the basis of their respective retention times and relative concentrations, were recorded (Table 1 below).

Effect of Weed on Root Length, Root/Shoot Ratio and Nodule-Forming

Characteristics of Melilotus parviflora. *Melilotus parviflora* Desf. growing wild with (considered as treated in the investigation) and without (considered as control) cogongrass was selected to study the effect of this weed on its root length, root/shoot ratio, and nodule characteristics. After careful uprooting of the plants, number and weight of nodules, root length, and root/shoot ratio were recorded (Table 2 below). Nodules of each of the sites were carefully washed with double distilled water (DDW), and then the N_2 fixation rate following the acetylene reduction activity was determined. Acetylene was injected into vials containing weighed nodules so as to give a partial pressure of 0.1 atmosphere. Samples of gas phase were drawn after 45 min and their acetylene and ethylene concentrations were assessed by gas chromatography (GC). Acetylene reduction was calculated as per gram fresh weight of the nodules. All trials were made in triplicate.

Effect of Weed on Soil Mycoflora. For analysis of the effect of the weed on mycofloral components, 12 localities, with (considered as treated) and without (considered as control) cogongrass were selected. Soils for fungal analyses were collected by carefully inserting vertically a sterilized glass tube. These tubes were immediately plugged to avoid any contamination and then were stored at 0–4°C. The soil plating technique was employed to isolate fungal colonies. For preparation of the soil suspension, 1 g of soil was shaken with 100 ml of DDW. Three replicates were made by pouring 1 ml of the aliquot from each of the soil suspensions into Petri plates containing 20 ml of cooled and melted Czapek's Dox yeast extract agar medium (Dickinson, 1971) supplemented with streptomycin. Culture plates were thus made and incubated in the culture room at 25 ± 3°C. Total population of fungal colonies was calculated using the following formula:

$$\text{Total number of fungal colonies} = \frac{\text{Mean No. of fungal colonies} \times \text{dilution factor}}{\text{weight of dry soil}}$$

Effect of Weed on Nutrient Availability. To study the effect of this weed on nutrient availability, three contiguous regions, one with the weed present, and the others 1.5 m and 3 m away from it were selected. Soil samples from the rhizosphere (to a depth of 15 cm) of these three sampling sites were collected, air dried, sieved, stored in paper bags and analyzed following standard procedures (Allen, 1989) for pH, electrical conductivity, organic matter, Cu^{2+}, Zn^{2+}, K^{+}, Na^{+}, Mg^{2+}, Ca^{2+}, Cl^{-}, and PO_4^{-3}.

Effect of Weed on Seed Germination and Growth. For this, the effect of different test solutions (TS1, TS2, TS3, and TS4) on seed germination and seedling characteristics of radish (*Raphanus sativus* var. pusa desi), mustard (*Brassica juncea* CV PR 45), fenugreek (*Trigonella foenum-graecum* var. pusa early bunch), and tomato (*Lycopersicon esculentum* var. pusa ruby), the commonly cultivated crops of the region, were investigated. These leachates were

prepared by taking 15 g of each of the plants' parts; leaf and root/rhizome, either alone (for TS1 and TS3) or mixed with garden soil (for TS2 and TS4), were soaked, the former in 100 ml of DDW and the latter in 1:5 soil–DDW for 72 hr. Each of these was filtered and made to 100 ml with DDW. These leachates were used in the experiments without any further dilution. For germination studies, 50 seeds of the respective crop plants were sown on filter paper moistened with DDW (control for plant leachate), garden soil leachate (control for soil leachate), or test solutions, each in equal volume and placed in 15-cm-diameter Petri plates. To maintain uniform moisture status in Petri plates, a cotton pad soaked in DDW or any of the test solutions was placed below the filter paper. Observations on root and shoot length were made every 24 hr up to seven days. Each treatment was replicated thrice. During the period of experimentation, the temperature regime of 22 $\pm$ 5°C and the diurnal regime of light conditions were maintained. After seven days, fresh and dry weights (after keeping at 60°C for 24 hr) of seedlings were recorded (see Table 5 below).

RESULTS

Phenolic Fraction Analysis through HPLC. The presence of this weed brought about distinct qualitative and quantitative changes in the phenolic fractions of the soil samples of different sampling sites (1–3) and the test solutions (TS1, TS2, TS3, and TS4) analyzed (Table 1). In all, 18 phenolic fractions were detected from eight extracts, of which fractions 6 and 14 were common to all. Fraction 8 was present in all the extracts except TS1, and fraction 3 was present in all except sampling site 3 and TS4. However, since these fractions also were detected in control soil, it could be surmised that these must be present in traces but did not show up on the HPLC. On the contrary, fraction 9, although absent in control soil and TS4, was present in rest of the extracts. Furthermore, the relative concentrations of fractions 3, 8, and 9 decreased with the increase in distance from the immediate vicinity (rhizosphere zone) of the weed. The relative concentrations of fractions 3, 5, and 7 were higher in plant extracts (TS1 and TS3) than in soil extracts (TS2 and TS4), while the reverse was true of the quantitative distribution of fraction 8. However, the phenolic fractions 4, 12, and 17 present in the soil with the weed were found to be absent in the test solutions (except TS1 with fraction 4). Phenolic fractions 1, 2, 11, and 13 could be detected only in the HPLC profiles of the soil extracts. It should be noted that even the concentrations of fractions that were common to all the extracts (fractions 6 and 14) exhibited a relative increase wherever the influence of the weed plant part was present.

