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ALLELOPATHIC EFFECTS OF *CENCHRUS CILIARIS* L. AND *BOTHRIOCHLOA PERTUSA* (L.) A. CAMUS

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Abstract

Cenchrus ciliaris L., and *Bothriochloa pertusa* (L) A. Camus are perennial range grasses growing from plains upto 1000m in hot and dry tropical and subtropical regions of the world including Pakistan. Both these grasses are preferred for pasture due to easy germination, fast growth, good palatability and better productivity. However, the pasture generally declines after few years. The present study was conducted to see if allelopathy might be responsible for the declination of pastures. Studies made with using aqueous extracts and added mulches from different plant parts indicated that extracts from various parts and mulches invariably inhibited the germination, radicle growth, dry weight and moisture contents of test species used in different bioassays and experiments. The toxicity depended upon the parts assayed, test species used, soaking duration and physiological parameter. Above ground parts, especially leaves, were more toxic than roots. The toxicity enhanced with increasing soaking duration and amount of plant material. However, the toxicity of shoots declined with constant leaching of plant material. Shoot mulches added to soil retarded the germination and dry weight of test species. It was observed that allelopathy operates through water soluble toxins. However, further study is needed to see the role of root exudates, rains leachates and to identify phytotoxins.

Introduction

Cenchrus ciliaris L., and *Bothriochloa pertusa* (L.) A. Camus are perennial range grasses growing from plains upto 1000m in tropical and subtropical region of the world including Pakistan. These grasses are preferred for their prompt germination and establishment of seedlings, better forage production and good palatability and their ability to with stand grazing and drought pressure. It has been observed that pure pastures of these grasses need reseeding after 4-5 years even under favourable conditions (Brown, 1966). Vyas (1965) reported that these grasses dominate in grassland communities that change to *Cenchrus* or *Bothriochloa* type of grassland under grazing. Bishop *et al*., (1974) attributed the decline of *Cenchrus* pasture to competition. Akhtar *et al*., (1978) in a preliminary study hinted upon the possibility of allelopathy by these grasses. Hussain *et al*., (1982) stated that competition alone can not be responsible for the reported decline of growth of *Cenchrus ciliaris* and *Bothriochloa pertusa*. They further pointed out the possibility of allelopathy by these grasses in mixed cultures.

Allelopathy operates in nature in many species including grasses through water soluble toxins that reach to the immediate habitat by various mechanisms (Hoque *et al*., 2003; Kadioglu & Yanar, 2004; Hussain *et al*., 2004, 2005; Nusr & Shariati, 2005; Ko *et al*., 2005; Iman *et al*., 2006; Batlang & Shushu, 2007; lannucci, 2007; Thapaliyal *et al*., 2007; Samreen *et al*., 2009; Hisashhi *et al*., 2009). Allelopathy is a complex process that operates along with competition in nature to suppress and finally exclude the susceptible associated species from the common habitat. It has been frequently observed that allelopathically

potential chemicals are present almost in all plant parts which are released in to the habitat under specific environmental conditions. Keeping in view the aggressive nature of both these grasses, suppression of associated species and self declination of pastures, the present study was conducted to see if allelopathy might play any role in the aggression and self declination of *Cenchrus ciliaris* and *Bothriochloa pertusa*.

Materials and Methods

1. Relative toxicity of plant parts: Glass ware, thoroughly washed with tap water was rinsed with doubled distilled water and sterilized at 180^{o}C for 4 hrs. Filter papers and other heat labile material were autoclaved at 15 psi for 30 minutes. Seeds were placed on two folds of Whatman filter paper No. 1. The dishes were moistened with sufficient amount of extract or distilled water for test or making control, respectively. The dishes, sealed with parafim M, were incubated at 26^{o}C for 72 hrs. Germination and radicle growth was recorded after 72 hrs. This would be referred to as "Standard filter paper bioassay" in the subsequent experiments.

Mature dried plants of either *Cenchrus ciliaris* or *Bothriochloa pertusa* were separated into inflorescences, leaves, stems and roots. Ten gm of every part was soaked in 100 ml of double distilled water for 12 h and filtered. These extracts were then tested against *Pennisetum americanum*, *Setaria italica* and *Lactuca sativa* seeds. Ten seeds of each species were placed in triplicate using standard filter paper bioassay for control and test. After 72 h, germination and radical growth of the test species was measured. The experiment was repeated.

2. Differential toxicity against different species: Five g dried crushed shoots of either *Cenchrus* or *Bothriochloa* were soaked in 100ml of distilled water for 12 h and filtered. The extracts were used against 25 different test species (Table 2) using standard filter paper bioassay. *Convolvulus arvensis, Salvia plebeia, Plantago lanceolata, Taraxacum officinale, Rumex dentatus, Calendula arvensis* and *Capsella bursa-pastoris* were incubated for 7 days; *Senebiera didyma* and *Allium cepa* were incubated for 96 h while rest of the species were incubated for 48h. All the test species had 10 replications with 10 seeds each. The experiment was repeated.

3. Effect of soaking duration: Five gm dried crushed shoots of *Cenchrus* or *Bothriochloa* were soaked in 100 ml distilled water for 6, 12, 24, 48 and 96 hrs at 25^{o}C and filtered. These extracts were used against *Brassica campestris, Setaria italica* and *Lactuca sativa* in a standard filter paper bioassay. Each treatment had 10 replicates, each with 10 seeds. The germination and radicle growth was recorded after 48h incubation at 26^{o}C

4. Effect of constant leaching on toxicity: Five g dried crushed shoots of *Cenchrus* or *Bothriochloa* were soaked in 100 ml double distilled water and filtered after 12 hrs. This was referred to as Ist extraction. The same shoots were dried at 60^{o}C for 72 hrs and re-soaked in 100 ml double distilled water for 12 hrs and filtered to get the 2nd extraction. The shoots after 2nd extraction were used in a similar way for 3rd, 4th and 5th extractions. For each extraction independent bioassay was run for 48 hrs at 26^{o}C using standard filter paper bioassay. *Brassica campestris, Setaria italica* and *Lactuca sativa* were used as the test species. Control and test were replicated 10 times, each with 10 seeds.

5. Effect of hot water extract: The extracts from *Cenchrus* and *Bothriochloa* were obtained by boiling 5gm of shoots in 100ml double distilled water for 10 minutes and filtered. The extracts were cooled to room temperature and diluted to 1:5 or 1:10 (V/V, extract / Hoaglands nutrient solution) to provide the test concentrations. In Control extract was replaced with distilled water. One-month-old healthy uniform seedlings of *Setaria italica*, *Sorghum vulgare* and *Oryza sativa* were transferred to glass vials containing 10 ml of test or control solutions. Twenty plants of each test species were individually transferred to each of the vials and allowed to grow for 2 to 10 days at room temperature (25± ^{o}C) under 16 hrs photoperiod. The glass vials were plugged with cotton and covered with brown paper to avoid light entry into the vials. On the 2nd, 4th, 6th, 8th and 10th day of treatment, fresh weight of shoots and roots were determined. They were then oven dried at 65^{o}C for 72h for dry weight determination. Each treatment had 4 seedlings. Percent moisture was calculated on oven dry weight basis.

