

BIOSTIMULANT AND PLANT GROWTH REGULATOR: APPLICATIONS AND SIGNALING MECHANISM

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Biological Suppression of *Parthenium hysterophorus* by Some Strong Allelopathic Weeds

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Abstract

In the present investigation Shoot Cut Bioassay, Seedling Bioassay, Chlorophyll, Nitrogen, and Protein Content, was analysed on *Parthenium hysterophorus* by the shoot leachates of *Cassia occidentalis* and *Calotropis procera* at 25%, 50%, 75% and 100% including control. It has been observed that at high concentration of *Cassia* shoot leachates against *Parthenium*; the phytotoxic damage rating scale was maximum and was found to be statistically significant, followed by *Calotropis*. Similar results were also observed in total chlorophyll, nitrogen and protein content of *Parthenium*. Data revealed that *Cassia* can be a promising weed to curb the population of *Parthenium*.

Keywords: Allelochemicals, Biochemical interaction, Coffee weed, Congress grass, Small Crown flower plant.

Introduction:

Parthenium weed is an aggressive annual herb, colonising roadsides, fallow land and overgrazed pastures where grass cover is having less growth. It does not normally establish in vigorous pastures or intact native vegetation. As it can only reproduce and spread by seed, the seed ecology of *Parthenium* weed contributes greatly to its success in semi-arid environment. *Parthenium hysterophorus* L. is a branching, annual

herb with pale green lobed leaves, a deep taproot, and an erect stem with several branches. It can grow to more than two metres high. It grows best on alkaline, clay-loam to heavy clay soils in areas where rainfall is greater than 500 mm per year. *Parthenium* weed is a serious problem. In grazing country, it can dominate pastures under continued heavy grazing and has the potential to exclude useful forage plants, thus decreasing pastures productivity,

carrying capacity and land values. *Parthenium* weed has been shown to be related to health problem for some people living or working in close proximity to it. Individuals in contact with *Parthenium* can develop sensitivity to the plant, which may then manifest as an allergy-type response. Many plants release some chemicals called allelochemicals during different growth stages which may affect the growth and development of adjoining plants. The phenomenon of one plant having detrimental effect on another plant through the production or release of toxic chemicals has been termed Allelopathy. The first observation that antagonistic competitor plants could replace *P. hysterophorus* and therefore had potential for biological control appears to have been made by Singh, 1983 who noted that *Cassia uniflora* moved into areas previously occupied by *Parthenium* weed, in the Maharashtra State of India. Aqueous foliar leachates of *Azadirachta indica* and *Prosopis juliflora*, inhibit seed germination of *Parthenium* by more than 95 percent (Dhawan et. al., 1994). It has been observed that *Tephrosea purpurea*, *Croton bonplandianum*, *Sida cordifolia* and other species of *Cassia* have been found to suppress *Parthenium*. Based on the field survey it has been observed that *Cassia occidentalis* and *Calotropis procera* is showing strong allelopathic potential against *Parthenium*. *Cassia occidentalis* Linn. commonly called as coffee weed belongs to the family Caesalpiniaceae. It is an erect, annual shrub. The leaves are pubescent and are

3 to 6 cm long. The inflorescence is axillary cyme. The flowers are purplish in colour and its flowering time is July to September. Fruit is a legume. *Calotropis procera* (Willd.) Dryand. ex W. Ait. commonly called as milk weed belongs to the family Asclepiadaceae. It is an annual, erect shrub. Milky latex is present in stem and leaves of plants. The inflorescence is polychasial cyme. The flowers are purplish white in colour and its flowering time is July to November. Fruit is an Etaerio of follicle. Therefore, this study is performed to assess the potential of above selected weeds on the growth and development of *Parthenium*.

