Pak. J. Bot., 43(1): 341-350, 2011.

ALLELOPATHIC EFFECTS OF RAIN LEACHATES AND ROOT EXUDATES OF *CENCHRUS CILIARIS* L. AND *BOTHRIOCHLOA PERTUSA* (L.) A. CAMUS

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Abstract

Studies on the allelopathy of *Cenchrus ciliaris* L., and *Bothriochloa pertusa* (L.) A. Camus showed that simulated and natural rain leachates from shoots and root exudates were inhibitory to various test species. The rain leachates and root exudates had differential toxicity against the *Brassica campestris, Lactuca sativa, Setaria italica* and *Pennisetum americanum* in various bioassays. Low concentration of inhibitors were either non inhibitory or were stimulatory to the germination and/or growth of tested species. Chromatography revealed the presence of chlorogenic, ferulic, caffeic, *p*-OH-benzoic, *p*-coumaric, vanillic, syringic and gentisic acids in natural rain leachates, all being proven allelopathic agents. It is concluded that the phytotoxicity was depending upon the concentration of rain leachates and root exudates, test species involved, and physiological parameters measured. Germination appeared to be a poor indicator of phytotoxicity than radicle growth.

Introduction

Cenchrus ciliaris L., and *Bothriochloa pertusa* (L.) A. Camus, both perennial range grasses are preferred for their better germination, seedling establishment and high palatability. They gain dominance by excluding associated species from the common habitat (Vyas, 1965; Brown, 1966). Bishop *et al*., (1974) observed that pure pastures of *Cenchrus ciliaris* decline after few years even under favourable habitat conditions probably owing to soil sickness. Later on Akhter *et al*., (1978), Hussain *et al*., (1982) and Hussain & Ilahi (2009) hinted upon the possibility of allelopathy by both these grasses.

Allelopathy plays important role in invasion, vegetation patterning, exclusion of associated species and reduction in productivity of many plants in natural and agroecosystem (Rice, 1984). Inhibition of germination, radicle growth, and other biochemical processes by many grasses including *Dichanthium annulatum* (Dirvi & Hussain, 1978), *Imperata cylindrica* (Hussain & Abidi, 1991, Anjum *et al*., 2005), *Panicum* (Begum & Hussain, 1980), *Sorghum* (Hussain & Gadoon, 1981) and *Eragrostis* (Hussain *et al*., 1984) in relation to productivity and growth have been worked out. Most studies have employed aqueous extracts from shoots for investigating allelopathic effects in laboratories. Rain leachates and root exudates are among the various possible mechanisms for releasing phytotoxins by allelopathic plants including grasses (Inam *et al*., 1989; Hussain & Abidi, 1991; Hussain *et al*., 2004, 2005; Javaid *et al*., 2005; Javid & Anjum, 2006; lannucci, 2007; Otusanya *et al*., 2008; Kato-Noguchi *et al*., 2009; Hussain & Ilahi, 2009). The decaying plant litter renders soil unfavorable for growth of susceptible species due to release of phytotoxins (Boz, 2003; Hussain & Gadoon, 1981; Cheema *et al.,* 1997; Batlang & Shushu, 2007).

Although, it has been demonstrated that *Cenchrus ciliaris* and *Bothriochloa pertusa* interfere with other grasses through roots (Hussain *et al*., 1982) and that aqueous extracts from shoots exhibit allelopathy (Hussain & Ilahi, 2009), yet the role of rain leachates and root exudates from living/dead parts of both these grasses in manifesting allelopathy in relation to self declination of pure pastures is not fully worked out. Furthermore, the phytotoxins responsible for its allelopathy are unknown. The present study addresses the same questions to explain the allelopathic mechanism of release of phytotoxins and to identify phytotoxins in both grasses.

Materials and Methods

1. Effect of simulated rain leachates: One hundred gm dried crushed shoots of either *Cenchrus ciliaris* or *Bothriochloa pertusa* were spread separately in large glass funnels. Two litres double distilled water was taken in 4, 500 ml separation flasks that served as Reservoir flask. The distilled water dripped from these reservoirs flasks through shoots in the collecting funnels and designated as "simulated rain leachate". This leachate (RL-I) was again dripped through the same shoots for the 2nd (RL-2)) and 3rd time (RL-3). The speed of dripping water was adjusted to 500 ml/4 h. During this time the funnels were frequently rotated manually to give uniform distribution. The RL-3 leachate was further concentrated to 5 and 10 times by evaporating at 50 ^{0}C in rotavapor. Seeds of *Brassica campestris*, *Lactuca sativa* and *Setaria italica* were sown in these simulated rain leachates following standard filter paper bioassay (Hussain & Abidi, 1991). Control were made with double distilled water. Every treatment for each species was replicated 5 times, each with 10 seeds. The dishes were incubated at 26^{o}C for 72h. The experiment was repeated 3 times.

