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ALLELOPATHIC POTENTIAL OF COLUMBUS GRASS (*SORGHUM ALMUM*) (PIPER) PARODI

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Sorghum almum (Piper) Parodi (Columbus grass), adapted to tropical and subtropical climates on variety of soils is yet to be introduced as a range grass in Pakistan. Besides self-inhibition, it retarded growth of *Pennisetum americanum* and *Setaria italica* by root exudates in mixed cultures. Aqueous extracts from various parts of the plant, root exudates collected in laboratory and soil taken from underneath Columbus grass exhibited phytotoxicity against *Brassica campestris, Cenchrus ciliaris, Lolium multiflorum, Pennisetum americanum, Setaria italica, Panicum antidotale* and *Sorghum almum.* Shoot extract reduced germination, fresh and dry biomass, water-contents and survival of the test species. Phytotoxicity depended upon the part assayed, test species used and physiological process involved. The introduction of Columbus grass would be ecologically unsuitable as pure or in mixed cultures with the above-mentioned species due to allelopathy.

INTRODUCTION

Sorghum almum (Piper) Parodi (Columbus grass), a native of Africa is adapted to tropical and subtropical climates on variety of soils [24]. It is introduced into dryer parts of Argentina and America [2]. Though the species is not found in Pakistan but experiments are in progress at the Pakistan Forest Institute, Peshawar for its introduction as a range grass.

The grass is preferred for its extreme drought resistence; easy germination, establishment and rapid growth producing enough palatable forage; high seed out put and sand binding capacity [2, 24].

Biochemical inhibition by Italian Rye-grass [20,21], *Cenchrus* and *Chrysopogon* [3], prairie grasses [4], *Chloris* and *Panicum* [17], *Sorghum almum* [22] and *Dichanthium annulatum* [8] under favourable physical environment have been reported. Columbus grass, besides showing self-declination of the pasture [9, 24], affected the growth of associated grasses and cultivated crops [5,10,13,16,18].

Biochemical ecology of introduced and weedy species must be worked out in relation to the local existing species. Since, *Sorghum almum,* is expected to be introduced as a range grass in Pakistan, therefore, keeping in mind the significance of allelopathy [19], the present investigation was conducted to find its allelopathic potential.

EXPERIMENTAL PROCEDURES AND RESULTS

Pot Interference Experiment. Interfering ability of *Sorghum almum* was tested by growing it with *Pennisetum americanum, Setaria italica* and *Sorghum* itself in either root separated or mixed culture following pot experiment of Akhtar *et al.* [3]. Seedlings were raised from seeds and thinned to 5 healthy uniform seedling per each half of the pot with 5 replicates. Experiment was commenced on June 23, 1978, and above ground parts were harvested after 3 months. Height, fresh and dry weights were determined.

Height, fresh and dry biomass of all the test species was significantly reduced in root mixed cultures by Columbus grass. Columbus grass, besides self-inhibition, was slightly affected by the other species sown in mixtures (Table 1).

Plot Interference Experiment. A 4×2 m loamy field plot was thoroughly watered a few days before starting the experiment to remove the possible residual toxicity due to previous plants. *Sorghum almum* was grown in mixed cultures with *Setaria italica* in alternate rows following Dirvi and Hussain [8]. Each species from mixed culture was extended on either side to make monocultures, serving as control. Thinning was done to avoid intraspecific competition. Water and Hoagland's solution was provided fortnightly to eliminate physical competition. Experiment was started on June 23, 1978, and regularly weeded by hand. After 4 months, height, fresh and dry weights of 5 plants, selected randomly in triplicate from mono- and mixed-cultures were determined.

Setaria italica showed reduced height, fresh and dry biomass in mixed cultures while Columbus grass remained unaffected, suggesting a possible inhibition by roots of *Sorghum almum* (Table 2).

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Table 1. Height, fresh and dry weights of interacting species in pot interference experiment.

