Allelopathic effects of different phenological stages of *Cassia occidentalis* L. on *Parthenium hysterophorus* L.

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Abstract

The effect of leachates of different phenological stages of *Cassia occidentalis* L. on *Parthenium hysterophorus* L. was studied. Seed germination, radicle length, plumule length and fresh and dry weight of *Parthenium* were recorded. Pigments and protein contents were also measured to evaluate relation between biochemical and biophysical parameters of *Parthenium* under stress. The leaf leachate of *Cassia* in different developmental stages and pod and seed leachates exhibited varying effect. The *Cassia* leachate obtained from the leaves of vegetative and flowering stages was more phytotoxic. It caused maximum inhibition of germination and seedling growth of *Parthenium.* Biochemical parameters, viz. protein and chlorophyll were also influenced. Root and shoot growth and fresh and dry weight decreased in almost all the treatments. The inhibition of germination was in order of vegetative > flowering > fruiting > fruit ripening > pod > seed while 25 and 50% concentrations of seed leachate stimulated the germination. The 100% concentration of leaf leachate from vegetative stage completely inhibited the germination while highest concentration of leaf leachate from vegetative, flowering, fruiting and fruit ripening stages caused the death of the plants. The average inhibition of parameters of seedlings in pot culture was in order of fruiting > vegetative > fruit ripening > flowering > pod > seed.

Keywords: Cassia occidentalis; Leachate; *Parthenium hysterophorus*; Phenological stages

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Introduction

Allelochemicals have a key role in plant defense. They influence both biodiversity and composition of plant communities. Production of allelochemicals varies with phenological stages of plants and different plant parts accumulate potential allelochemicals in particular stages of development.

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Cassia species are widely distributed herbaceous weeds common in India wastelands. *Cassia tora* and *C. occidentalis* cover a large area of wastelands from the north during the rainy season while *C. sericea* is common in the south. *Cassia* species can be used for the biological control of *Parthenium hysterophorus*. Plant parts of *C. tora* have allelochemicals which inhibit seed germination of *Parthenium* (Kumar and Bhan, 1997; Senthil et al., 2004; Singh and Thapar, 2002). The allelopathic potential of *Cassia* at different phenological stages is largely unknown.

The aim of the present study is to assay the allelopathic potential of different phenological stages of *Cassia occidentalis* on *Parthenium hysterophorus.*

Materials and Methods

All the experiments were conducted in the Department of Botany, University of Allahabad, Allahabad located at 24° 47' and 50° 47' N latitude and 81° 9' and 82° 21' E Longitude, 78 m above the sea level.

Preparation of leachates

The leaves of *C. occidentalis* at different phenological stages and pods and seeds were collected from July to October, 2007 and 2008. The sampled leaves of different phenological stages, viz. vegetative, flowering, fruiting, fruit ripening and pods and seeds of the plants were soaked in distilled water in the ratio of 1:5 (w:v) and kept in refrigerator at 8 °C for 48 hours. The leachates were filtered with muslin cloth and Whatman No. 1 filter paper. They were raised to original volume by adding distilled water and were treated as leachates of 100% concentration. Leachates of graded concentration of 10, 25 and 50% were prepared by diluting the mother leachates. These leachates were transferred in capped bottles and kept in refrigerator at 7- 8 °C (Richardson and Williamson, 1988) and applied finally for bioassay tests.

Measurement of pH

pH of the leachate was determined with the help of pH meter. pH of the leaf, pod and seed leachate was between 6.5 and 7.1.

Determination of water potential

Water potential of *Parthenium* was determined following Chardokov (1948). Two parallel sets of test tubes containing equal volumes of graded concentrations of sucrose solution were taken. One set of test tubes were colored slightly by dissolving small crystals of dye, Methylene blue in them. The addition of dye does not change water potential significantly. In other set with no dye *Parthenium* shoot was immersed in graded concentration of sucrose solution. After 30 minutes the shoot was removed and a small drop of equivalent dye colored solution was added to the test tube.

If the colored drop rises, the solution in which the shoot was immersed has become denser indicating the shoot have absorbed water. The shoot had lower (more negative) water potential than the original solution. If the drop sinks, the solution has become less dense having absorbed water from the shoot. The solution then had a lower water potential than the original tissue. If the drop diffuses out into the solution without rising or sinking, then no change in concentration occurs and the water potential of the solution is equal to that of the tissue. When several solutions of different concentrations are used, there is usually one in which drop neither sinks nor floats (Knipling and Kramer, 1967).

At Pressure (p) = 0 Ψs is equal to Ψ and thus the average Ψ of the tissue.

$\Psi s = CiRT$

where, Ψs = osmotic potential, Ψ= water potential, C = concentration of solution express in molality (moles of solute per Kg water) and i = a constant that account for ionization and /or other deviation from the perfect solution.

For sucrose i= 1.0, R = gas constant (0.00831 kg MPa $mol^{-1}K^{-1}$) and T = absolute temperature (K) = °C (C+273) = 25 + 273 = 298 °K.

Water potential of leachates of *Cassia* and *Parthenium* shoot were compared by the same method. The two parallel sets of test tubes containing leachates of 100% concentrations were taken. In one set of tubes small crystals of Methylene blue were added. In other set shoots of *Parthenium* were immersed for 30 minutes. One drop of colored leachate was added in the respective tubes with equivalent concentration of leachate. Colored drops in all tubes rose indicating that the *Parthenium* shoot had absorbed water and the leachate had no osmotic effect on the *Parthenium* shoot.