2. Effect of natural rain leachates: To confirm the findings of simulated rain leachates experiment, another experiment was performed by using natural rain leachates. One hundred g dried shoots of either *Cenchrus* or *Bothriochloa* were spread in large funnels and kept under natural slow drizzling rain. For control, rainwater was collected directly in the flasks. The collection of rainwater (control) or rain leachates (for making test) was started when it had rained for about one hour to avoid aerial contaminants. The funnels containing shoots along with collecting flasks were kept on one m high bench to avoid any contamination by splashing water from soil. This slow drizzle almost took 12 hrs during December for collecting sufficient "Natural rain leachates". Since these rain leachates were very dilute, therefore a portion of this leachate was concentrated by 5 times (X5) using rotavapor at 50^{o}C. These rain leachates were used against *Brassica campestris, Setaria italica* and *Lactuca sativa* in standard filter paper bioassay following Hussain & Abidi (1991). Two types of controls consisting of simple rainwater and double distilled water (DDW) were made to assess the quality of simple rain water. All treatments were replicated 3 times, each with 100 seeds of respective test species. Germination and radicle growth was recorded after 72 h incubation at 26^{o}C.

3. Effect of root exudates: A six month old plant of either *Cenchrus* or *Bothriochloa* was up rooted; their roots were thoroughly washed first with tap water followed by distilled water to remove soil contaminants. It was then transferred to 14x5 cm sterilized glass vials containing Hoagland's nutrient solution. The glass vials were covered with black paper to prevent light entry into the vials. There were 4 replicates, which were kept under16 h photoperiod at room temperature (25–30°C). After 6 weeks plants were removed, solutions from all glass vials were mixed, filtered and designated as "Test solution". A bioassay was run by growing *Setaria italica, Brassica campestris* and *Lactuca sativa* in test solutions using standard filter paper bioassay (Hussain & Abidi, 1991). Control was made by using Hoagland's nutrient solution. There were 10 replicates, each with 10 seeds. Germination and radicle growth was recorded after 72 h incubation at 26°C.

4. Effect of soil leaching: Twenty four, 20 cm earthen, pots were filled with equal volume of similar loamy soil. The pots were divided into three groups of eight each. In two of the groups, 10 seedlings of either *Cenchrus* or *Bothriochloa* were raised per pot. Third group of 8 pots was left without any grass, which served as control. All the pots were kept under natural conditions in net house for two months (March-April); and thereafter hanged as "Upper Series of donor plants". A "Lower Series" of receptor pots consisted of 32, 10 cm plastic pots, each with 2 seedlings of *Pennisetum americanum*. These 32 pots of the "Lower Series" were divided into 8 groups of 4 pots each and treated as follows. Half strength Hoagland's solution was provided to avoid nutrient deficiency and to see if nutrients might eliminate allelopathic effects.

i. Watered twice a week with leachates received from four *Cenchrus* donor pots of upper series.
ii. Watered as in treatment i and also given 50 ml half strength Hoagland's solution.
ii. Watered twice a week with leachates received from four *Bothriochloa* donor pots of upper series.
iv. Watered as in treatment iii and also given 50 ml half strength Hoagland's solution.
v. Watered twice a week with leachate received from four pots of upper donor series, but without any grass in them.
vi. Watered as in treatment v and also given 50 ml half strength Hoagland's solution.
vii. Watered with tap water twice a week.
viii. Watered as in treatment vii and also given 50 ml half strength Hoagland's solution weekly.

After 4 weeks the test species were harvested for dry weight determination. During the entire experimental period the pots received uniform natural conditions.

5. Identification of phytotoxins: Identification of phytotoxins was made following methods described by Lodhi & Rice (1971) and Hussain & Abidi (1991). Natural rain leachates collected earlier was concentrated to 1/3rd of its original volume in rotavapor and acidified with 1N HCl to pH 2-3. To this concentrate, double amount of ether was added. The mixture was vigorously re-flux shaken for at least 30 min., in separation flask and thereafter separating flask was left till the separation of etherial and aqueous layers. The ether layer was saved and aqueous fraction was re-shaken for another two extractions. All the three ether fractions were combined and concentrated. The dry residue, taken in methanol, was spotted on Whatman chromatographic No.1 paper strips along with standard compounds including caffieic acid, ferulic acid, *p*-OH-benzoic acid, *p*-coumaric acid, chlorogenic acid, ellagic acid, vanillic acid, benzoic acid and quercetin. The chromatograms were run in 6% acetic acid (6% V/V acetic acid) and BAW (n-butanol: acetic acid:water = 63:10:27 ml) solvent systems in descending order. The chromatograms were inspected under long (366 nm) and short (254 nm) UV light and sprayed with spraying reagent for further confirmation. The different colours and Rf values of standard and unknown compounds were compared for identification.