6. Effect on the seed viability: Plants extract was obtained by soaking 10g shoots in 100ml double distilled water for 12 hrs. Seeds of *Brassica campestris*, *Lactuca sativa* and *Setaria italica* were soaked for 15 hours in extracts (test) or double distilled water (control) at 25^{o}C with following 12 treatments.

i. Seeds soaked and grown in distilled water.
ii. Unsoaked seeds grown in distilled water.
iii. Seeds soaked and grown in *Cenchrus* extract.
iv. Seeds soaked and grown in *Bothriochloa* extract.
v. Seeds soaked in *Cenchrus* extract and grown in distilled water.
vi. Seeds soaked in *Bothriochloa* extract and grown in distilled water.
vii. Seeds soaked in *Cenchrus* extract, washed and grown in distilled water.
viii. Seeds soaked in *Bothriochloa* extract, washed and grown in distilled water.
ix. Seeds soaked in *Cenchrus* extract, washed and grown in *Cenchrus* extract.
x. Seeds soaked in *Bothriochloa* extract, washed and grown in *Bothriochloa* extract.
xi. Unsoaked seeds grown in *Cenchrus* extract.
xii. Unsoaked seeds grown in *Bothriochloa* extract.

Seeds in each treatment were incubated at 26^{o}C for 72h in a standard filter paper bioassay. Each species in each treatment had 10 replicates, each with 10 seeds. The percent germination was recorded at the end of the experiment.

7. Mulching experiment: Sand was thoroughly washed, dried and sterilized at 180^{o}C for 12h. It was taken in 70 x 50cm pots. Seeds of *Pennisetum americanum*, *Sorghum vulgare* and *Setaria italica* were tested in following treatments.

I. Sand + I g pieces of filter papers. This served as control.
II. Sand + I g unwashed shoots of *Cenchrus*.
III. Sand + I g unwashed shoots of *Bothriochloa*.
IV. Sand + I g washed shoots of *Cenchrus*.
V. Sand + I g washed shoots of *Bothriochloa*.
VI. Sand + I g filter papers soaked in *Cenchrus* extract and dried.
VII. Sand + I g filter papers soaked in *Bothriochloa* extract and dried.

For treatments IV and V, shoots were washed in running water for 4 hours, then dried at 60°C for 72 hours and used. In the case of treatments VI and VII, extracts were obtained by boiling 5 g of shoots in 100 ml distilled water for 10 minutes. In these extracts, small pieces of filter papers were soaked for 4 hours and dried at 60°C for 48 hours. These filter papers were then used in the experiment.

Shoots or fine pieces of treated filter paper were spread on the top of sand surface. Fifty ml distilled water was added to each pot. The test species were replicated 4 times, each with 10 seeds. After 5 days incubation at 26°C, the germination was recorded and seedlings were thinned to 4 per pot. The pots were then transferred to 16 hours light period. Each pot was provided with 10ml of full strength Hoagland's solution at week's interval. After 2 weeks, plants were harvested and dried at 60°C for 72 hours for dry weight determination.

In another experiment equal volume of loamy soil was taken in 15x10 cm pots, lined with polythene bags to avoid leaching through the pots. In every pot 25gm dried loosely crushed shoots of either *Cenchrus* or *Bothriochloa* were spread on the top. Control pots had no shoots. All the treatments were in triplicate. These pots were kept under natural conditions (August and September) and watered regularly. Weeds were removed regularly by hand. After two months soil was collected from all the treatments, litter removed and mixed. It was dried at room temperature and used in the following experiments.

a. Soil-extract bioassay: Twenty g dried test or control soil, sieved through 2mm mesh, was mixed in 100 ml of double distilled water and shaken for 12 hours to get soil extract which was used against *Brassica campestris, Lactuca sativa* and *Setaria italica* in a standard filter paper bioassay. In each case 5 replicates, each with 10 seeds were used. The dishes were incubated at 26°C for 48 hours.

b. Soil-bed bioassay: The test and control soils were spread separately in 1cm layer in 15cm diam., dishes and saturated with double distilled water and then allowed to come to the field capacity. One sheet of Whatman filter paper No. 1 was kept on the top. Each dish had 100 seeds of *Brassica campestris, Lactuca sativa* or *Setaria italica* in triplicate and incubated at 26°C for 72 hours.

All the results were compared with control using t-tests. Germination was statistically analyzed using z-test (Cox, 1967).

Results

1. Relative toxicity of plant parts: The germination of *Pennisetum* was reduced only by *Cenchrus* leaf extract (Table 1); while that of *Setaria* was significantly inhibited by all the extracts, except *Cenchrus* stem extracts (Table 1). The extracts from inflorescences, leaves and stems significantly inhibited germination of *Lactuca*. The radicle growth of all the test species in *Cenchrus* extracts was inhibited more by the above ground parts than roots. *Setaria* and *Lactuca* were the most susceptible species (Table 1).

All the extracts from *Bothriochloa* significantly inhibited the radicle growth, especially those of *Setaria* and *Lactuca* in inflorescences, leaves and stem extracts. Root extracts were generally less inhibitory than others. It was obvious that extracts from above ground parts, especially from *Cenchrus*, were inhibitorier than roots. It was therefore, concluded that in all the subsequent studies above ground parts may be tested further.

Table 1. Relative phytotoxicity of various parts of *Cenchrus ciliaris* and *Bothriochloa pertusa* against the germination (%) and radicle growth (mm) of the test species.