The water potential of leachates was always higher (less negative) than that of seedlings of recipient plant. The water potential of shoots of *Parthenium* was 0.55 MPa $mol^{-1}K^{-1}$. The water potential of leaf leachate of *Cassia* obtained from all the phenological stages and

pod and seed leachates was higher (less negative) than that of *Parthenium*.

Germination bioassay

A set of 20 viable seeds of *Parthenium* in replicate of three were soaked in 10 ml of leachates obtained from the leaves of different phenological stages, i.e., vegetative, flowering, fruiting, fruit ripening stages and pods and seeds for 8 hours. The seeds soaked in distilled water for the same duration were taken as control. The seeds of *Parthenium* were placed in equal distance in Petri plates (diameter 29 cm, depth 1.5 cm) lined with filter papers moistened in 10 ml of respective leachate/water. The Petri plates were moistened with 3 ml of respective leachate/extract/water according to the treatments as and when required. After 3 DAS the germination up to 10 DAS and length of radicles and plumules up to 7 DAS were recorded regularly at the intervals of 24 hrs.

Seedling bioassays

The viable seeds of *Parthenium* were collected from the field. The seeds at the rate of 5 seeds/pot were sown in plastic pots (diameter 7cm, depth 9cm) filled with well sieved soil prepared by mixing of farmyard manure, garden soil and sand (1:1:1) during July to October, 2007 and 2008 in culture room (Temperature= 25° ± 2 °C, Photoperiod= 15 hrs and Relative Humidity= 60%). The pots were watered as and when required. After 7d thinning of plants was done and one plant/pot was maintained. 20 day old healthy seedlings of uniform height were selected for the seedling bioassay. The pots with seedlings were grouped according to the treatments. The seedlings were irrigated with graded concentration of different leachates of *Cassia*. The seedlings treated with water were taken as control. Equal amount of water and leachate was used for irrigation. The experiment was conducted in replicates of 10 up to 30 days. The seedlings were uprooted, washed under tap water to remove the soil sticking on the roots. The seedlings were sampled for biophysical and biochemical parameters. Length of root and height of shoot were recorded. The fresh (FW) and dry weight (DW) of seedlings were measured. The dry weight was measured after drying the samples for 80 °C in oven for 48 hours.

Chlorophyll content

The amount of chlorophyll was determined following the method of Arnon (1949). Ten mg of first fully expanded fresh leaves of each plant under treatments were ground with neutral sand and 10 ml of 80% acetone and centrifuged at 3000 rpm for 10 minutes. Supernatant was used to measure optical density. Optical density was measured at 645 nm and 663 nm. Chlorophyll a, b and total chlorophyll were calculated as follow:

Total chlorophyll (mg/g) = $\frac{20.2\times OD_{645}+8.02\times OD_{663}}{1000\times W}\times V$

Chlorophyll a (mg/g) = $\frac{12.7\times OD_{663}-2.69\times OD_{645}}{1000\times W}\times V$

Chlorophyll b (mg/g) = $\frac{22.9\times OD_{645}-4.68\times OD_{663}}{1000\times W}\times V$

where V = volume of the supernatant in ml, W = fresh weight of the leaves in gm and OD = Optical density.

Quantitative estimation of protein

Quantitative estimation of protein was done following the method of Lowry et al. (1951). Ten mg of first fully expanded fresh leaves of each plant under treatments was homogenized with 1 ml of 1N NaOH for 5 minutes at 100 °C. Then 5 ml of alkaline copper reagent was added. After 10 minutes at room temperature 0.5 ml of Folin-Ciocalteau reagent was added and mixed in a tube. The absorbance 650 nm was measured after 30 minutes. The amount of protein was calculated with reference to standard curve of bovine serum albumin.

Statistical analysis

Analysis of variance was carried out for all the data generated from this experiment, employing one way ANOVA test using GPIS software 1.13 (GRAPHPAD, California, USA).

Results

Petri dish assays

The leachates from the leaves of *Cassia* plants of different developmental stages inhibited the seed germination of *Parthenium hysterophorus.* The leaf leachate of vegetative stage caused maximum inhibition of germination. The highest concentration of leaf leachate of vegetative stage resulted in complete failure of seed germination. The leachate obtained from the leaves of fruit ripening stage of *Cassia* was less inhibitory to germination. Inhibitory effects of leachate decreased with the advancing development stages of *Cassia* plants.

Pod leachate recorded higher level of seed germination as compared with leaf leachates. The seed leachate exhibited varying degree of response. The minimum inhibition was recorded in lowest concentration of seed leachate. The moderate concentrations (25 and 50%) stimulated seed germination which declined to level of control in 100% concentration of seed leachate. The leaf and pod leachate exhibited

Table 1
Effects of leaf leachate of vegetative stage of *Cassia occidentalis* L. on germination and seedling growth of *Parthenium hysterophorus* L.