Results

1. Effect of simulated rain leachates: There were no significant differences in the germination of the test species except in the concentrated leachates of *Cenchrus*. Germination of *Setaria* and *Brassica* was slightly reduced in concentrated leachates of *Brothriochloa* (Table 1). The radicle growths of all the test species were significantly ($p<0.05$) inhibited in all the treatments (Table 1). The radicle growths of *Brassica campestris* and *Lactuca sativa* gradually decreased with increasing concentration of leachates (from RL-1 to RL-3; and from x5 to x10 concentration of RL-3). The radicle growth of *Setaria italica* although was inhibited in RL-I treatments, but was less affected in the concentrated leachate of RL-3.

2. Effect of natural rain leachates: Natural rain leachates, especially concentrated, from both these grasses significantly ($p<0.05$) inhibited the germination and radicle growth of all the test species (Table 2). *Lactuca sativa* and *Setaria italica* were affected more than *Brassica campestris.* No differences in radicle growths were observed either in distilled or simple rain water suggesting that rain water had no inhibitory effects. The results of present and those of simulated rain leachates bioassay confirm each other's findings. *Setaria italica* behaved exactly in the same way as it did in the simulated rain leachate bioassay. It was suggested that leaching from shoots or litter is an important mechanism of allelopathy in both these grasses.

3. Effect of root exudates: When seeds of test species were grown in nutrient solution that had previously supported growth of these two grasses, the germination of *Brassica campestris* and *Setaria italica* was significantly inhibited by *Cenchrus* root exudates while *Bothriochloa* root exudates inhibited germination of *Brassica campestris* only. The radicle growths of all the test species was significantly ($p<0.05$) inhibited (Table 3).

4. Soil leaching experiment: Fig. 1 represents the total dry weights of two seedlings of *P. americanum* in 8 different treatments. It was obvious that the dry weights of test plants that received leachates from either grass (Treatments i & iii) were significantly reduced ($p<0.05$) even in the presence of nutrient solution (Treatments ii & iv). This suggests that the nutrients could not eliminate the toxic effects. Differences between treatments v & vii and between vi & viii indicated that soil had sufficient nutrients, therefore treatments v and vi were considered as true control for the treatments i and iii. Treatments v & vi differed significantly ($p>0.05$) from other treatments. Differences between treatments i & iii and ii & iv showed that *Cenchrus* was more inhibitory than *Bothriochloa*.

5. Identification of phytotoxins: Paper chromatography suggested the presence of *p*-OH-benzoic acid, *p*-coumaric acid, caffeic acid, vanillic acid, ferulic acid, syringic acid and gentisic acid. All these compounds are known allelopathic agents (Rice, 1984; Lodhi & Rice, 1971; Lodhi, 1975; Hussain & Abidi, 1991; Hussain *et al*., 1992; Inam *et al*., 1987, 1989) that exhibit allelopathy against susceptible species.

Table 1. Effect of simulated rain leachates on germination and radicle growth of test species.
(RL-1, RL-2 and RL-3 are the number of leaching; while 5x and 10x is further concentration of RL-3).

Test species	Control	*Cenchrus ciliaris* rain leachates					*Bothriochloa pertusa* rain leachates				
		RL-1	RL-2	RL-3	RL-3		RL-1	RL-2	RL-3	RL-3	
					5X	10X				5X	10X
	Germination (%)										
Brassica campestris	86 a	82 a	76 a	85 a	72 b	b ---	88 a	74 a	78 a	82 a	80 a
Lactuca sativa	96 a	94 a	94 a	92 a	6 b	6 bc	94 a	86 a	92 a	92 a	96 a
Setaria italica	84 a	66 b	58 c	52 c	62 bd	62 bd	84 a	68 b	61 b	72 c	72 c
	Radicle growth (mm)										
Brassica campestris	5.28 a	4.60 a	4.30 a	4.32 a	3.76 b	c -----	4.96 a	3.36 a	3.94 bc	3.25 c	1.75 d
± SD % of Control	1.85	1.61	1.17	1.12	0.85	-----	0.97	0.84	0.58	0.40	0.32
	100	87.12	81.43	81.81	71.21	-----	93.90	63.63	74.62	61.55	32.95
Lactuca sativa	6.20 a	6.08 ab	5.06 bc	5.50 c	3.10 d	e ------	5.52 ab	4.80 bc	4.72 b	4.22 c	2.50 d
± SD % of Control	0.38	0.77	0.86	0.61	0.46	------	1.03	0.65	0.12	0.48	0.15
	100	98.06	81.93	88.70	50.00	------	88.88	78.88	76.12	68.06	41.93
Setaria italica	10.72 a	3.28 bd	1.50 c	1.14 c	5.40 bd	4.54 d	4.68 bd	3.34 bcd	2.26 c	4.42 d	4.36 d
± Sd % of control	3.66	1.21	0.56	0.80	1.21	1.80	0.54	1.10	0.25	0.48	0.27
	100	30.59	13.99	10.63	50.37	42.33	30.22	30.00	21.08	41.23	40.67