Species	Extract/Part	*Pennisetum americanum*		*Setaria italica*		*Lactuca sativa*	
		Test	% of control	Test	% of control	Test	% of control
		Germination (%)					
Cenchrus ciliaris	Control	98.0a	100.00	21.0a	100.00	45.0a	100.00
	Inflorescences	89.0b	90.81	00.00_b	00.00	5.0b	11.11
	Leaves	64.0c	65.23	3.0c	14.28	00.00c	00.00
	Stems	90.0bd	91.74	26.0e	123.80	13.0d	28.88
	Roots	92.0abd	93.87	21.ae	100.00	46.5ad	1C3.55
Bothriochloa pertusa	Control	100.0a	100.00	47.0a	100.00	93.00a	100.00
	Inflorescences	90.0a	90.00	17.0b	36.17	10.00b	10.75
	Leaves	100.0a	100.00	13.0bc	27.65	3.00c	3.09
	Stems	96.0a	96.6	10.0bd	21.45	17.00d	18.29
	Roots	100.0a	100.00	27.0e	57.14	100.00ae	107.18
		Radicle growth + SD (mm)					
Cenchrus ciliaris	Control	44.2 ± 6.2a	100.00	2.0 ± 0.5a	100.00	5.1 ± 1.2a	100.00
	Inflorescences	6.4 ± 1.5a	14.40	00.00b	00.00	0.1 ± 0.05b	1.98
	Leaves	1.4 ± 0.5c	31.00	0.1 ± 0.05c	5.00	00.00c	00.00
	Stems	27.6 ± 2.3d	62.40	1.5 ± 0.5acd	75.00	0.3 ± 0.5bd	5.90
	Roots	33.0 ± 3.4de	74.40	1.7 ± 0.2acde	85.00	4.0 ± 0.7d	78.20
Bothriochloa pertusa	Control	64.3 ± 5.7a	100.00	1.8 ± 0.30a	100.00	11.5 ± 2.10a	100.00
	Inflorescences	32.5 ± 4.5b	50.54	1.2 ± 0.20a	66.66	0.04 ± 0.05b	30.49
	Leaves	38.6 ± 4.8c	60.00	1.2 ± 0.10a	66.66	0.2 ± 0.01bc	10.73
	Stems	40.7 ± 3.7cd	63.29	0.9 ± 0.05b	50.00	1.0 ± 0.02d	8.68
	Roots	51.4 ± 5.6e	70.92	1.8 ± 0.20a	100.00	7.2 ± 2.1d	62.60

Means followed by the same letters are not significantly different from each other at p=0.05
Each value represents the mean of 10 replicates, each with 10 seeds with two repetitions.

2. Differential toxicity against different species: The germination of all the test species was significantly inhibited in *Cenchrus* extract except *S. didyma, H. vulgare, Trifolium repens, T. aestivum* and *Convolvulus arvensis.* Similarly, *Bothriochloa* extract inhibited the germination of most of the test species with the exception of *S. didyma, R. dentatus, B. pertusa, B. napus, H. vulgare, T. repens* and *T. aestivum* (Table 2). The radicle growth of all the tests species was inhibited in extracts from both the grasses. *Lactuca sativa, S. plebeia B. napus, Chrysopogon aucheri, Hyparrhenia rufa, Allium cepa, Brassica campestris* and *Bothriochloa pertusa* were the most sensitive tests species in *Cenchrus* extracts. While *B. campestris, Allium cepa, S. plebeia, L. sativa* and *Chrysopogon aucheri* were sensitive to *Bothriochloa* extracts. It was concluded that the extracts from both the grasses exhibited differential toxicity against the various test-species including self-toxicity. Germination and radicle growth were independently affected.

3. Effect of soaking duration on phytotoxicity: The inhibitory effects on germination and radicle growth enhanced with increasing duration of soaking (Table 3) in all the test species. In nature the shoots might release more toxins provided sufficient time is permitted for leaching. *Cenchrus* extracts appeared to be inhibitorier than *Bothriochloa*.

4. Effect of constant washing on toxicity: Germination of all the test species in all the extracts was significantly reduced upto 3rd extraction. The inhibitory effects gradually got reduced from first extraction through 5th. The maximum inhibited germination occurred in extract from 1st extraction than in others (Table 4). The radicle growth of all test species was significantly retarded in the first few bioassays. But in the 5th bioassay the radicle growths almost equaled to the control (Table 4). The toxicity of extracts decreased with constant washing (extractions 1st through 5th). The results revealed that constant washing of plant material decreased the toxicity due to leaching of toxins.

5. Effect of hot water extract: The dry weight (Table 5) and moisture contents (Table 6) of shoots and roots of *S. italica* decreased in the test conditions on the second day of the treatment. However, there was no definite trend between the duration of treatment and reduction of dry weights or between shoot and root biomass. In some of the cases, the dry weights almost equaled to the control values. The differences in moisture contents were clearer than the differences in dry weight (Table 6).

In *S. vulgare* the dry weight of shoot exhibited no change during the first 2 days in extracts from both the grasses. However, it slightly decreased on the 4th and inhibition became significant on the 10th day in 1:10 concentration (Table 7). The dry weights of roots were reduced on the 4th and following days of treatment at both the concentrations. However, no linear relationship between the duration of treatment and reduction in dry weight could be observed. The moisture contents of shoot and root declined significantly on the 2nd and subsequent days of treatment at both the concentrations (Table 8).

The dry weights of shoots of rice seedlings decreased on the 2nd day that became significant on the 6th day (Table 9). The root dry mass initially declined in all the test conditions but eventually either got stimulated or equaled to that of control in all the treatments. The moisture contents of shoot and root significantly decreased on 2nd and following days in both the concentration (Table 10). However, on the 10th day it either excelled or equaled to control in all the treatments.

Table 2. Differential phytotoxicity of *Cenchrus ciliaris* and *Bothriochloa pertusa* shoot extracts against 25 different species.

No.	Test species	*Cenchrus ciliaris* extract		*Bothriochloa pertusa* extract	
		Germination	Radicle growth	Germination	Radicle growth
1.	*Allium cepa*	37.80	17.70	64.63	31.57
2.	*Bothriochloa pertusa*	52.48	29.80	93.23*	63.10
3.	*Brassica campestris*	44.16	18.67	17.85	9.23
4.	*Brassica napus*	25.50	2.04	91.8*	45.9
5.	*Calendula arrensis*	57.89	43.47	56.14	42.02
6.	*Capsella bursa- pastoris*	94.33*	69.48	86.79*	79.22
7.	*Cenchrus ciliaris*	50.84	50.80	69.86	63.90
8.	*Chrysopogon aucheri*	54.32	23.07	54.32	24.85
9.	*Cicer aurietinum*	64.04	46.92	85.9*	41.95
10.	*Conyza sp.*	90.0*	42.42	80.0*	36.36
11.	*Dichanthium annulatum*	75.40	42.64	80.32	34.80
12.	*Hordeum vulgare*	97.95*	85.75*	97.95*	82.52*
13.	*Hyparrhenia rufa*	23.80	10.00	41.16	50.00
14.	*Lactuca sativa*	6.45	2.31	6.45	1.78
15.	*Lolium multiflorum*	82.43	42.93	78.37	42.65
16.	*Pennisetum americanum*	77.20	73.80	77.20	43.13
17.	*Plantago lanceolata*	54.30	24.50	61.40	35.60
18.	*Rumex dentatus*	77.14	64.00	91.42*	74.00
19.	*Salvia plebeia*	70.90	21.80	65.4	31.69
20.	*Senebiera didyma*	98.11*	56.75	94.33*	80.18
21.	*Setaria italica*	64.00	46.90	70.50	50.50
22.	*Sorghum vulgare*	84.52	53.81	79.76	81.62
23.	*Taraxacum officinale*	77.40	65.12	85.1*	61.57
24.	*Trifolium repens*	95.74*	78.06	103.19*	88.77*
25.	*Triticum aestivum*	90.62*	58.29	92.1*	54.32

Each value is a mean of 10 replicates, each with 10 seeds; all values expressed as % of their control. All the values are at least significant at p=0.05 except those with astrike (*).