Nodule Experiments. In the present investigation nodule characteristics, root length, and root/shoot ratio of *Melilotus parviflora* were significantly

TABLE 1. HPLC ANALYSIS OF PHENOLIC FRACTIONS OF SOILS AND TEST SOLUTIONS[a]

Phenolic fraction		Relative concentration (percent)							
		Soil				Test Solution			
No.	Retention time (min)	CS	SS1	SS2	SS3	TS1	TS2	TS3	TS4
1.	0.308	0.208							
2.	1.408	9.35							
3.	1.558	15.12	18.00	7.04		11.51	3.62	19.02	
4.	1.60			6.84	8.90	3.62			
5.	1.79					34.40	4.78	38.40	7.94
6.	2.10	25.57	26.80	38.60	29.20	21.30	19.07	16.75	63.32
7.	2.30					6.92	1.49	4.64	
8.	2.46	36.85	27.20	20.21	18.37		2.73	2.12	11.70
9.	2.80		17.00	15.40	14.13	5.27	7.20	3.23	
10.	3.07					4.74	3.41		4.04
11.	3.27	4.05							
12.	3.60		3.90	2.42	3.68				
13.	3.73	2.62							
14.	4.05	1.95	6.13	7.34	11.07	1.04	2.38	10.09	1.14
15.	4.25					10.70			
16.	4.35	2.63					49.80		2.84
17.	5.10			1.49	3.40				
18.	5.70				2.90				

[a] CS, control soil; SS1, sampling site 1; SS2, Sampling site 2; SS3, Sampling site 3; TS1, Leaf leachate; TS2, Soil + leaves leachate; TS3, Root/rhizome leachate; TS4, Soil root/rhizome leachate.

TABLE 2. ROOT LENGTH, ROOT/SHOOT RATIO, AND NODULE CHARACTERISTICS OF *Melilotus parviflora* GROWING WITH (TREATED) AND WITHOUT (CONTROL) COGONGRASS

Characteristics	Control	Treated
Nodule number	58.50 ± 19.01	26.00 ± 7.88**[a]
Nodule weight (g)	0.0073 ± 0.0026	0.0041 ± 0.0017*
N_2 fixation (μmol C_2H_4/g fresh wt)	10.9588 ± 3.4078	2.1814 ± 0.8494***
Root length (cm)	10.540 ± 1.390	7.460 ± 2.48*
Root/Shoot ratio	0.367 ± 0.101	0.228 ± 0.055**

[a] *$0.05 \leq P < 0.1$, **$0.01 \leq P < 0.05$, ***$0.001 < P < 0.01$.

affected by the weed (Table 2). Nodule weight, root length ($0.05 \leq P < 0.1$), nodule number, root/shoot ratio ($0.01 \leq P < 0.05$) and nitrogen fixation ($P < 0.1$) were reduced in plants occurring with the cogongrass.

Soil Mycoflora Studies. The fungal components of soil were found to be affected by this weed. Significant differences in the number of colonies per gram of soil of four species of *Aspergillus* were recorded in soils associated with the weed (Table 3). Thus, in comparison to control, reductions in the number of colonies were as follows: 50.13% in *Aspergillus fumigatus* ($P < 0.25$), 36% in *A. niger* ($P < 0.25$), 49.35% in *A. candidus* ($0.05 < P < 0.07$); whereas augmentation (65.27%) was recorded in *A. flavus* ($0.05 < P < 0.07$).

Soil Nutrient Studies. Data from Table 4 suggest that no significant changes were brought about by the weed in the concentration of nutrients in the soil samples analyzed.