Observation	Species	Sowing condition	Interfering species		
			Pennisetum	*Setaria*	*Sorghum*
Height (cm)	Test species	Root separated	14.2	6.3	19.2
		Root mixed	8.1	3.5	16.1
		Control (%)	57.04*	55.55*	83.85*
	Sorghum almum	Root separated	24.30	19.90	22.40
		Root mixed	21.60	19.50	19.00
		Control (%)	88.88	98.00	80.48
Fresh weight (g)	Test species	Root separated	5.80	00.80	11.70
		Root mixed	2.40	00.20	9.00
		Control (%)	41.38*	25.00*	70.60*
	Sorghum almum	Root separated	8.00	25.80	16.50
		Root mixed	4.60	22.50	15.80
		Control (%)	57.50*	87.20*	97.75
Dry weight (g)	Test species	Root separated.	2.70	0.30	5.80
		Root mixed	0.80	0.10	4.50
		Control (%)	29.63*	33.33*	77.58*
	Sorghum almum	Root separated	3.80	11.80	8.40
		Root mixed	3.10	8.10	8.00
		Control (%)	91.57	68.47*	95.23

Each value is a mean of 5 replicates, each with 5 plants.
*Significant at P = 0.05

Table 2. Height, fresh and dry weights of the interacting species in plot experiment.

Observations	Sowing conditions	Interacting species	
		Sorghum	*Setaria*
Height (cm)	Monoculture	185.50	63.60
	Mixed culture	180.00	55.00
	Control (%)	97.56	84.47*
Fresh weight (g)	Monoculture	477.10	17.50
	Mixed culture	329.20	5.30
	Control (%)	68.55*	30.28†
Dry weight (g)	Monoculture	85.30	8.60
	Mixed culture	78.10	3.60
	Control (%)	91.55	41.86*

Each value is the mean of 3 replicates, each with 5 plants.
*Significant at P = 0.05, †Significant at P = 0.01.

Allelopathic Studies

Inflorescences, shoots (leaves and stems) and roots of mature plants of *S. almum* were dried at room temperature (25–30°) in shade. Aqueous extracts were prepared by soaking 5 g powdered material in 100 ml double distilled water for 24 hr at 25° and filtered. Extracts were stored at 5–10° when not in use.

Petri dishes (9 cm dia) with two layers of Whatman filter paper No. 1 seed-beds were used as substrate for the seeds of test species. Tests and controls were made by soaking the seed beds with extract and distilled water respectively. Petri dishes were sealed with parafilm 'M' to avoid moisture loss. The dishes were incubated at 26° for 48 hr followed by recording germination and radicle growth. There were always 5 replicates, each with 10 seeds unless otherwise stated. This procedure would be referred to as standard filter paper bioassay in the present study. Results were statistically analysed using Z and t tests [7].

Aqueous Extract Bioassay. Aqueous shoot extract of Columbus grass was used against *Brassica campestris, Cenchrus ciliaris, Lolium multiflorum, Panicum antidotale, Pennisetum americanum, Setaria italica* and *Sorghum almum* in standard filter paper bioassay.

The extracts significantly inhibited germination and radicle growth of all the test species. *Pennisetum, Lolium Brassica* and *Setaria* were more affected than others. The in-

Table 3. Effect of aqueous shoot extract on germination and radicle growth of the test species.

Test species	Control	Test	Control (%)
	Germination (%)		
Brassica campestris	70.00	16.00	22.85†
Cenchrus ciliaris	66.00	50.00	75.75*
Lolium multiflorum	92.00	8.00	8.69†
Panicum antidotale	50.00	16.66	33.32†
Pennisetum americanum	68.00	14.00	20.58†
Setaria italica	92.00	40.00	43.47†
Sorghum almum	28.00	14.00	50.00*
	Average radicle growth ±SD		
Brassica campestris	37.80±0.69	0.20±0.02	5.29†
Cenchrus ciliaris	6.84±1.04	5.22±1.19	76.31*
Lolium multiflorum	7.84±1.34	0.22±0.06	2.72†
Panicum antidotale	1.26±0.01	0.28±0.07	22.22†
Pennisetum americanum	19.74±2.16	0.30±0.04	1.51†
Sorghum almum	1.53±0.22	0.54±0.03	33.33†

Each value is the mean of 5 replicates, each with 10 seeds.
*Significant at P= 0.05, †Significant at P= 0.01

Table 4. Effect of root exudate of *Sorghum almum* on germination (%) and radicle growth (mm) of the test species.