Table 3. Effect of soaking duration (Hrs) on the toxicity of extracts against germination (%) and radicle growth + SD (mm) of the test species.

Test Species	Control	Soaking durations (Hrs) 6 Test	6 % of Control	12 Test	12 % of Control	24 Test	24 % of Control	48 Test	48 % of Control	96 Test	96 % of Control
***Cenchrus ciliaris* extract**											
					Germination (%)						
Brassica campestris	91a	82a	90.10	83a	91.20	84a	92.30	45b	49.30	29c	31.86
Setaria italica	93a	82a	91.39	84a	90.32	84a	92.30	84a	90.30	13b	13.97
Lactuca sativa	94a	78.00	82.98	80.0	85.11	76b	80.85	42	44.68	40	42.55
					Radicle growth ± SD (mm)						
Brassica campestris	10.90a	6.54b	60.00	6.54b	60.18	6.15b	56.42	0.45c	4.12	0.30d	2.75
±SD	±3.53	±1.41		±1.52		±1.25		0.13		±0.08	
Setaria italica	21.23a	20.50a	96.56	19.14a	90.15	9.89b	46.58	0.24c	1.13	0.19c	0.89
± SD	±2.40	±2.70		2.16		2.5		0.08		0.01	
Lactuca sativa	19.56	18.11	92.59	17.90	91.52	13.25	67.74	10.11	51.69	7.99	40.84
±SD	3.22	2.06						1.99		1.20	
***Bothriochloa pertusa* extract**											
					Germination (%)						
Brassica campestris	91a	86a	94.50	70b	76.92	93a	102.19	70b	76.92	42c	46.15
Setaria italica	93a	92a	98.92	89a	95.69	88a	94.04	62b	66.66	14c	15.05
Lactuca sativa	94	80	85.11	82	87.23	76	80.85	70	74.47	45	47.87
					Radicle Growth ± SD (mm)						
Brassica campestris	10.90a	4.91b	45.04	3.80bc	34.86	3.09c	28.34	1.45d	13.30	0.42e	3.85
± SD	±3.53	±0.08		±0.09		±0.05		±0.46		±0.11	
Setaria italica	21.23a	18.72b	88.17	17.04b	80.26	16.64b	78.37	2.02c	9.51	0.14d	0.65
± SD	±2.40	±2.79		±2.13		±3.25		±1.02		±0.05	
Lactuca sativa	19.56	18.59	95.04	17.85	91.26	15.01	76.73	14.95	76.43	12.59	64.37
±SD	3.22	2.30		2.11		2.00		1.29		1.30	

Means followed by the same letters are not significantly different from each other at P=0.05
Each value is the mean of 10 replicates, each with 10 seeds.

Table 4. Effect of constant leaching on the toxicity of extracts on the germination and growth of the test species.

No. extraction	Control	Radicle growth (mm)				Germination (%)		
		Cenchrus ciliaris extract		*Bothriochloa pertusa* extract		Control	*Cenchrus ciliaris*	*Bothriochloa pertusa*
		Test	Control	Test	Control			
				Brassica campestris				
1st	10.49 ± 2.6	1.87± *0.*5a	17.82*a	0.33 ± 0.4	3.14*a	84	37*a	15*a
2nd	8.63 ± 2.5	2.82 ± 0.6	32.67a	2.24 ± 0.7	25.98*b	81	41*a	42*b
3rd	11.99 ± 2.1	5.63 ± 1.2	46.95*b	6.82 ± 2.1	57.71*c	100	79*b	81*c
4th	9.33 ± 2.4	6.24 ± 1.7	66.88*b	7.54 ± 2.0	80.81*c	87	86b	83c
5th	9.33 ± 2.6	9.23 ± 2.7	98.92c	9.88 + 3.1	105.95d	87	86b	85c
				Lactuca sativa				
1st	9.50 ± 2.5	1.12 ± 0.5	11.79*a	0.50 ± 0.05	5.26*a	94	20*a	12*a
2nd	9.80 ± 2.3	1.42 ± 0.5	14.49*a	0.55 ± 0.05	5.61*a	94	25*a	10*a
3rd	9.84 ± 2.4	9.40 ± 2.6	96.44b	9.58 ± 2.3	97.36b	93	90b	94b
4th	7.63 ± 2.4	7.44 ± 2.1	97.51b	6.43 ± 1.7	84.27c	91	95b	78*c
5th	7.63 ± 1.9	7.68 ± 2.2	100.66b	6.34 ± 1.8	83.09c	91	88b	82*c
				Setaria italica				
1st	15.26 ± 2.11	7.28 ± 2.11	47.71	8.35 ± 2.11	57.72	100	45*a	56*a
2nd	17.01 ± 1.99	8.00 ± 2.00	47.03	8.50 ± 2.2	49.97	100	50*a	63*a
3rd	16.25 ± 2.11	9.99 ± 1.99	61.48	10.99 ± 2.0	67.08	100	69*b	73*a
4th	15.92 ± 2.01	10.26 ± 2.53	64.45	11.98 ± 2.3	75.25	100	75*b	80c
5th	15.30 ± 2.35	12.97 ± 2.16	84.77	13.55 ± 2.1	88.56	100	94c	98c

* and ** Significantly different from control at 0.05 and 0.01 level of probability.

Means followed by the same letters are not significantly different from each other at p=0.05 level of probability.

Each value is the average of 10 replicates, each with 10 seeds. (For number of extraction see text).

Table 5. Effect of aqueous extracts from shoots of *Cenchrus ciliaris* and *Bothriochloa pertusa* on the dry weight of shoots and roots of *Setaria italica*.

Extract concentration	Day	Shoot dry weight ± SD (mg)			Root dry weight ± SD (mg)		
		Control	*Cenchrus ciliaris*	*Bothriochloa pertusa*	Control	*Cenchrus ciliaris*	*Bothriochloa pertusa*
1:05	2	12.87 ± 2.13	6.57 ± 1.23**	10.75 ± 1.20	2.50 ± 0.51	1.60 ± 0.05*	1.57 ± 0.63*
	4	14.80 ± 2.41	6.97 ± 1.45*	7.30 ± 1.37**	3.77 ± 0.79	1.57 + 0.04**	1.47 ± 0.69**
	6	10.55 ± 1.92	10.00 ± 2.10	9.37 ± 21.10	1.77 ± 0.13	1.77 ± 0.12	1.65 ± 0.64
	8	13.42 ± 1.25	6.95 ± 1.73*	5.92 ± 1.24**	3.30 ± 0.21	1.30 ± 0.70**	1.12 ± 0.53**
	10	9.30 ± 1.41	7.97 ± 1.89	6.55 ± 1.47*	2.65 ± 0.13	2.90 ± 0.32	1.70 ± 0.71*
1:10	2	10.07 ± 1.62	10.85 ± 1.75	11.57 ± 2.01	2.05 ± 0.15	1.77 ± 0.16*	2.00 ± 0.62
	4	8.87 ± 1.20	6.40 ± 1.1*	8.07 ± 1.78	1.97 ± 0.16	1.67 ± 0.17	1.85 ± 0.71
	6	13.82 ± 2.10	6.62 ± 1.12**	10.50 ± 2.00	3.30 ± 0.87	1.05 ± 0.10**	1.67 ± 0.39*
	8	12.62 ± 1.29	7.87 ± 0.95**	8.07 ± 1.95*	2.25 ± 0.85	1.95 ± 0.07	1.10 ± 0.45*
	10	13.32 ± 1.01	9.37 ± 1.40*	9.12 ± 1.57*	5.75 ± 1.02	2.22 ± 0.90**	1.12 ± 0.48**

*= Significantly different from control at p=0.05; **= Significantly different from control at p=0.01
Each value is a mean ± SD of 4 replicates, each with one seedling.