Treatments	Germination % DAS				Length (cm) 3 DAS		4 DAS		7 DAS	
	2	3	4	7	R	P	R	P	R	P
C	68± 1.00	76.33± 2.51	76.33± 2.51	83.66± 2.51	1.03± 0.03	0.80± 0.01	1.07± 0.02	1.30± 0.03	1.50± 0.04	2.55± 0.05
T_1	0± 0[b]	23.67± 1.52[b]	48± 3b	51.66± 2.51b	0.17± 0.01b	0.13± 0.02by	0.27± 0.01b	0.17± 0.02b	0.30± 0.01by	1.03± 0.04b
T_2	0± 0[b]	0± 0[by]	28± 3by	59.66± 2.51by	0.00by	0.00by	0.00by	0.00by	0.10± 0.02by	0.87± 0.03by
T_3	28.33± 1.52[byn]	35.66± 2.51[byn]	40± 3bxn	44.33± 3.51bxn	0.00by	0.30± 0.01byn	0.10± 0.01byn	0.37± 0.02byn	0.40± 0.03byn	0.90± 0.05by
T_4	0± 0b[q]	0± 0[byq]	0± 0bynq	0± 0bynq	0.00by	0.00byq	0.00byq	0.00byq	0.00bynq	0.00bynq

Data are the means of 3 replicates ± standard deviation. [b]P<0.01, versus C; [y]P<0.01 versus T_1; [n]P<0.01 versus T_2; [q]P<0.01 versus T_3. DAS = Days after sowing, C=control, T_1=10%, T_2=25%, T_3=50%, T_4=100% leachate, R = Radicle, P=Plumule

Table 2
Effects of leaf leachate of flowering stage of *Cassia occidentalis* L. on germination and seedling growth of *Parthenium hysterophorus* L.

Treatments	Germination % DAS				Length (cm) 3 DAS		4 DAS		7 DAS	
	2	3	4	7	R	P	R	P	R	P
C	68± 1.00	76.33± 2.51	76.33± 2.51	83.66± 2.51	1.03± 0.03	0.80± 0.01	1.07± 0.02	1.30± 0.03	1.50± 0.04	2.55± 0.05
T_1	0± 0b	36.00± 1.00b	55.66± 1.52b	59.66± 1.52b	0.13± 0.01b	0.13± 0.03b	0.17± 0.03b	0.50± 0.2b	0.23± 0.03b	1.53± 0.04b
T_2	0± 0b	20.00± 2.00by	21.66± 2.08by	60.00± 3.00b	0.20± 0.02b	0.17± 0.01b	0.23± 0.01bx	0.47± 0.03b	0.50± 0.02by	0.77± 0.04by
T_3	28.33± 1.52byn	23.66± 1.52by	31.66± 1.52byn	35.66± 1.52byn	0.23± 0.01byn	0.23± 0.02byn	0.33± 0.02byn	0.37± 0.03byn	0.42± 0.01byn	0.80± 0.04by
T_4	0± 0bq	4.00± 1.00bynq	4.33± 0.57bynq	7.66± 1.52bynq	0.00bynq	0.00bynq	0.00bynq	0.03± 0.01bynq	0.00bynq	0.07± 0.01bynq

Data are the means of 3 replicates ± standard deviation. [b]P<0.01, versus C; [y]P<0.01 versus T_1; [n]P<0.01 versus T_2; [q]P<0.01 versus T_3. DAS = Days after sowing, C=control, T_1=10%, T_2=25%, T_3=50%, T_4=100% leachate, R = Radicle, P = Plumule

concentration dependent inhibition of seed germination. The order of inhibition of seed germination in *Parthenium* in leaf, pod and seed leachates was: vegetative > flowering > fruiting > ripening > pod > seed (Table 1-6).

The lower (10%) concentration of leachate from the leaves of vegetative stage of *Cassia* was less inhibitory to seedling growth as compared with that of higher (50%) concentration. Moderate concentration (25%) arrested the radicle and plumule growth up to 4 days after sowing (DAS) (Table 1). The leachate from the leaves of flowering stage arrested the growth of radicle up to 7 DAS while the growth of plumule was faster. The inhibition of plumule growth was higher under the influence of increased concentration of leachate. The leaf leachates of 25 and 50% concentrations were less inhibitory to radicle growth as compared with that of leaf leachate of 10% concentration. Highest concentration of leaf leachate caused maximum inhibition of seedling growth (Table 2). The leaf leachate of fruiting stage at 10% was inhibitorier than moderate (25 and 50%) concentration. The 100% concentration of leaf leachate of fruiting stage caused maximum inhibition of seedling growth (Table 3). The different concentration of leaf leachate of fruit ripening stage of *Cassia* followed trend of fruiting stage (Table 4). The inhibitory effect of pod leachate was concentration dependent. The inhibition of seedling growth increased with

Table 3
Effects of leaf leachate of fruiting stage of *Cassia occidentalis* L. on germination and seedling growth of *Parthenium hysterophorus* L.

Treatments	Germination %				Length (cm)					
	DAS				3 DAS		4 DAS		7 DAS	
	2	3	4	7	R	P	R	P	R	P
C	68±	76.33±	76.33±	83.66±	1.03±	0.80±	1.07±	1.30±	1.50±	2.55±
	1.00	2.51	2.51	2.51	0.03	0.01	0.02	0.03	0.04	0.05
T_1	0±	40.00±	60.00±	64.00±	0.30±	0.18±	0.36±	0.26±	0.42±	1.20±
	0b	2.00by	2.00b	2.00b	0.01b	0.01b	0.02b	0.02b	0.03b	0.02b
T_2	0±	23.66±	32.66±	60.33±	0.60±	0.30±	1.00±	0.72±	1.11±	1.18±
	0b	1.52by	2.08by	0.57b	0.02by	0.01by	0.01by	0.02by	0.03by	0.03b
T_3	0±	12.33±	24.00±	40.00±	0.40±	0.30±	0.70±	0.60±	1.00±	1.13±
	0b	2.51byn	3.00byn	2.00byn	0.01byn	0.01by	0.02byn	0.02byn	0.03byn	0.04b
T_4	0±	12.00±	16.00±	31.66±	0.20±	0.10±	0.40±	0.50±	0.90±	1.00±
	0b	2.00byn	2.00bynq	1.52bynq	0.02bynq	0.01bynq	0.03bnq	0.03bynq	0.04bynp	0.03bynq