Test species	Germination (%)			Radicle growth (mm)				
	Control	Test	Control (%)	Control	±SD	Test	±SD	Control (%)
Brassica campestris	46.66	40.00	85.72*	1.60	0.52	1.16	0.56	72.50*
Cenchrus ciliaris	16.66	6.66	39.97†	0.96	0.67	0.43	0.03	44.79**
Lolium multiflorum	70.00	60.00	85.71*	2.63	0.40	2.86	0.60	108.74
Pennisetum americanum	86.66	86.66	100.00	31.76	8.57	24.00	5.82	75.56*
Setaria italica	96.66	96.66	100.00	22.56	4.33	19.03	4.46	84.35*
Sorghum almum	80.00	13.33	16.66†	4.10	1.05	0.16	0.05	3.90**

Each value is the mean of 3 replicates, each with 10 seeds.
*Significant at P = 0.05, †Significant at P = 0.01

hibition was due to water-soluble toxins present in the grass extract. The susceptibility of the species varied (Table 3).

Relative Toxicity of Grass Parts. Extracts from inflorescences, shoots and roots were tested against the aforementioned test species using standard filter paper bioassay.

Germination and growth of all the test species was significantly retarded except germination of *Panicum* in root extract and of *Pennisetum* in inflorescence and root extracts (Fig. 1). Above ground parts, especially the shoots, were more inhibitory than roots. Germination and radicle growth of the test species was not equally affected.

Root Exudate Bioassay. Six-month old plants of Columbus grass, after thoroughly washing their roots, were individually transplanted into 17×7 cm sterilized glass vials with 300 ml Hoagland's solution in triplicate. The vials were completely wrapped with brown paper to avoid algal growth due to light. These vials containing plants were kept under 16 hr photoperiod at room temperature (25–30°). After 10 days, the solutions from all the vials were filtered after mixing and referred to as test solution. Seeds of *Brassica campestris, Cenchrus ciliaris, Lolium multiflorum, Pennisetum americanum, Setaria italica* and *Sorghum almum* were grown in the test solution for 48 hr at 26°. Controls were made with Hoagland's solution.

Germination and radicle growth of all the test species, except germination of *Pennisetum* and *Setaria,* was significantly inhibited by root exudates (Table 4). Columbus grass itself was more susceptible followed by *Cenchrus.* The observed inhibition was due to presence of some toxic compounds in the solution.

Aqueous Culture Bioassay. Shoot extract was mixed with equal volumes of Hoagland's nutrient solution to get test solution. Control was made similarly by replacing extract with double distilled water. Twenty ml of either test or control solution was taken in 8×1.6 cm glass vials. Fifteen-day old uniform healthy seedlings of *Pennisetum americanum* and *Setaria italica* were used as the test species. Roots were thoroughly washed first with tap water followed by distilled water. A single seedling of each test species was then transferred to each of the vial. The vials were plugged with cotton and wrapped with brown paper to cut light supply into the vials. Each species in each treatment having 10 replicates was kept under 16 hr photoperiod for 5 days. Survival was recorded daily. After determination of fresh weight, the seedlings were dried at 60° for 72 hr for dry weight and moisture-content determination.

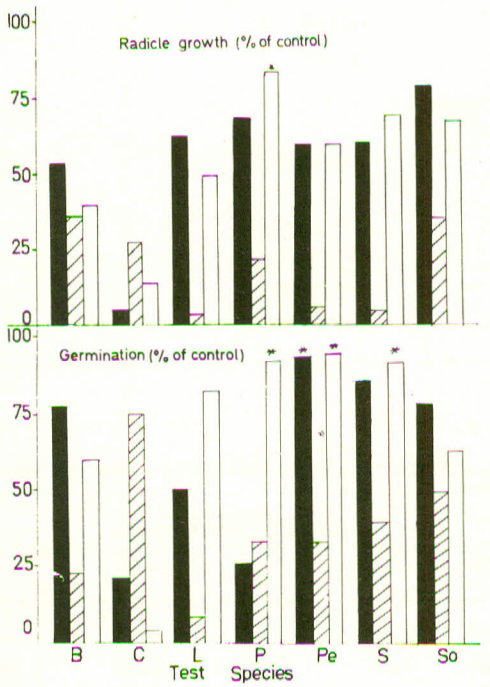

Fig. 1. Effect of aqueous extracts of inflorescence (solid), shoot (hatched) and roots (blank) on germination and radicle growth of test species. [Each value is a mean of 5 replicates, each with 10 seeds, expressed as % of control. *All values significant at P = 0.05 except (*)].
(B=*Brassica campestris*, C=*Cenchrus ciliaris*, L=*Lolium multiflorum*, P=*Panicum antidotale*, Pe=*Pennisetum americanum*, S=*Setaria italica*, So=*Sorghum almum*).