Materials and Methods

The upper parts of shoot tips were collected from the selected botanic agents/plants. 100 grams of shoot tips were soaked in 500ml of double distilled water each under aseptic conditions for 9 days and placed in conical flasks in a refrigerator at $8 \pm 1^{\circ}\text{C}$. The aqueous leachates were filtered through three layers of muslin cloth/cheese cloth to remove debris. The filtrate was then re-filtered through one layer of Whatmann no. 1 filter paper. Leachates of 25%, 50%, 75% and 100% concentration were prepared with sterilized distilled water and used for bioassay.

Shoot Cut Bioassay

Shoots of *Parthenium* (5.0 cm) with one or two inflorescences were taken. The inflorescences are washed in tap water. Then they were dipped in 1% NaOCl solution for 3 minutes. The tips of the shoots were immediately washed in sterilized distilled water to remove any

residual trace of the chemical. An inclined cut was made at the tip and the shoot were placed in test tubes containing 10 ml of shoot leachates of 25%, 50%, 75% and 100% concentrations of botanic agents. The test-tubes were sealed with cotton buds and aluminium foil to make it air tight. Effect of leachates was observed after 72 hours at room temperature. Phytotoxic damage was recorded on the basis of rating scale of 0-5, where 0 = no effect, 1 = slight chlorosis and slight necrosis, 3 = acute chlorosis and marked necrosis/drooping of entire twig, 4 = falling of flowers and leaves/high necrosis and chlorosis, 5 = acute chlorosis and very high necrosis leading to death of the whole shoot.

Seedling Bioassay

Seedling of *Parthenium* were raised in plastic pots containing sterilized soil, sand and peat (1:1:1) and placed at room temperature $25 \pm 1^\circ\text{C}$. These seedlings were sprayed with shoot leachates of 25%, 50%, 75% and 100% concentrations of botanic agents. Observations regarding the toxicity of seedlings were made after 72 hours. Phytotoxic damage was recorded on the basis of rating scale of 0 – 4 where 0 = no effect, 1 = slight chlorosis, 2 = marked chlorosis, 3 = drooping of seedling, 4 = death of seedling.

Chlorophyll Estimation

Chlorophyll content of *Parthenium hysterophorus* was estimated according to Arnon's, 1949. 40mg (0.04gm) of *Parthenium* leaves were treated with 25%, 50%, 75% and 100% concentration

of shoot leachates of botanic agents. The treated *Parthenium* leaves were placed in black plastic bottles containing 10ml of 80% acetone and then it was sealed with adhesive tape at its mouth so that acetone may not get evaporated and kept undisturbed in a refrigerator for 5 – 6 days at $8 \pm 1^\circ\text{C}$ temperature. After 6 days optical density was recorded by spectrophotometer at different wavelength i.e. 480, 510, 630, 645, 652, and 665 nm. Using following formula amount of Chl 'a', Chl 'b' and total chlorophyll was calculated and tabulated in tables. Chl 'a' = 15.6 (O.D. at 665nm) – 2.0 (O.D. at 645nm) – 0.8 (O.D. at 630nm)

$$\text{Total chlorophyll} = \frac{\text{O.D. at } 652\text{nm}}{34.5} \times 1000$$

$$\text{Chl 'b'} = \text{Total chl} - \text{Chl 'a'}$$

Estimation of Nitrogen

Nitrogen was estimated by following the method of Snell and Snell, 1955. 100 mg (0.1 gm) of *Parthenium* leaves were treated with 25%, 50%, 75% and 100% concentration of shoot leachates of botanic agents. Then, the treated *Parthenium* leaves were placed in 50 ml conical flask and mixed with 2ml of conc. H_2SO_4 and then it was heated on hot plate at 40°C . When volume reduces to half of the original volume, 1.5ml of 30% H_2O_2 was added. Then the solution was heated gently at $10 - 20^\circ\text{C}$ till the clear extract was obtained. The content was then transferred in 100 ml volumetric flask and the volume was made up to the mark with distilled water. After preparation of acid extract of plant

material, the nitrogen was estimated as follows:

1.0 ml of prepared acid extract from plant material was taken in 50 ml volumetric flask. To these 10 drops of 10% NaOH and 10 drops of 10% sodium silicate was added and the solution was diluted up to the mark. 1.0 ml of freshly prepared nessler's reagent was added to the flask, the colour intensity was measured by colorimeter after 15 minutes at transmittance of 420 nm using a reagent blank as reference. With the help of standard curve prepared with 100 ppm NH₄Cl solution the amount of N₂ in the sample was found out.