Means followed by the same letters with in a row are not significantly different from each other at $p=0.05$.

Table 2. Effect of Natural rain leachates of *Cenchrus ciliaris* and *Bothriochloa pertusa* on germination and radicle growth of test species. (Ix and 5x show the concentration of the rain leachates).

Test species	Distilled water control	Simple rain water	Rain Leachate			
			Cenchrus ciliaris		*Bothriochloa pertusa*	
			1X	5X	1X	5X
	Germination (%)					
Brassica campestris	86.33a	81.00 a	83.33 a	9.00 b	92.00 a	29.00 c
% of control		94.1	96.52	10.43	106.57	33.59
Lactuca sativa	96.67	96.00	91.66	63.33 a	89.66	67.00 a
% of control		99.31	94.82	65.51	92.75	69.31
Setaria italica	73.00 b	74.00 b	61.00 b	55.00 c	48.66 c	50.00 c
% of control		101.37	83.56	75.34	66.66	68.99
	Radicle Growth ± SD (mm)					
Brassica campestris	5.40 ± 0.27a	4.90 ± 0.2 a	2.77 ± 0.67 b	0.26 ± 0.08 c	3.27 ± 0.39 b	0.88 ± 0.04 c
% of control	100.00	90.74	51.30	4.81	60.56	16.30
Lactuca sativa	6.92 ± 0.11	6.88 ± 0.21	2.50 ± 0.60	0.22 ± 0.05	3.76 ± 0.07	0.35 ± 0.02
% of control		99.42	36.13	3.18	54.33	5.06
Setaria italica	5.19 ± 0.22 a	4.76 ± 0.72 a	3.78 ± 0.84 b	2.98 ± 0.53 b	2.13 ± 0.58 c	3.32 ± 0.90 c
% of control	100.00	91.71	72.83	57.42	41.05	63.57

Means followed by the same letters with in row are not significantly different from each other at $p=0.05$

Table 3. Effect of root exudates of *Cenchrus ciliaris* and *Bothriochloa pertusa* on the germination and radicle growth of the test species.

Test species	Control	Root exudates			
		Cenchrus ciliaris		*Bothriochloa pertusa*	
		Test	% Of control	Test	% Of control
	Germination (%)				
Brassica campestris	89	82	92.13*	76	85.39*
Lactuca sativa	85	80	94.11	82	96.47
Setaria italica	100	87	87.00**	92	92.00
	Radicle Growth ± SD (mm)				
Brassica campestris	9.34 ± 2.4	6.68 ± 1.5	73.44**	4.83 ± 1.3	51.71**
Lactuca sativa	8.35 ± 1.7	6.47 ± 1.7	77.48**	6.02 ± 1.8	72.09*
Setaria italica	23.86 ± 3.6	9.45 ± 1.9	39.60**	15.77 ± 2.3	66.09**