Data are the means of 3 replicates ± standard deviation. $^{b}P<0.01$, versus C; $^{y}P<0.01$ versus T_1; $^{n}P<0.01$ versus T_2; $^{p}P<0.05$, $^{q}P<0.01$ versus T_3. DAS = Days after sowing, C=control, T_1=10%, T_2=25%, T_3=50%, T_4=100% leachate, R = Radicle, P = Plumule

Table 4
Effects of leaf leachate of fruit ripening stage of *Cassia occidentalis* L. on germination and seedling growth of *Parthenium hysterophorus* L.

Treatments	Germination %				Length (cm)					
	DAS				3 DAS		4 DAS		7 DAS	
	2	3	4	7	R	P	R	P	R	P
C	68±	76.33±	76.33±	83.66±	1.03±	0.80±	1.07±	1.30±	1.50±	2.55±
	1.00	2.51	2.51	2.51	0.03	0.01	0.02	0.03	0.04	0.05
T_1	0±	40.33±	60.00±	64.33±	0.36±	0.22±	0.38±	0.38±	0.46±	1.27±
	0b	2.51b	2.00b	1.52b	0.03b	0.01b	0.03b	0.02b	0.02b	0.03b
T_2	0±	24.00±	32.00±	59.66±	0.60±	0.32±	1.10±	0.80±	1.22±	1.21±
	0b	1.00by	1.00by	2.51b	0.02by	0.01by	0.03y	0.04by	0.05by	0.04b
T_3	0±	11.66±	23.66±	39.66±	0.44±	0.32±	0.74±	0.72±	1.16±	1.26±
	0b	1.52byn	1.52byn	2.51byn	0.02bxn	0.01by	0.02byn	0.03by	0.04by	0.05b
T_4	0±	12.33±	16.00±	31.66±	0.22±	0.12±	0.38±	0.64±	0.76±	1.06±
	0b	0.57byn	1.00bynq	1.52bynq	0.03bynq	0.03bynq	0.02byn	0.06byn	0.02bynq	0.06bynq

Data are the means of 3 replicates ± standard deviation. $^{b}P<0.01$, versus C; $^{x}P<0.05$, $^{y}P<0.01$ versus T_1; $^{n}P<0.01$ versus T_2; $^{q}P<0.01$ versus T_3. DAS = Days after sowing, C=control, T_1=10%, T_2=25%, T_3=50%, T_4=100% leachate, R = Radicle, P = Plumule

concentration of pod leachate (Table 5). All concentrations of seed leachate except 25% stimulated the plumule growth at 7 DAS. The seed leachate caused inhibition of radicle growth except in the treatment with 25% concentration of leachate (Table 6).

Pot assays

Chlorophyll and protein contents of *Parthenium* plants decreased when treated with leachates of *Cassia* of different phenological stages. Maximum reduction of chlorophyll and protein contents were recorded in seedlings of *Parthenium* treated with leachates of fruiting stage of *Cassia*. The seedlings treated with highest concentration of leaf leachates of vegetative, flowering, fruiting, and fruit ripening stage of *Cassia* did not survive. The decrease in chlorophyll and protein contents was concentration dependent. The pod and seed leachates of *Cassia* exhibited different trends. The *Parthenium* plants survived in all concentrations of pod and seed leachates of *Cassia*. Minimum amount of total chlorophyll was recorded in *Parthenium* treated with pod

Table 5
Effects of pod leachate of *Cassia occidentalis* L. on germination and seedling growth of *Parthenium hysterophorus* L.

Treatments	Germination %				Length (cm)					
	DAS				3 DAS		4 DAS		7 DAS	
	2	3	4	7	R	P	R	P	R	P
C	68± 1.00	76.33± 2.51	76.33± 2.51	83.66± 2.51	1.03± 0.03	0.80± 0.01	1.07± 0.02	1.30± 0.03	1.50± 0.04	2.55± 0.05
T_1	40.33± 0.57b	64.33± 1.52b	67.66± 1.52b	72.33± 2.51b	0.80± 0.02b	0.90± 0.03b	0.93± 0.04b	1.30± 0.02	0.97± 0.05b	2.77± 0.02b
T_2	11.66± 1.52by	51.66± 1.52by	60.33± 3.51by	65.66± 2.08bx	0.47± 0.01by	0.00by	0.63± 0.02by	0.80± 0.03by	0.70± 0.04by	2.37± 0.05by
T_3	40.33± 1.52bn	39.66± 2.51byn	44.33± 0.57byn	52.33± 2.51byn	0.33± 0.01byn	0.47± 0.02byn	0.40± 0.02byn	1.17± 0.04byn	0.43± 0.02byn	2.23± 0.07bym
T_4	11.66± 1.52byq	23.66± 1.52bynq	27.66± 1.52bynq	28.00± 1.00bynq	0.20± 0.01bynq	0.53± 0.02bynp	0.33± 0.01bynp	0.67± 0.03bynq	0.36± 0.01byn	0.87± 0.04bynq

Data are the means of 3 replicates ± standard deviation. $^{b}P<0.01$, versus C; $^{y}P<0.01$ versus T_1; $^{m}P<0.05$, $^{n}P<0.01$ versus T_2; $^{p}P<0.05$, $^{q}P<0.01$ versus T_3. DAS = Days after sowing, C=control, T_1=10%, T_2=25%, T_3=50%, T_4=100% leachate, R = Radicle, P = Plumule

Table 6
Effects of seed leachate of *Cassia occidentalis* L. on germination and seedling growth of *Parthenium hysterophorus* L.