Seed Germination and Growth Experiments. All the four leachates affected variously the seed germination and seedling characteristics of the different seeds tested (Table 5). Inhibition in percentage seed germination and shoot length in

TABLE 3. FUNGAL COMPOSITION OF SOILS WITH (TREATED) AND WITHOUT (CONTROL) COGONGRASS

Name of fungus	Number of Colonies of[a]	
	Control soil	Treated soil
Aspergillus flavus Link	108	288**
A. fumigatus Fresenius	375	187*
A. niger Van Teighem	750	480*
A. candidus Link	616	312**
A. terreus Thom	375	187
A. nidulans (Eidam) Winter		200[b]
A. humicola Chaudhuri	200	200
A. luchuensis Inui	300[b]	
Curvularia pallescens Boedijn	100[c]	100[b]
Penicillium javanicum Van Beyma	200[c]	
P. chrysogenum Thom	658	562
Trichoderma viride Pers ex Fries	100[b]	266
Cladosporium cladosporides (Fries.) De Vries		200[b]
Rhizopus nigricans Ehrenberb	300[c]	
Fusarium oxysporum (Schl ex Fr.) Snyder et Hansen	200[c]	
Epicoccum purpurascens Ehrenb ex Schlecht		200[b]
Aurobasidium pullulans (de Bary) Arnaud	600[b]	
Alternaria alternata (Fr.) Keissler		200[b]
Yeast-like fungi	300[b]	

[a] *$P < 0.25$, **$0.05 < P < 0.07$.
[b] Colonies detected only in one replicate.
[c] Colonies detected only in two replicates.

TABLE 4. CHEMICAL CHARACTERISTICS OF SOILS FROM THREE DIFFERENT SAMPLING SITES[a]

Chemical characteristics	Soil samples collected from		
	Rhizosphere zone of cogongrass	1.5 m away from cogongrass	3 m away from cogongrass
pH	8.227 ± 0.0808	8.070 ± 0.098**[d]	8.353 ± 0.341
EC[b] (μmho/cm)	427.293 ± 82.75	795.120 ± 335.90*	710.830 ± 226.250*
OM (%)[c]	0.879 ± 0.039	1.016 ± 0.204	0.963 ± 0.100
Cl^- (%)	0.013 ± 0.0028	0.021 ± 0.008*	0.041 ± 0.030*
PO_4^{-3} (mg/100 g)	0.958 ± 0.141	1.017 ± 0.117	1.189 ± 0.091**
Cu^{2+} (mg/100 g)	0.189 ± 0.083	0.107 ± 0.031*	0.111 ± 0.008*
Zn^{2+} (mg/100 g)	1.356 ± 0.505	1.147 ± 0.087	0.549 ± 0.177
Na^+ (mg/100 g)	40.417 ± 15.97	58.00 ± 40.420	110.670 ± 68.030*
K^+ (mg/100 g)	34.833 ± 3.02	56.16 ± 17.710*	33.750 ± 19.540
Mg^{2+} (mg/100 g)	33.660 ± 6.80	32.50 ± 10.64	33.730 ± 3.400
Ca^{2+} (mg/100 g)	313.133 ± 49.47	313.56 ± 126.57	304.230 ± 38.100

[a] See text for details.
[b] Electric conductivity.
[c] Organic matter.
[d] *$0.05 < P < 0.1$, **$P < 0.05$.

tomato was brought about in all test solutions. In general, tomato was found to be the most affected by the test solutions. In fact, except for dry weight (TS1, TS2, and TS3) and root length (TS2, TS3, and TS4), all other parameters studied showed maximum inhibition in tomato. TS1 inhibited most of the seedling characteristics of all the seeds studied except a marginal increase in dry weight in fenugreek and tomato. Inhibition by TS2 was least in radish as compared to other systems investigated. Similarly, TS3 suppressed all the seedling characteristics except dry weight in radish, mustard, and tomato, where it showed an increase. TS4 did not decrease the fresh weight of the seedlings of any of the seeds tested. Even the dry weight was not reduced except for the significant reduction (23.21%) noted in tomato. The germination response of different seeds varied: increases in radish and fenugreek and reductions in mustard and tomato were observed. It may be noted that the reduction in values of all the characters by TS1 and TS3 was markedly significant as compared to TS2 and TS4, respectively. Moreover, inhibition in TS2 and TS3 was more than in TS1 and TS4.

DISCUSSION

These data clearly establish that cogongrass produces phenolic compounds, especially those represented by phenolic fractions 5, 7, 9, 10, and 12, which get incorporated in the soil and cause an allelopathic effect on other plant spe-

TABLE 5. EFFECT OF DIFFERENT COGONGRASS LEACHATES[a] ON SEED GERMINATION AND SEEDLING CHARACTERISTICS OF DIFFERENT CROP SEEDS