* & ** Significantly different from control at p= 0.05 & 0.01

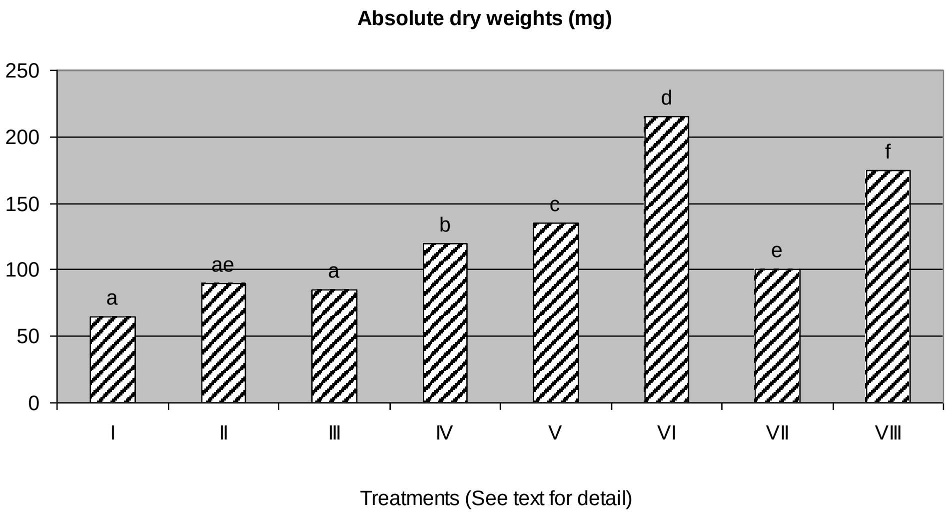

Fig. 1. Effect of Soil leaching on the dry weight of *Pennisetum americanum.* Each bar represents the total dry weights of 4 replicates, each with two seedlings. Bars with similar letters are not significantly different from each other.

Discussion

The present study revealed that simulated and natural rain leachates reduced the germination and growth of test species with almost same level of inhibition in the presence of added nutrient solutions. Furthermore, germination and growth of various test species in distilled water and simple rain water remained unaffected with almost similar results confirming that rain water had no inhibitory effects. The findings agree with other workers (Hussain *et al*., 1979, 1988, 1992; Inam & Hussain, 1988; Inam *et al*., 1989), who also reported inhibitory effects of artificial and natural rain leachates from other plants. It is frequently reported that low concentration of phytotoxins generally either remain non inhibitory or might stimulate germination and seedling growth of same or different test species (Hussain & Ilahi, 2009; Samreen *et al*., 2009; Thapaliyal *et al*., 2007; Hoque *et al*., 2003) and similar situation was observed in the present study. The present study further concludes that root exudates from both these grasses decreased the germination and growth of test species in various bioassays. Similar to our findings Iannucci (2007), Kato-Noguchi *et al*., (2009) and Hussain & Ilahi (2009) also observed similar inhibition of test species by root exudates. Similarly in soil leaching experiment, the receptor test seedlings exhibited significantly decreased dry weight whenever they were irrigated with water leaching through *Cenchrus* or *Bothriochloa* pots. The added nutrients neither eliminated nor reduced the toxicity of root exudates. On the contrary, Otusanya *et al*., (2008) and Dirvi & Hussain (1979) stated that roots exudates were not inhibitory to test species due to their low concentration of toxins.

Rain leachates proved inhibitory due to presence of some water soluble phytotoxins. Chromatography revealed that natural rain leachates contained chlorogenic, feruilic, caffeic, *p*-OH-benzoic, *p*-coumaric, vanillic, syringic and gentisic acids, which are strong inhibitors of germination and growth of susceptible plants (Rice, 1984; Lodhi & Rice, 1971; Lodhi, 1975; Inam *et al*., 1989; Hussain & Abidi, 1991; Hussain *et al*., 1992). These water soluble toxins released by donor plants accumulate in the soil where they retard the germination and growth of susceptible species. The associated species are negatively affected by variety of mechanisms including impaired water and mineral absorption by roots, transpiration, photosynthesis and other adverse biochemical changes. Furthermore, allelopathic substances delay and retard the division and elongation of meristematic cells (Hussain *et al*., 1984; Hussain & Ilahi, 2009) that might also be cause of retarded growth of test species.

The present study supports the previous findings (Hussain & Ilahi, 2009) regarding the differential phytotoxicity of aqueous extracts and rain leachates and root exudates due concentration of rain leachates, test species used and the growth parameters measured. It was also concluded that germination was a poor indicator for measuring phytotoxicity in allelopathic studies and the findings are supported by other studies (Hussain & Ilahi, 2009; lannucci, 2007; Kato-Noguchi *et al*., 2009). It was also observed that leachates from *Cenchrus* were more toxic than *Bothriochloa* due to species specificity as reported by similar studies (Samreen *et al*., 2009; Kato-Noguchi *et al*., 2009; Hussain & Ilahi, 2009; Javaid & Anjum, 2006; Dirvi & Hussain, 1979). The present findings suggest that the observed self declination of pastures of these grasses is due partly to their auto-allelopathy. Although, phytotoxins in natural rain leachates have been identified, yet further study is needed to see the fate and persistence of phytotoxins in soil and their possible effects on various physiological processes of susceptible species. It would also be interesting to envisage the phytotoxicity of these grasses against microbes and weedy species as a candidate as biocontrol agent (Cheema *et al*., 1997).

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(Received for publication 15 February 2010)