Treatment	Germination %				Length (cm)					
	DAS				3 DAS		4 DAS		7 DAS	
	2	3	4	7	R	P	R	P	R	P
C	68± 1.00	76.33± 2.51	76.33± 2.51	83.66± 2.51	1.03± 0.03	0.80± 0.01	1.07± 0.02	1.30± 0.03	1.50± 0.04	2.55± 0.05
T_1	60.00± 3.00b	51.66± 0.57b	52.00± 2.00b	68.00± 1.00b	0.93± 0.06a	0.70± 0.03a	1.17± 0.05a	1.50± 0.02b	1.30± 0.03b	2.63± 0.07b
T_2	44.00± 1.00bx	92.33± 2.51by	91.66± 1.52by	92.00± 2.00by	0.57± 0.02by	0.90± 0.04ay	1.00± 0.03y	1.17± 0.05by	1.53± 0.02y	2.27± 0.06y
T_3	64.00± 2.00byn	91.66± 0.57by	91.66± 0.57by	91.66± 2.51by	0.83± 0.03bxn	1.00± 0.03bym	0.90± 0.03bym	1.60± 0.04bxn	1.20± 0.03byn	2.70± 0.07bn
T_4	64.33± 0.57bynq	75.66± 1.52ynq	79.66± 1.52ynq	79.66± 1.52ynq	0.47± 0.03bynq	0.73± 0.04nq	0.80± 0.02bynp	1.13± 0.05byq	1.10± 0.02bynq	2.97± 0.05bynq

Data are the means of 3 replicates ± standard deviation. $^{a}P<0.05$. $^{b}P<0.01$, versus C; $^{x}P<0.05$, $^{y}P<0.01$ versus T_1; $^{m}P<0.05$, $^{n}P<0.01$ Versus T_2; $^{p}P<0.05$, $^{q}P<0.01$ versus T_3. DAS = Days after sowing, C=control, T_1=10%, T_2=25%, T_3=50%, T_4=100% leachate, R = Radicle, P = Plumule

leachate of 50% concentration while lower concentration increased the chlorophyll contents but increase was recorded in the highest concentration of leachate. The seed leachate of 50% concentration stimulated the chlorophyll contents but other concentrations decreased the amount. Successive decrease of protein content was evident in the treatments with pod leachate. The seed leachate of 25% concentration stimulated the protein contents which declined in higher concentration (50 and 100%) treatments to the level lower than that of control.

The leaf leachate of vegetative stage of *Cassia* stimulated the root growth but inhibited shoot growth. The number of leaves per plant decreased to the minimum with leachate of 25% concentration. The root growth drastically increased in the seedlings treated with 50% concentration of leachate while shoot growth was minimum. Lower concentration of leaf leachate (10 and 25%) exhibited decreasing trend of fresh weight (FW) and dry weight (DW) of root and shoot. The leaf leachate of 50% concentration increased FW of root but decreased DW. The same concentration enhanced FW and DW of shoot as compared with lower concentration of the leachate (Table 7).

The leaf leachate of lower (10%) and higher (50%) concentrations of flowering and fruit ripening stage stimulated the root growth but inhibited shoot growth. The number of leaves per plant successively decreased in treatments with leaf leachate of flowering and fruit ripening stage (Table 8 and 10). Maximum decrease in

Table 7
Allelopathic effects of leaf leachate of vegetative stage of *Cassia occidentalis* L. on chlorophyll, protein and biophysical parameters of *Parthenium hysterophorus* L.

Treatments	Chla (mg/g)	Chl b (mg/g)	Chl a+b (mg/g)	Protein mg/100mg	RL (cm)	SL (cm)	No. of Leaves /plant	Root (mg/plant)		Shoot (mg/plant)	
								FW	DW	FW	DW
C	2.76± 0.02	1.33± 0.02	4.09± 0.03	9.36± 0.015	12.65 ±1.40	6.55± 0.70	12.50± 1.70	106.25± 4.20	28.50± 3.90	824.25± 12.10	147.50± 10.74
T_1	2.04± 0.0057b	1.48± 0.025b	3.75± 0.03b	9.24± 0.025b	17.25± 1.90c	4.40± 0.46b	9.50± 1.82	99.50± 6.38b	14.25± 1.78b	359.00± 13.72b	55.25± 4.32b
T_2	1.820± 035by	1.15± 0.03by	2.97± 0.015by	8.24± 0.02by	14.60± 1.84	4.25± 0.34b	7.50± 1.34c	63.50± 3.26by	27.50± 2.42by	138.00± 5.34by	21.25± 2.00by
T_3	1.61± 0.025byn	0.87± 0.02byn	2.48± 0.02byn	7.20± 0.035byn	18.20± 2.00c	3.35± 0.30b	10.00± 1.28	182.25± 5.12byn	26.25± 2.30byn	823.25± 11.22byn	137.75± 4.24y
T_4	D	D	D	D	D	D	D	D	D	D	D

Data are the means of 3 replicates ± standard deviation. [a]*P*<0.05. [b]*P*<0.01, [c]*P*<0.001,versus C; [x]*P*<0.05, [y]*P*<0.01 versus T_1; [n]*P*<0.01 versus T_2. C=control, T_1=10%, T_2=25%, T_3=50%, T_4=100% Leaf leachate; D=Dead plants, RL=Root length, SL=Shoot length, FW=Fresh weight, DW=Dry weight

Table 8
Allelopathic effects of leaf leachate of flowering stage of *Cassia occidentalis* L. on chlorophyll, protein and biophysical parameters of *Parthenium hysterophorus* L.