Protein content

The protein content in plant sample was calculated by multiplying percentage nitrogen content of plant sample by the factor of 6.25.

$$\text{Percentage of Protein} = \% \text{ of Nitrogen} \times 6.25$$

Statistical analysis

Statistical analysis of the data recorded was done. The design is Factorial Completely Randomized Design (FCRD) and conclusion was drawn from the data on the basis of Twoway Analysis of Variance (ANOVA) technique. The software used is INDOSTAT, version 98. The calculated values were compared with tabulated value at 5% level of significance (Fisher, 1935) for the appropriate degree of freedom.

Results and Discussion

Shoot Cut Bioassay

Table-1 revealed that maximum phytotoxic damage on the rating scale

was inflicted in 100% shoot leachates of *Cassia occidentalis* i.e. 5.00 against shoots of *Parthenium*, followed by P2 in which phytotoxic damage on the rating scale was found to be 4.20. In control, no effect was observed. In 75% and 50% concentration similar results were observed. Minimum inhibition was observed in 25% concentration that is 3.2 and 1.3 in P1 and P2 plant, respectively. It was observed that shoot leachates of 9th day, at 100% concentration after 72 hours shows maximum phytotoxic damage on the rating scale, and they differ significantly.

Seedling Bioassay

It was observed in table-2 that maximum phytotoxic damage on the rating scale was inflicted in 100% shoot leachates of *Cassia occidentalis* i.e. 4.00 against shoots of *Parthenium*, followed by P2 in which phytotoxic damage on the rating scale was found to be 3.20. In control, no effect was observed. In 75% and 50% concentration 2.8 and 1.8 damage rating scale was observed in P1 and P2 plant, respectively. Minimum inhibition was observed in 25% concentration that is 1.0 and 0.3 in P1 and P2 plant, respectively. It was observed that shoot leachates of 9th day, at 100% concentration after 72 hours shows maximum phytotoxic damage on the rating scale, and they differ significantly.

Chlorophyll Estimation

Chlorophyll 'a', 'b' and Total Chlorophyll

Table - 3 revealed that maximum inhibition in Chl 'a' of *Parthenium* was

by 100% shoot leachate of *Cassia* (P1) i.e. 8.20, followed by *Calotropis* (P2) in which 12.56 Chl 'a' was observed. Highest value was observed in control i.e. 21.24. 100% concentration was found to be significant over other concentrations. Table – 3 depicted that maximum inhibition in Chl 'b' of *Parthenium* was by shoot leachate of *Cassia* i.e. 2.34, followed by *Calotropis* in which 3.00 Chl 'b' was observed. In control 3.42 Chl 'b' was observed. 100% concentration was found to be significant. Maximum inhibition in total chlorophyll of *Parthenium* was observed by 100% shoot leachate of *Cassia* i.e. 10.54, followed by *Calotropis* in which 15.56 total chlorophyll was observed. Highest total chlorophyll was observed in control i.e. 24.66.

Estimation of Nitrogen and Protein content

Table-3 revealed that maximum inhibition in percent nitrogen of *Parthenium* was by 100% shoot leachate of *Cassia* i.e. 3.15, followed by 75%, 50% and 25% in which 3.48, 3.88 and 4.33 nitrogen percentage was observed, respectively. Different data was observed in P2 plant. At 100% shoot leachate of *Calotropis* nitrogen percentage was found to be i.e. 5.81, followed by 75%, 50% and 25% in which 6.00, 6.87 and 7.89 nitrogen percentage was observed, respectively. Highest percent nitrogen was observed in control i.e. 5.85. 100% concentration is significant over other concentrations. Table-3 revealed that maximum inhibition in protein content of