Survival of *Pennisetum* and *Setaria* was 30 and 62.5% respectively on the third day and it decreased further on the following days (Table 5). Extract, besides reducing the fresh weight, inhibited water absorption by the roots, thus significantly reducing water contents of the seedlings (Table 5). The low survival, reduction in biomass and water contents of the seedlings was the result of phytotoxicity of Columbus grass.

Sand Culture Experiment. Equal volume of washed river sand, sterilized at 170° for 4 hr, was taken in 8×6 cm pots. Ten seeds of *Pennisetum americanum* and *Setaria italica* were sparately sown in each of the pot. Test and control solutions, used for watering the pots, were made by mixing equal volumes of Hoagland's solution or double distilled water respectively. Germination was recorded after 4-day incubation at 26° and seedlings thinned to 4 per pot. Pots were then transferred to 16 hr photoperiod at room temperature (25–30°). Fresh, dry weight and moisture contents of the tops of 10) replicates were determined after 3 weeks.

Aqueous extract besides reducing germination, fresh and dry biomass, significantly reduced moisture contents of both the test species in the presence of nutrients, suggesting the inhibition of water absorption by roots (Table 6).

Soil Residual Toxicity. Soil was collected up to 9-cm depth from areas with or without Columbus grass and treated after Dirvi and Hussain [8]. Soil extract and soil-bed bioassays were run against the aforementioned test species following Dirvi and Hussain [8].

Table 5. Effect of aqueous shoot extract on fresh and dry weight (g), moisture (%) and survival (%) of the test species.

Test species	Conditions	Fresh weight (g)	Dry weight (g)	Moisture (%)	Survival (%/days) 1	2	3	4	5
Pennisetum americanum	Control	350.87	105.88	231.38	100	100	100	100	100
	Test	307.22	98.37	200.31	100	90	30	20	20
	Control (%)	87.55*	93.00	86.56*	100	90	30	20†	20†
Setaria italica	Control	587.19	109.28	437.32	100	80	80	80	80
	Test	350.79	100.70	248.35	100	50	50	10	10
	Control (%)	59.74†	92.14	58.78†	100	62.5*	62.5*	12.5†	12.5†

Each value is the mean of 10 replicates, each with one seedling.
*Significant at P = 0.05, †Significant at P = 0.01

Table 6. Effect of aqueous shoot extract on germination, growth and water contents of the test species in sand culture experiment. Germination is mean of 10 replicates each with 10 seeds while other values are mean of 10 replicates each with 4 seedlings.

Test species	Observations	Control	Test	Control (%)
Pennisetum americanum	Germination (%)	87.00	70.00	80.45*
	Fresh weight (mg)	103.29	73.32	70.98*
	±SD	15.22	8.95	
	Dry weight (mg)	12.11	11.34	93.64
	±SD	1.32	1.86	
	Moisture contents (%)	752.93	546.56	72.59*
	±SD	43.65	50.12	
Setaria italica	Germination (%)	82.00	72.00	87.80*
	Fresh weight (mg)	30.99	12.38	39.94*
	±SD	5.64	2.21	
	Dry weight (mg)	4.56	4.16	91.22
	±SD	0.74	1.01	
	Moisture contents (%)	559.86	197.59	35.29*
	±SD	41.86	32.93	

*Significant at P = 0.05.

Table 7. Effect of *Sorghum almum* soil extract on germination (%) and radicle growth (mm) of the test species.