Treatments	Chl a (mg/g)	Chl b (mg/g)	Chl a+b (mg/g)	Protein mg/100mg	RL (cm)	SL (cm)	No. of Leaves /plant	Root (mg/plant)		Shoot (mg/plant)	
								FW	DW	FW	DW
C	2.76± 0.02	1.33± 0.02	4.09± 0.003	9.30± 0.015	12.65± 1.40	6.55± 0.70	12.50± 1.70	106.25± 4.20	28.50± 3.90	824.25± 12.10	147.50± 10.74
T_1	2.42±. 0.02b	1.38± 0.035c	3.80± 0.02b	9.16± 0.015b	13.30± 1.64	5.70± 0.60	10.50± 1.32	101.75± 5.00	24.25± 2.42	686.25± 10.48b	109.75± 8.68
T_2	1.96± 0.021by	1.42± 0.03	3.38± 0.015by	8.80± 0.011by	12.00± 1.32	5.25± 0.49	9.00± 1.08	74.50± 6.58c	18.00± 2.83b	465.75± 6.52by	70.25± 3.62
T_3	2.13± 0.01byn	1.43± 0.025b	3.56± 0.005byn	7.96± 0.025byn	15.40± 2.46	4.45± 0.45b	8.50± 0.20	90.75± 3.08b	21.00± 1.98c	497.25± 5.88byn	76.00± 5.30
T_4	D	D	D	D	D	D	D	D	D	D	D

Data are the means of 3 replicates ± standard deviation. [a]*P*<0.05. [b]*P*<0.01, [c]*P*<0.001,versus C; [x]*P*<0.05, [y]*P*<0.01 versus T_1; [n]*P*<0.01 versus T_2. C=control, T_1=10%, T_2=25%, T_3=50%, T_4=100% Leaf leachate; D=Dead plants, RL=Root length, SL=Shoot length, FW= Fresh weight, DW=Dry weight

shoot growth and reduction in number of leaves was recorded in treatment with leaf leachates of fruiting stage. The FW and DW of root and shoot decreased in all treatments but leaf leachate of fruiting stage caused maximum reduction in FW and DW of root (Table 9). The FW and DW of shoot in the treatment with 25% concentration with leaf leachate of fruit ripening stage exhibited increase over treatment with 10% and 50% concentration of leachate (Table 10).

The treatment with pod leachate of lowest concentration stimulated root growth, number of leaves per plant, FW of root and DW of root and shoot but successively decreased in the treatments with other concentrations of leachates (Table 11). A variation of narrow range in number of leaves per plant was recorded in *Parthenium* plants treated with pod and seed leachates of *Cassia*. The seed leachate of 25% increased the number of leaves per plant which successively decreased at higher concentrations. The seed leachate decreased FW and DW of root and shoot. The FW and DW of shoot were minimum with seed leachate of 50% concentration (Table 12). The inhibition of growth parameters of seedlings in pot assay was in order of fruiting > vegetative > fruit ripening > flowering stage > pod leachate > seed leachate.

Table 9
Allelopathic effects of leaf-leachate of fruiting stage of *Cassia occidentalis* L. on chlorophyll, protein and biophysical parameters of *Parthenium hysterophorus* L.

Treatments	Chl a (mg/g)	Chl b (mg/g)	Chl a+b (mg/g)	Protein (mg/100 mg)	RL (cm)	SL (cm)	No. of Leaves /plant	Root (mg/plant)		Shoot (mg/plant)	
								FW	DW	FW	DW
C	2.76± 0.02	1.33± 0.02	4.09± 0.03	9.36± 0.015	12.65± 1.40	6.55± 0.70	12.50± 1.70	106.25± 4.20	28.50± 3.90	824.25± 12.10	147.50± 10.74
T_1	2.30± 0.03b	1.41± 0.02	3.71± 0.04b	8.16± 0.015b	12.90± 1.65	4.65± 0.20b	9.50± 1.80	99.75± 3.46b	25.00± 2.68	576.25± 7.56b	92.25± 6.90b
T_2	1.58± 025by	1.34± .015	2.92± 0.035by	7.60± 0.02by	10.15± 1.22	4.30± 0.93by	7.50± 0.98c	66.75± 2.98by	16.00± 1.10by	374.50± 4.26by	65.50± 3.15by
T_3	1.33± 0.005byn	0.98± 0.50czo	2.31± 0.035byn	6.96± 0.015byn	11.70± 1.60	2.55± 0.08byn	4.50± 1.02bz	89.00± 2.88bz	24.00± 2.02o	347.50± 2.98byn	63.00± 4.33by
T_4	D	D	D	D	D	D	D	D	D	D	D

Data are the means of 3 replicates ± standard deviation. $^{a}P<0.05$. $^{b}P<0.01$, $^{c}P<0.001$,versus C; $^{x}P<0.05$, $^{y}P<0.01$, $^{z}P<0.001$ versus T_1; $^{m}P<0.05$, $^{n}P<0.01$, $^{o}P<0.001$ versus T_2. C=control, T_1=10%, T_2=25%, T_3=50%, T_4=100% Leaf leachate; D=Dead plants, RL=Root length, SL=Shoot length, FW=Fresh weight, DW=Dry weight

Table 10
Allelopathic effects of leaf leachate of fruit ripening stage of *Cassia occidentalis* L. on chlorophyll, protein and biophysical parameters of *Parthenium hysterophorus* L.