Table 6. Effect of aqueous extracts from shoot of *Cenchrus ciliaris* and *Bothriochloa pertusa* on the moisture contents of shoots and roots of *Setaria italica* of 4 seedlings expressed as % dry weight ± SD.

Extract concentration	Day	Shoot moisture contents (%)			Root moisture contents (%)		
		Control ± SD	*Cenchrus* ± SD	*Bothriochloa* ± SD	Control ± SD	*Cenchrus* ± SD	*Bothriochloa* ± SD
1:05	2	346.40 ± 20.16	294.68 ± 15.61**	248.84 ± 16.21*	344.00 ± 21.45	368.75 ± 19.71	315.87 ± 18.38*
	4	450.17 ± 15.32	159.86 ± 10.15*	253.18 ± 17.90**	388.74 ± 20.51	315.87 + 17.85	169.49 ± 11.74**
	6	378.67 ± 17.72	128.50 ± 11.20**	154.40 ± 11.30**	421.13 ± 22.72	322.62 ± 16.94*	225.76 ± 10.23*
	8	543.58 ± 24.30	115.83 ± 13.13**	118.99 ± 9.72**	618.74 ± 27.83	459.62 ± 19.23*	364.44 ± 13.44**
	10	406.18 ± 18.50	182.13 ± 13.13**	121.37 ± 9.81**	566.98 ± 19.75	350.86 ± 17.97	276.47 ± 12.14*
1:10	2	497.77 ± 21.52	260.83 ± 18.54*	397.19 ± 21.31*	393.00 ± 20.12	430.00 ± 19.23	352.00 ± 13.44
	4	683.57 ± 27.61	303.13 ± 20.34*	385.71 ± 22.40**	244.00 ± 18.22	255.37 ± 15.26	255.41 ± 12.15
	6	439.57 ± 17.82	83.40 ± 7.85**	343.33 ± 22.71*	487.88 ± 17.55	297.62 ± 13.61*	434.33 ± 13.51*
	8	337.06 ± 16.73	136.51 ± 11.45**	261.61 ± 17.52*	677.78 ± 23.43	496.15 ± 14.16	550.00 ± 17.67*
	10	260.07 ± 20.33	166.67 ± 12.95**	211.23 ± 18.33*	328.57 ± 21.75	307.87 ± 15.95	224.44 ± 9.95*

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

Table 7. The effect of aqueous extracts from shoot of *Cenchrus ciliaris* and *Bothriochloa pertusa* on the dry weights of shoots and roots of *Sorghum vulgare*.

Extract concentration	Day	Shoot dry weight ± SD (mg)			Root dry weight ± SD (mg)		
		Control	*Cenchrus*	*Bothriochloa*	Control	*Cenchrus*	*Bothriochloa*
1:05	2	42.50 ± 3.54	45.17 ± 3.17**	43.17 ± 4.13*	17.50 ± 1.13	9.57 ± 1.11**	22.50 ± 3.13
	4	43.52 ± 3.56	36.42 ± 3.13*	40.05 ± 4.01	17.00 ± 1.21	8.27 + 0.95**	9.05 ± 1.12**
	6	48.45 ± 3.95	45.00 ± 3.94	42.50 ± 4.62*	16.20 ± 1.34	13.50 ± 1.12*	8.70 ± 0.79**
	8	48.02 ± 3.95	43.77 ± 3.81	38.67 ± 4.11*	10.00 ± 1.09	7.50 ± 0.72*	9.50 ± 0.72**
	10	55.47 ± 4.32	54.20 ± 4.73	28.12 ± 3.71**	17.02 ± 1.02	17.02 ± 1.02	4.45 ± 0.12**
1:10	2	45.50 ± 3.73	41.35 ± 4.11	52.12 ± 5.10*	13.62 ± 1.62	12.50 ± 1.21	11.47 ± 1.76
	4	43.50 ± 3.31	37.05 ± 3.17*	33.62 ± 3.36*	16.82 ± 1.72	9.27 ± 0.85*	8.47 ± 1.76*
	6	42.47 ± 4.13	37.50 ± 2.97*	41.40 ± 4.13*	10.82 ± 1.21	8.77 ± 0.71*	9.10 ± 0.83*
	8	44.32 ± 4.45	37.02 ± 3.41*	40.00 ± 4.10*	12.50 ± 0.98	8.20 ± 0.79	4.57 ± 0.03**
	10	49.50 ± 4.67	26.70 ± 2.59**	25.90 ± 2.39*	12.50 ± 1.00	8.95 ± 0.65*	8.77 ± 0.69*

* and ** Significantly different from control at p=0.05 and p=0.01
Each value is based on 4 replicates, each with one seedling.

Table 8. The effect of aqueous extracts from shoot of *Cenchrus ciliaris* and *Bothriochloa pertusa* on the moisture contents (%) of shoots and roots of *Sorghum vulgare* of 4 seedlings, expressed as % dry weights SD.

Extract concentration	Day	Shoot moisture contents (%)			Root moisture contents (%)		
		Control ± SD	*Cenchrus* ± SD	*Bothriochloa* ± SD	Control ± SD	*Cenchrus* ± SD	*Bothriochloa* ± SD
1:05	2	365.41 ± 26.13	319.36 ± 21.63	317.31 ± 21.63*	182.59 ± 10.11	122.19 ± 11.92*	162.55 ± 12.34
	4	417.12 ± 27.39	346.67 ± 25.13*	395.01 ± 21.95	433.84 ± 24.21	314.29 + 26.04*	183.98 ± 13.37**
	6	430.59 ± 25.11	406.66 ± 27.74	336.64 ± 23.51*	404.30 ± 25.14	257.77 ± 24.46**	249.69 ± 20.12**
	8	411.22 ± 25.95	186.07 ± 11.29**	152.18 ± 12.34**	425.00 ± 32.27	384.65 ± 23.17*	210.78 ± 19.01**
	10	355.65 ± 21.3	331.22 ± 21.94	168.17 ± 11.78**	391.58 ± 20.19	355.21 ± 19.57	324.15 ± 22.95
1:10	2	333.13 ± 20.17	314.63 ± 20.16	330.22 ± 23.24	279.52 ± 17.98	117.60 ± 10.11**	220.45 ± 20.13*
	4	436.03 ± 21.33	421.73 ± 22.66*	319.63 ± 20.79*	472.24 ± 25.81	301.19 ± 27.21*	426.55 ± 25.37*
	6	408.40 ± 22.75	353.04 ± 20.10*	366.96 ± 21.93*	411.44 ± 24.75	258.14 ± 19.93**	339.55 ± 24.21*
	8	341.50 ± 23.14	265.98 ± 17.73*	270.44 ± 20.17*	655.73 ± 30.51	391.46 ± 24.24**	302.13 ± 19.18**
	10	382.52 ± 19.73	171.53 ± 11.53**	185.13 ± 18.17*	303.20 ± 18.93	220.11 ± 22.15*	201.13 ± 17.52*