Crop seeds	Source of test solution	Seed germination	Root length[b]	Shoot length[b]	Fresh weight[c]	Dry weight[c]
Radish	L[a]	0.00	−22.37	−4.09	−11.11	−15.59
	S(L)	+10.00	−1.37	+2.39	+6.58	+12.67
	R	−14.90	−81.12	−64.55	−46.43	+12.47
	S(R)	+2.22	−8.13	−5.30	+2.52	+16.48
Mustard	L	−2.08	−36.30	−6.59	−16.10	−15.47
	S(L)	+2.12	−33.24	−18.85	−11.43	−6.77
	R	0.00	−94.25	−62.25	−44.52	+51.69
	S(R)	−2.04	−22.40	−1.40	+2.63	−0.99
Fenugreek	L	−8.88	−21.54	−34.70	−12.00	+7.11
	S(L)	−2.27	−10.55	−18.70	+3.43	−9.45
	R	−14.58	−49.36	−10.56	−30.29	−9.15
	S(R)	+4.34	−3.48	−8.60	+9.54	+1.02
Tomato	L	−43.24	−67.21	−77.87	−51.24	+4.42
	S(L)	−20.00	−17.91	−48.20	−17.14	−3.60
	R	−39.47	−90.64	−72.51	−53.25	+16.50
	S(R)	−15.15	+7.40	−97.78	+5.15	−23.21

[a] See text for details.
[b] Average percent decrease (−) or increase (+) in length as compared to control.
[c] Average percent decrease (−) or increase (+) in weight as compared to control.
[d] L, Leaf leachate; S(L), soil + leaf leachate; R, root/rhizome leachate; S(R), Soil + root/rhizome leachate.

cies. This also is supported by the fact that even the concentrations of those compounds that are common to control soils and test solutions were higher in the soils collected either from or near the rhizosphere of the weed. Furthermore, that these compounds move from the weed plant to the soil through the medium of water (as leachates were prepared in the aqueous medium) explains the success of the weed in lawns, orchards, and cultivated fields where irrigation is frequent. Furthermore, since the contribution of allelochemics is more by the root/rhizome than by the leaves, etc. (Table 1), and the percentage germination and seedlings is concentration-dependent, it is not difficult to conclude that the weed succeeds through allelochemics in the rhizosphere zone. As a matter of fact, this may be the most important means to totally eliminate any other plant species from its vicinity, as no buried or sown propagules would be able to establish themselves beyond the germination stage. This is borne out also by the observation that both root and shoot lengths of the germinated seeds tested were inhibited by the test solutions. Furthermore, the relative decrease in the

concentrations of phenolic fractions in the different sampling sites suggests that the zone of influence of the weed is much beyond the immediate vicinity of the weed. That the allelopathic effect is very efficient is suggested also by the reduction in the nodule characteristics and thus the N_2 fixation capacity of the legume *Melilotus parviflora*, as well as by an overall reduction in the soil mycoflora. The inhibitory effect of the weed on rhizosphere mycoflora may account for the insignificant change in the nutrient status of the rhizosphere soil of the weed, as the release of the nutrients would be affected under such conditions. Although this is in contrast to the observations of Glass (1973) and Mersie and Singh (1988), it proves that cogongrass is a very effective competitor because, in spite of its aggressiveness toward other plant species, the weed does not create stress for itself by changing the substratum. Interestingly, despite such allelopathic potential, the weed seems to be selective, as exhibited by the data on seed germination and seedling characteristics (Table 5). However, as opined by Williams and Hoagland (1982), since such a selection can also be because of seed characteristics such as size or seed coat permeability or other physiological characteristics, any inference in this regard requires further investigation.

The data in Table 1 show that the concentrations of the common phenolic fractions were higher in field soil leachates than in any of the test solutions. This could be due to the constancy of availability of the phenolic compounds for longer periods as compared to the test solutions, where the time allowed for leaching was shorter and the quantity of plant parts per gram of soil was less than the plant biomass available in the sampling sites. Furthermore, a decrease in concentration of phenolic fractions (Table 1) and percent germination and seedling characteristics in TS2 and TS4 as compared to TS1 and TS3, is now shown. This suggests selective release of the compounds in the medium. Similarly, this may be the explanation for the detection of phenolic fractions 5, 7, and 10 in the test solutions and 9 and 12 in the soils from the sampling sites. This observation is of significance since the quantity leached and made available to bring allelopathic effects should be crucial for understanding the mode of interference. The presence of fractions 4, 12, and 17 only in the soil with the weed (except TS1 with fraction 4) but their absence in the test solutions where fractions 5, 7, and 10 are present, could be due to phased release (quantitative as well as qualitative) of both these groups of compounds, as well as their stability outside the tissue of the plant. As suggested by Rice (1984), the role of mycoflora may also be important in the breakdown of the compounds in the soil system and may explain the differences in the phenolic fractions as noted above. Furthermore, it is very likely that the compounds released later are resistant to such processes as compared to those released earlier. Detailed studies on these aspects are necessary before any final comment is made on this aspect.

These investigations also brought out that tomato seeds can be used as an

assay system for such studies. A major outcome of the present investigation has been importance of HPLC analysis of soils and leachates as a tool for rapid and routine delimitation of the causes or the mode of achieving interference success by a weed. However, there remains the need to characterize the allelochemics to understand the interference mechanism.

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