Treatments	Cha (mg/g)	Chl b (mg/g)	Chl a+b (mg/g)	Protein (mg/100mg)	RL (cm)	SL (cm)	No. of Leaves /plant	Root (mg/plant)		Shoot (mg/plant)	
								FW	DW	FW	DW
C	2.76± 0.02	1.33± 0.02	4.09± 0.03	9.36± 0.015	12.65± 1.40	6.55± 0.70	12.50± 1.70	106.25± 4.20	28.50± 3.90	824.25± 12.10	147.50± 10.74
T_1	2.76± 0.06	1.15± 0.02b	3.91± 0.025b	9.12± 0.025b	12.75± 1.32	5.0± 0.37c	11.00± 1.40	101.75± 5.00	26.00± 3.25	447.20± 9.05b	71.75± 6.42b
T_2	1.87± 0.02by	1.34± 0.01y	3.21± 0.035by	8.92± 0.025by	11.5± 1.08	4.8± 0.25b	10.50± 1.14	96.75± 3.96	24.75± 3.90	460.75± 3.12b	100.25± 7.85by
T_3	1.42± 0.03byn	1.25± 0.017byn	2.67± 0.005byn	7.88± 0.011byn	13.4± 1.27	5.2± 0.62c	10.00± 1.15	74.75± 4.00byn	21.75± 3.50	400.25± 7.08b	72.75± 5.90bn
T_4	D	D	D	D	D	D	D	D	D	D	D

Data are the means of 3 replicates ± standard deviation. $^{a}P<0.05$. $^{b}P<0.01$, $^{c}P<0.001$,versus C; $^{x}P<0.05$, $^{y}P<0.01$ versus T_1; $^{m}P<0.05$, $^{n}P<0.01$ versus T_2. C=control, T_1=10%, T_2=25%, T_3=50%, T_4=100% Leaf leachate; D=Dead plants, RL=Root length, SL=Shoot length, FW=Fresh weight, DW=Dry weight

Discussion

The water potential (Ψ) of leaf, pod and seed leachates of *Cassia* was higher (less negative) than that of shoots of *Parthenium.* The higher water potential of leachates causes endosmosis. Thus the test plants were not subjected to water deficit due to higher concentration of leachates. The allelochemicals present in leachates affected germination and seedling growth (Narwal et al., 1992; Chon et al., 2003).

The allelochemicals present in leachates might have altered some of the physiological processes responsible for the plant growth. The inhibition of cell division and elongation resulted in reduction of growth (Bagavathy and Xavier, 2007). The inhibition of growth was concentration dependent. The accumulation of allelochemicals due to increased concentration of leachate resulted in suppression of plant growth (Singh et al., 2008; Singh et al., 2009a; Singh et al., 2009b).

Leaf leachates are most effective in regulating the growth of seedlings as compared with the pod and seed leachates because of potential allelochemicals. Allelochemicals influence chlorophyll content (Moreland and

Table 11
Allelopathic effects of pod leachate of *Cassia occidentalis* L. on chlorophyll, protein and biophysical parameters of *Parthenium hysterophorus* L.

Treatments	Chl a (mg/g)	Chl b (mg/g)	Chl a+b (mg/g)	Protein mg/g FW	RL (cm)	SL (cm)	No. of Leaves /plant	Root (mg/plant)		Shoot (mg/plant)	
								FW	DW	FW	DW
C	2.76± 0.02	1.33± 0.02	4.09± 0.03	9.36± 0.01	12.65± 1.40	6.55± 0.70	12.50± 1.70	106.25± 4.20	28.50± 3.90	824.25± 12.10	147.50± 10.74
T_1	2.64± 0.015b	1.52± 0.03b	4.16± 0.015a	9.16± 0.02b	12.40± 0.96	6.75± 0.70	13.00± 1.80	115.00± 5.00	29.50± 3.86	775.50± 10.20b	154.25± 10.90
T_2	2.46± 0.005by	1.08± 0.02by	3.54± 0.04by	8.76± 0.01by	11.85± 1.05	6.25± 0.82	11.50± 1.45	101.00± 4.32y	27.25± 4.00	693.50± 8.50by	131.75± 8.98z
T_3	1.58± 0.025byn	0.91± 0.04byn	2.49± 0.015byn	7.88± 0.02byn	9.65± 0.88	5.10± 0.48cz	11.00± 1.60	77.00± 3.44byn	23.75± 2.32	669.50± 5.98byo	107.25± 6.05byo
T_4	2.04± 0.045bynq	1.04± 0.04bynq	3.08± 0.03bynq	6.28± 0.04bynq	8.50± 1.10	4.85± 0.49byo	10.50± 1.30	75.25± 4.05byn	19.50± 2.20	625.25± 7.80bynq	106.25± 6.68byo

Data are the means of 3 replicates ± standard deviation. [a]P<0.05. [b]P<0.01, [c]P<0.001,versus C; [x]P<0.05, [y]P<0.01 versus T_1; [m]P<0.05 , [n]P<0.01, [o]P<0.001 versus T_2; [p]P<0.05, [q] P<0.01 versus T_3. C=control, T_1=10%, T_2=25%, T_3=50%, T_4=100% Leaf leachate; D=Dead plants, RL=Root length, SL=Shoot length, FW=Fresh weight, DW=Dry weight

Table 12
Allelopathic effects of seed leachate of *Cassia occidentalis* L. on chlorophyll, protein and biophysical parameters of *Parthenium hysterophorus* L.