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

Table 9. Effect of aqueous extracts from shoot of *Cenchrus ciliaris* and *Bothriochloa pertusa* on the dry weights of shoots and roots of rice seedlings.

Extract concentration	Day	Shoot dry weight ± SD (mg)			Root dry weight ± (mg)		
		Control ± SD	*Cenchrus* ± SD	*Bothriochloa* ± SD	Control ± SD	*Cenchrus* ± SD	*Bothriochloa* ± SD
1:05	2	18.12 ± 1.13	10.00 ± 0.50	8.40 ± 0.75*	9.00 ± 0.75	6.87 ± 0.31*	8.90 ± 0.07
	4	12.50 ± 0.93	8.90 ± 0.73*	11.90 ± 0.78	9.32 ± 0.73	10.52 + 1.00*	6.62 ± 0.35**
	6	17.02 ± 1.21	8.70 ± 0.63*	9.52 ± 0.51*	7.50 ± 0.55	6.87 ± 0.34**	6.62 ± 0.39**
	8	9.52 ± 0.79	7.50 ± 0.75*	9.67 ± 0.83	7.37 ± 0.68	6.75 ± 0.49	6.52 ± 0.42
	10	12.50 ± 1.01	11.32 ± 1.02	11.77 ± 0.87	5.00 ± 0.21	4.30 ± 0.53	6.77 ± 0.17
1:10	2	13.97 ± 1.13	9.22 ± 0.93*	8.47 ± 0.94*	6.50 ± 0.34	5.00 ± 0.34	5.87 ± 0.21
	4	13.00 ± 1.11	9.27 ± 0.85*	8.40 ± 0.93*	9.52 ± 0.50	7.50 ± 0.19*	6.87 ± 0.38*
	6	15.00 ± 1.72	12.50 ± 0.99	7.50 ± 0.63**	6.25 ± 0.75	6.02 ± 0.11	6.22 ± 0.41
	8	11.67 ± 1.05	10.00 ± 0.55	11.52 ± 1.02	8.35 ± 0.77	7.50 ± 0.17	7.50 ± 0.51
	10	12.92 ± 1.04	10.60 ± 0.67	13.75 ± 1.12	8.52 ± 0.89	6.05 ± 0.31*	4.27 ± 0.39**

*= Significantly different from control at p=0.05; **= Significantly different from control at p=0.01
Each value is based on mean of 4 replicates, each with one seedling.

Table 10. Effect of aqueous extracts from shoots of *Cenchrus ciliaris* and *Bothriochloa pertusa* on the shoots and roots moisture contents (%) of 4 Rice seedling, expressed as % of dry weights ± SD

Extract concentration	Day	Shoot Moisture contents ± SD			Root moisture contents ± SD		
		Control ± SD	*Cenchrus* ± SD	*Bothriochloa* ± SD	Control ± SD	*Cenchrus* ± SD	*Bothriochloa* ± SD
1:05	2	365.41 ± 30.16	319.36 ± 27.01*	317.31 ± 19.97*	189.57 ± 11.39	122.19 ± 11.76*	162.55 ± 13.19
	4	417.12 ± 20.65	346.67 ± 26.51*	395.01 ± 21.39	433.84 ± 20.16	314.79 + 23.49*	249.69 ± 17.47**
	6	430.59 ± 28.51	406.66 ± 25.16	336.64 ± 23.07*	404.30 ± 21.47	257.77 ± 21.44**	210.78 ± 17.47**
	8	411.22 ± 31.75	186.07 ± 17.23**	152.18 ± 11.67**	425.00 ± 26.73	384.86 ± 23.10	324.15 ± 21.55**
	10	355.65 ± 20.63	331.22 ± 19.36	168.17 ± 13.79**	391.58 ± 20.16	355.21 ± 24.48	220.45 ± 21.55**
1:10	2	333.13 ± 20.18	314.63 ± 20.12	330.22 ± 20.15	279.52 ± 18.71	117.60 ± 12.16**	420.45 ± 23.11
	4	436.03 ± 22.09	421.73 ± 24.29*	319.63 ± 21.15	472.24 ± 23.17	301.19 ± 27.08*	426.55 ± 26.10*
	6	408.40 ± 27.11	353.04 ± 21.93*	366.96 ± 30.10*	411.44 ± 25.19	258.14 ± 25.11**	339.55 ± 18.89*
	8	341.50 ± 25.15	268.98 ± 20.11	270.44 ± 27.33*	655.73 ± 31.29	301.46 ± 20.19**	302.20 ± 21.90**
	10	382.52 ± 19.78	171.53 ± 16.75**	185.13 ± 9.13**	303.20 ± 20.90	220.11 ± 13.77**	201.13 ± 20.07**

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

Table 11. Effect of aqueous extracts of *Cenchrus ciliaris* and *Bothriochloa pertusa* on the viability of seeds of test species.

Treatments	Test species					
	Brassica campestris		*Lactuca sativa*		*Setaria italica*	
	Test	% of Control	Test	% of Control	Test	% of Control
I (Control)	78	100.0	100	100	92	100.0
II	75	96.15	98	98	96	104.34
III	19**	24.36	57	57	76**	82.61
IV	27**	34.62	59	59	61**	66.30
V	63*	80.77	62	62	65**	70.65
VI	60*	76.92	64	64*	68**	73.91
VII	55**	70.51	74	74*	77**	83.69
VIII	61*	78.21	72	72*	71*	77.17
IX	33**	42.31	50	50**	80*	86.96
X	26**	33.33	54	54**	75**	81.52
XI	55**	70.51	58	58*	87	94.56
XII	63*	80.76	62	62*	66**	71.73

*= Significant at p= 0.05; **= Significant at p= 0.01

Each value is a mean of 10 replicates, each with 10 seeds. (See details of treatments in the text).

6. Effect on seed viability: Table 11 represents the following results. Treatment I was taken as the true control for the experiment.