Test species	Germination (%)			Radicle growth (mm)				
	Control	Test	Control (%)	Control	±SD	Test	±SD	Control (%)
Brassica campestris	54.00	46.00	85.18*	2.36	0.63	1.82	0.31	77.11†
Cenchrus ciliaris	24.00	22.00	91.66	1.64	0.66	1.28	0.83	78.04*
Lolium multiflorum	84.00	90.00	107.14	9.54	2.64	10.14	2.01	106.28
Panicum antidotale	30.00	14.00	46.66†	1.26	1.00	0.50	0.04	39.68†
Pennisetum americanum	100.00	94.00	94.00	12.74	0.02	10.02	1.22	78.64*
Setaria italica	94.00	94.00	100.00	14.16	2.77	13.30	1.20	93.92
Sorghum almum	28.00	10.00	35.71†	1.54	0.62	0.22	0.03	14.28†

Each value is a mean of 5 replicates, each with 10 seeds.
*Significant at P = 0.05 †Significant at P = 0.01

(a) Soil Extract Bioassay: Germination of *Brassica, Panicum* and *Sorghum* and growth of all the test species, except *Lolium* and *Setaria,* was significantly inhibited by grass soil extract (Table 7). *Sorghum* itself was more affected than the others.

(b) Soil-Bed Bioassay: Grass soil, used as seed beds, significantly inhibited germination and radicle growth of all the test species, except germination of *Pennisetum* and *Setaria* (Table 8).

The observed inhibition was due to release of phytotoxins from Columbus grass into the soil, rendering it toxic for the growth of susceptible species. The toxicity was species related.

DISCUSSION

Biochemical inhibition of germination and growth of species is a widespread phenomenon in plants. Columbus grass reduced growth of test species in the root mixed cultures by toxic root exudates. The root exudate bioassay further confirmed the release and toxicity of root exudates. *Lolium, Cenchrus, Dichanthium* and tobacco similarly inhibited growth of test species by root exudates [3,8,15,20]. Rovira [23] and Woods [25] reported inhibitory effects of root exudates of many species.

The extraction of toxins by soaking grass material in distilled water suggested a possible toxin transport mecha-

Table 8. Germination (%) and radicle growth (mm) of test species grown on *Sorghum almum* soil beds.

Test species	Germination (%)			Radicle growth (mm)				
	Control	Test	Control (%)	Control	±SD	Test	±SD	Control (%)
Brassica campestris	83.33	63.33	75.99*	3.80	0.75	2.50	0.65	65.78*
Cenchrus ciliaris	40.00	16.66	41.65†	2.63	0.49	0.93	0.30	35.36†
Lolium multiflorum	83.33	66.66	79.99*	10.83	0.68	5.93	1.00	54.75†
Panicum antidotale	23.33	6.66	28.54*	0.96	0.02	0.20	0.01	20.83†
Pennisetum americanum	100.00	100.00	100.00	12.90	3.31	11.11	0.26	86.12*
Setaria italica	96.66	96.66	100.00	14.80	3.62	10.20	1.96	68.91†
Sorghum almum	26.00	6.66	25.61†	0.90	0.08	0.10	0.02	11.11†

Each value is a mean of 3 replicates, each with 10 seeds.
*Significant at P = 0.05
Significant at P = 0.01

nism that would operate in nature. Aqueous extracts from various parts of Columbus grass, like other grasses [3,4,6,8, 17,21], invariably inhibited germination and radicle growth of susceptible species. The inhibitory effects of grass soil or its extract was due to accumulation of toxins in the soil where they remained effective inducing toxicity. Soil taken from underneath *Lolium, Dichanthium* and tobacco [8,14,21] was significantly inhibitory to test species. As observed in the sand culture experiment, soil extract and soil-bed bioassays, the soil underneath Columbus grass was rendered toxic by toxins released either during its active growth as leachates, root exudates or during the deacy of the litter or it could be the combined effect of these processes.

The reduced moisture contents of the seedlings suggested a possible loss of water absorption capacity by roots in the extract. Dirvi and Hussain [8] and Hussain *et al.* [14] observed similar allelopathic loss of water absorption of test species by plant extract. Low water-contents reduced growth and survival of the test species by conditioning the physiological processes.

The present findings revealed that Columbus grass, besides exhibiting autotoxicity, was allelopathic against other test species. The inhibition mechanism was active in nature either through root exudates, leachates or and decay of the litter. The observed self-declination of Columbus grass pasture [10,24] and affected growth of associated species was primarily due to its allelopathy. Therefore, its introduction in Pakistan would not be beneficial as a range grass. However, it may be introdced after thorough allelopathic studies in relation to the the expected associates and prevailing climatic conditions of the area. Further studies are in progress to identify the inhibitors.

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