Treatments	Chl a (mg/g)	Chl b (mg/g)	Chl a+b (mg/g)	Protein (mg/g) FW	RL (cm)	SL (cm)	No. of Leaves /plant	Root (mg/plant)		Shoot (mg/plant)	
								FW	DW	FW	DW
C	2.76± 0.02	1.33± 0.02	4.09± 0.03	9.36± 0.01	12.65± 1.40	6.55± 0.70	12.50 1.70	106.25± 4.20	28.50± 3.90	824.25± 12.10	147.50± 10.74
T_1	1.89± 0.03b	1.62± 0.04b	3.50± 0.02b	8.84± 0.03b	9.00± 0.98b	6.60± 0.82	11.00± 1.36	102.25± 5.00	26.50± 3.00	816.25± 12.24	139.00± 8.65
T_2	2.64± 2.08by	1.27± 0.05y	3.91± 0.04by	10.66± 0.02by	11.20± 1.02	5.20± 0.62	13.00± 1.60	85.75± 4.98by	19.75± 2.80a	692.25± 9.06by	110.75± 11.28by
T_3	2.80± 0.04yn	1.41± 0.03 byn	4.21± 0.02 byn	6.76± 0.02 byn	10.45± 0.68	6.20± 0.72	10.50± 1.55	89.00± 4.90bx	25.75± 3.35	610.25± 11.28 byn	96.50± 6.92by
T_4	2.72± 0.01 ymp	1.13± 0.02 bynq	3.86± 0.01 byq	5.43± 0.01 bynq	8.65± 0.90 bm	5.85± 0.52	9.50± 1.04	74.75± 4.00 bymp	23.00± 2.72	700.75± 10.78 byq	107.75± 11.06 by

Data are the means of 3 replicates ± standard deviation. [a]P [a]P<0.05,[b]P<0.01, versus C; [x]P<0.05,[y]P<0.01 versus T_1; [m]P<0.05,[n]P<0.01 versus T_2; [p]P<0.05,[q]P<0.01 versus T_3. C=control, T_1=10%, T_2=25%, T_3=50%, T_4=100% Leaf leachate; D=Dead plants, RL=Root length, SL=Shoot length, FW=Fresh weight, DW=Dry weight

Novitzky, 1987; Bagavathy and Xavier, 2007). The decrease in chlorophyll pigment is dependent on the concentration of allelochemicals present in leachate (Eyini et al., 1989; Mamata and Mahadevappa, 1992; Singh and Thapar, 2002). Decrease in chlorophyll content under influence of allelochemicals present in leachate limits photosynthetic rate and accumulation of photoassimilate resulting in inhibited growth. The decrease in root and shoot growth andtheir fresh and dry weight may be possible strategies of plant to tolerate the environmental stress. Allelochemicals may cause excessive loss of electrolytes resulting in reduced chlorophyll (Alsaadawi et al., 1986; Jaykumar et al., 1990; Suseeladevi et al., 1992; Bajaj et al., 2004). The low amount of chlorophyll under the influence of allelochemicals might be due to inhibited biosynthesis and/or breakdown of chlorophyll. The test plants exhibited lower amount of chlorophyll pigments because of the potential allelochemicals present in leachate of donor plants (Batish et al., 2006). The rate of photosynthesis is proportional to the amount of chlorophyll present in the test plants which resulted in decreased plant growth. According to Jayakumar et al. (1992) phenolic compounds present in maize decreases height of plant and total chlorophyll in pea.

Allelopathins present in leachate reduced protein contents (Venkateshwarlu et al., 2001). The reduction of protein may be due to inhibition of biosynthesis and/or increased degradation of protein (Batish et al., 2006). The decrease in protein is dependent on concentration of leachate. The allelochemicals present in leachate exhibited different effect. The increase in protein contents by seed leachate of specific concentration may be due to allelochemicals which promote the biosynthesis or inhibit the breakdown of protein. The leachates of *Cassia* decreased the growth of target plants in pot culture. Root of seedlings in pot culture exhibited different responses to leaf leachates of *Cassia*. The leaf leachate of vegetative and flowering stage stimulated the growth of roots of plants in pot culture. It appears that leaf leachate stimulated the cell division and expansion of cells causing root growth. But dry weight of roots and shoots exhibited decreasing trends.

The leaf leachates of donor plant in early stages of development were phytotoxic in comparison with that of later stages. The leaves accumulate potential allelochemicals because they are the locus of biosynthesis. This is evident from the higher phytotoxicity of leaves as compared with that of pod and seed leachate. Allelochemicals from the leaves are translocated to various parts of plants (Klein and Yun, 1992). The plants in early phenological stages exhibit higher metabolic activities resulting in biosynthesis and accumulation of potential allelochemicals. In the early stages plants have to strengthen the defense system to establish the seedlings. During the flowering and fruiting stage the metabolic rate may be increased because of diversion of photoassimilate to fruits and seeds. In pot culture fruiting stage exhibited more phytotoxic effects. The increased metabolic rate resulted in increased biosynthesis and accumulation of potential allelochemicals. In pot culture the leaf leachate in fruiting stage was more phytotoxic than other stages. The varied response of leachates at different concentrations may be due to altered concentration of potential allelochemicals.

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