1. There were no differences in germination between treatments I & II for the test species, showing that soaking alone could not inhibit germination.
2. The maximum inhibited germination was observed in treatments III & IV ($p>0.01$) for the test species.
3. The treatments IX & X significantly ($p>0.01$) inhibited the germination due to presence of toxins in the extracts.
4. Treatments V &VI also showed significant inhibition; showing that reduction was due to extract and that seeds were not killed.
5. The germination in VII & VIII treatments was comparatively high, suggesting that the viability of seeds was not lost; however, germination was reduced.
6. There were insignificant differences in germination between treatments V & XI and between VI & XII.

The results suggest that the extracts did not killed the seeds, but significantly suppressed the germination, especially when seeds were either soaked and then grown in extract or grown in distilled water, or when they were soaked in extracts, washed and then grown in extract. In nature, when the seeds are subjected to constant supply of toxins from both these plants, their germination might be retarded.

7. Mulching experiment: Germination and dry weights of all the three test species was significantly reduced in treatments II, III, VI and VII containing phytotoxins. The germination and growth was slightly better in treatments IV & V (Table 12) where shoots were washed with water. When shoots were allowed to remain in soil for two months and the soil was used in soil bed and soil extract bioassays, it was seen that although germination of all the three test species remained almost unaffected, yet the radicle growth of all the test species was significantly ($p>0.05$) inhibited (Table 13). It was concluded that released toxins rendered soil unfavourable for the growth of test species.

Table 12. The effect of added shoot mulch on the germination and dry weight of test species.

Treatments	*Pennisetum americanum*		*Sorghum vulgare*		*Setaria italica*	
	Test	% of Control	Test	% of Control	Test	% of Control
	Germination (%)					
I (Control)	100.00	100	95.00	100.00	100	100
II	72.50	72.50*	70.00	73.68*	62	62**
III	75.5	75.50*	72.50	76.31*	70	70**
IV	70.00	70.00*	72.50	76.31*	78	78*
V	77.00	77.00*	85.00	89.47*	83	83*
VI	60.00	60.00*	45.00	47.36*	60	60**
VII	62.5	62.50*	60.00	63.15*	68	68**
	Dry weight ± SD (mg)					
I (Control)	22.62 ± 1.53	100a	62.10 ± 3.45	100a	59.20 ± 2.96	100
II	16.62 ± 1.71	74.80b	46.92 ± 2.15	75.55b	35.20 ± 3.0	59.46
III	15.90 ± 1.90	70.29b	42.42 ± 2.17	68.30b	45.29 ± 3.80	76.50
IV	19.35 ± 1.27	85.54c	56.75 ± 2.75	91.38c	50.12 ± 4.50	84.66
V	18.52 ± 1.65	81.87c	52.97 ± 3.12	85.29c	55.9 ± 4.8	94.43
VI	10.65 ± 1.34	47.08d	28.37 ± 2.50	47.29d	40.7 ± 3.01	68.75
VII	11.65 ± 1.43	51.50d	28.00 ± 2.17	45.08d	45.16 ± 5.20	76.28

*= Significantly different from control at p=0.05

Values followed by the same letters are not significantly different from each other at 0.05 level of probability. Each value is the average of 4 replicates, each with 10 seeds in the case of germination and dry weights are average per pot of 4 replicates, each with 4 seedlings. (See details of treatments in the text).

Table 13. Effect of added shoot mulch on the soil toxicity in soil extract and soil bed bioassays against the germination and radicle growth.

Test species	Bioassay	*Cenchrus ciliaris*			*Bothriochloa pertusa*	
		Control	Test	% of Control	Test	% of Control
		Germination (%)				
Brassica campestris	Soil extract	82	80	97.56	88	107.32
	Soil bed	90	89	98.89	86	95.56
Lactuca sativa	Soil extract	78	74	94.87	76	97.94
	Soil bed	80	76	95.00	74	92.50
Setaria italica	Soil extract	94	88	93.61	80	85.11
	Soil bed	98	90	91.84	78	79.59
		Radicle growth (mm)				
Brassica campestris	Soil extract	7.52 ± 1.14	5.76 ± 1.20	76.60*	5.82 ± 1.11	77.39*
	Soil bed	11.23 ± 1.95	4.63 ± 0.5	41.23**	5.55 ± 0.7	49.42**
Lactuca sativa	Soil extract	13.21 ± 1.29	6.59 ± 1.20	49.89**	7.01 ±1.02	53.07**
	Soil bed	15.98 ± 2.20	7.39 ± 2.10	46.77**	8.11± 2.11	50.75**
Setaria italica	Soil extract	14.93 ± 2.60	7.25 ± 2.30	48.56**	8.99 ± 2.8	60.21*
	Soil bed	15.88 ± 3.11	8.11 ± 1.90	51.07**	10.25 ± 2.01	64.55*

** =Significantly different from control at p=0.05

Each value is a mean of 5 replicates, each with 10 seeds in the case of soil extract bioassay and soil bed bioassay is mean of 3 replicates each with 10 seeds.

Discussion

Many grasses exhibit allelopathy against the associated species by releasing of water soluble phytotoxins through rain, dew or irrigation water. The present study observed that aqueous extracts from inflorescences, leaves, stems and roots obtained by soaking for

various durations invariably reduced germination, seedling growth, biomass and moisture contents of test species. It was also shown that different parts of the same plant had differential toxicity not only against the different test species but also against the various growth parameters. Hisashi *et al*., (2009) and Samreen *et al*., (2009) reported that growth of roots and shoots was independently affected by the same extract. Our findings in this regards agree with other studies (Hussain *et al*., 2004, 2005; Iman *et al*., 2007; Otusanya *et al*., 2008) who reported that various parts of same plant have differential phytotoxicity. Dirvi & Hussain (1978) reported that *Dichanthium* was more allelopathic than *Hyparrhenia* and that shoots were inhibitorier than roots. On the other hand rhizomes/ roots of *Imperata cylindrica* were more toxic than shoots (Hussain & Abidi, 1991). The leaves of *Datura* (Hussain *et al*., 1978) were more inhibitory than other parts. Similarly *Xanthium* plant parts had differential phytotoxicity against various test species (Inam *et al*., 1987). In the present study when 25 different test species were tested against same extracts it was seen that they responded differently from each other and germination and seedling growth were also independently affected. Many other workers (Hoque *et al*., 2003; Kadioglu & Yanar, 2004; Otusanya *et al*., 2008; Samreen *et al*., 2009; Hisashi *et al*., 2009) have also suggested differential inhibition of seedling growth and germination in different species. The findings show that germination of some test species was inhibited and that of other either remained unaffected or stimulated. It can be inferred that susceptible species might be eliminated from common habitat while resistant species might share the habitat. The present findings are in line with those of Hoque *et al*., (2003), Shaukat *et al*., (2003), Kadioglu & Yanar, (2004), Iannuci, (2007), Otusanya *et al*., (2008) and Samreen *et al*., (2009) who also reported similar findings for other species. It was interesting to note that the response of germination, seedling growth, biomass and moisture contents of test species towards the same extract was variable and this agree with Hisashi *et al*., (2009).

The observed low moisture contents of shoots and roots might be due to the inability of roots to absorb sufficient water from the growth medium or due to high transpiration rate due to allelopathic stress. Reduced root or shoot moisture contents create physiological drought like condition that imbalance the various biochemical functions to ultimately decrease the overall growth performance (Lodhi & Nickell, 1973; Dirvi & Hussain, 1978; Samreen *et al*., 2009). Allelopathy reduces the absorption of water of susceptible plants from the growth medium.

The present findings suggest that irrigation water, rains, dew and even soil moisture might release water soluble substances from living parts and/or from litter from both these grasses rendering the nearby soil unfavourable. The findings agree with those of Batlang & Shushu (2007) who stated that sunflower residue inhibited growth of *Vigna*. The toxicity of aqueous extract and release of phytoxins however was dependent upon the duration of soaking. Enhancing the soaking duration from 6 to 96 hrs might have facilitated either the release of more quantity or more types of phytotoxins or both from these grasses that augmented the phytotoxicity. Samreen *et al*., (2009) and Batlang & Shushu (2007) suggested that allelopathic stress depends upon concentration of allelopathic material. In nature irrigation, rain and soil moisture will continuously leach out phytotoxins from these grasses to deposit in the soil. Similarly, Samreen *et al*., (2009) also demonstrated that enhancing soaking duration increased phytotoxicity of aqueous extracts. This behavior was further confirmed in another experiment where constantly leaching plant chemicals lost phytotoxicity against the same test species. The aqueous extracts obtained after repeated leaching (1^{st} to 5^{th} extraction in this case) had

germination and radicle growth values almost equal to that of control in the 5th extraction. This experiment suggests that repeated leaching from litter might release maximum possible amount of phytotoxins but on the other hand it is also possible that the toxicity might be reduced or lost with constant leaching. There must be regular source of phytoxins to manifest allelopathy. It has been frequently observed in the present and many other studies (Achhreddy & Singh, 1984; Kadioglu & Yanar, 2004; Hoque *et al*., 2003; Shaukat *et al*., 2003) that low concentration of allelopathic substances either had no inhibition or stimulated germination and radicle growth. The findings are in line with those of Samreen *et al*., (2009) who reported that the toxicity of aqueous extracts from *Calotropis* depended upon the soaking duration and amount of material soaked.

The poor and delayed germination of test species under allelopathic stress might be due to either death of embryo or imposition of dormancy or inactivity of seeds (Nasr & Shariati, 2005). When this aspect was envisaged, it was found that embryo within seeds were not killed by aqueous extracts. Seeds retained viability which was confirmed by TTC test. Instead seeds went into state of dormancy or inactivation under allelopathic stress. Seeds when grown or treated with aqueous extracts (Treatments III & VI and IX & X) failed to germinate. Whenever, extracts were removed or washed (Treatments VII & VIII), they germinated better. In nature this response of seeds might provide them chance to survive under allelopathic stress. Furthermore, it is also possible that seeds might remain protected against microbial action in soil in the presence of allelopathic substances. This aspect can be exploited further by using extracts to prevent decay of seeds in soil (Rice, 1984; Nasr & Shariati, 2005). Seeds might germinate whenever conditions turn favourable due to loss of allelopathic substances from soil.

Aqueous extracts obtained at normal room temperature exhibited phytotoxicity. It was further desired to see the behavior of extract obtained through boiling. Although, it is highly unnatural to use boiling water for extraction of phytotoxins, yet many workers (Lodhi & Nickell, 1973) have utilized this technique. We observed that hot water extract invariably reduced the seedling biomass and moisture contents of *Sorghum vulgare*, *Setaria italica* and *Oryza sativa*. However, no definite trend could be established between the duration of the experimental period (2-10th day) and reduction in growth parameters. The sufferings of 1st two species were natural as they are mesophytes, but rice, which is hydrophyte, was also inhibited due to presence of phytotoxins in the extracts. Lodhi & Nickell (1973) observed that hot water extract from *Celtis* reduced the growth, moisture contents and gas exchange capacity of test species in a similar experiment. It was concluded that hot water extract from both these grasses not only reduced the time period for extraction of toxins but also retained phytotoxicity. The present findings regarding the toxicity of hot water extracts are supported by many similar studies (Lodhi & Nickell, 1973). It is obvious that soil or air temperature never reaches boiling point in nature but the summer temperature may fluctuate between 40-45^{o}C in the plains of Pakistan. It is quite possible that aqueous extracts from *Cenchrus ciliaris* and *Bothriochloa pertusa* under high temperature not only retain toxicity but might become concentrated in the soil due to evaporation of soil moisture. Furthermore, the phytotoxins might accumulate in the upper soil layers due to upward movement by evaporative power of air, thereby further intoxicating the soil for the susceptible plants.

The added shoot mulch of both these grasses retarded the germination and seedling growth of test species. However, soil extract when used neither suppressed germination nor seedling growth probably due to low concentration of inhibitors. Many workers (Achhereddy & Singh, 1984; Hoque *et al*., 2003; Hussain *et al*., 2004, 2005; Samreen *et*

al., 2009) have also reported ineffectively or stimulatory effects of aqueous extracts with low concentration of inhibitors in soil or bioassays. Batlang & Shushu (2007) reported that sunflower residue completely inhibited nodulation and flowering of *Vigna*. The addition of litter from both these grasses might render the soil unfavourable for the germination and growth of susceptible species provided a constant source of phytotoxins is available.

Both these grasses also exhibited auto-toxicity against themselves. Rice (1984) documented many plants with reported autotoxicity. The observed gain of dominance, replacement of other associated species and self declination of *Cenchrus* pasture with passage of time might partially be due to allelopathy (Hussain *et al*., 1982). Iman *et al*., (2006) also reported autotoxicity by some crops species.

It was also seen in various bioassays and experiments that *Cenchrus* was more toxic than *Bothriochloa*. Allelopathy depends upon the inherent capability of donor plant to synthesize and release phytotoxic substance. Many studies on allelopathy have concluded similarly regarding inter specific variations in the manifest of allelopathy (Hussain *et al*., 2004, 2005; Iman *et al*., 2006; Hisashi *et al*., 2009). Allelopathy is a complex ecological process operating within the environmental complex; therefore the fate of added litter and released phytotoxins depends on the related habitat conditions including the character of soil and climate. Although, it is concluded that both these grasses are strongly allelopathic at least against the present test species yet it becomes difficult to separate competition and allelopathy as the relative importance of these two phenomena are difficult to distinguish in nature (Dofdotter *et al*., 2002). Shoots generally appeared to be inhibitorier than other parts. The toxicity depended upon the parts assayed, test species and physiological parameter involved. However, further study is required to see the role of root exudates and rain leachates in manifesting allelopathy and to identify the phytotoxins responsible for allelopathy.

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