Parthenium was observed in the 100% shoot leachate of *Cassia* i.e. 19.68, followed by *Calotropis* in which 36.31 protein content was observed. Highest protein content was observed in control i.e. 36.56. The data differs significantly. The allelopathic properties of plants can be explained successfully as a tool for weed reduction. The chemical exudates from allelopathic plants are proposed to play a major role in the allelopathy mode of action. *Parthenium hysterophorus* L., a common problematic weed of wastelands as well as crop fields in India is well known of its lethal allelopathic effect on many agricultural crops including chickpea, kodo mustard, linseed etc. (Oudhia and Tripathi, 1998). It also has the potential to disrupt natural ecosystem and becoming a curse for biodiversity at many places in India. In order to manage this noxious weed in ecologically safe and viable manner, at present there is a trend towards searching for allelopathic plant species which has the potential to replace this weed through allelopathic mechanism. During field surveys it has been observed that at some sites *Cassia occidentalis*, *Calotropis procera*, *Chenopodium album*, *Croton bonplandianum*, *Abutilon theophrasti*, *Acacia arabica* have replaced this weed in patches. Mamatha and Mahadevappa (1992) based on their preliminary surveys have reported that *Cassia sericea*, *C. tora*, *Tephrosia purpurea* and *Croton bonplandianum* restricted *Parthenium* invasion in many states in India. The allelochemicals present in *Azadirachta indica* have inhibitory effect

on the germination and seedling growth of *Parthenium* and *Amaranthus*. There was a variability in germination percentage, shoot and root growth of seedling due to the treatments. The degree of phytotoxicity of senescent leaves was more as compared to green leaves and bark was more inhibitory to germination of *Parthenium* and *Amaranthus*.

Inhibition of *Parthenium* seed germination was absolute in aqueous foliar extracts of *Cassia occidentalis*, *Andrographis paniculata* and *Abutilon indicum*. There was no stimulatory effect of extracts on seed germination and seedling growth to test *P. hysterophorus*. All the species tested, inhibited the early seedling growth and fresh weight of the seedling. *Cassia sericea* recorded the highest reduction in seedling length i.e. 87.66 and 87.31% by leaf and whole plants extract, respectively at 50% concentration followed by *Hyptis suaveolens* (83.89 & 84.55%). These plants also caused maximum reduction in the seedling fresh weight of *Parthenium hysterophorus* (Senthil et. al., 2004). The grazing animals dislike *Cassia* due to this pungent odour and this

is considered as desirable traits, as it repelled the cattle and successfully replaced *Parthenium* even in unprotected land. Its rapid rate of growth, wide range of ecological adaptability, sufficient quantity of seed production and its utility are also considered for its choice to suppress this weed. *Cassia* also has the allelopathic action which hampers the growth of *Parthenium*. Further, research at the University of Agricultural Science, Bangalore confirmed that the plant botanical agents (BA's) could exert allelopathic impact and hinder germination and growth of *Parthenium* both in green house and in its natural habitat.

Conclusion

Evidence showed that higher plants release a diversity of allelochemicals into the environment which includes phenolics, alkaloids, long-chain fatty acids terpenoids and flavonoids. Once the most promising phytochemicals are identified along with their preference, they can be recommended for mass scale cultivation in *Parthenium* infested pockets for subsequent replacement of this noxious weed.

Table-1. Herbicidal potential of shoot leachates of *Cassia occidentalis* and *Calotropis procera* on bioactivity of *Parthenium hysterophorus* Shoot-Cut Bioassay

Allelopathic plants	Incubation periods and concentration ± Phytotoxic damage rating			
	9 th day extract after 72 hours exposure time			
	25%	50%	75%	100%
P ₁	3.2±0.2	4.3±0.3	4.3±0.7	5.0±0.0
P ₂	1.3±0.4	3.6±0.3	3.8±0.2	4.2±0.0
Control	0.0±0.0			

n = 10; Mean ± Standard Error; Shoot cut bioassay; 0 = No phytotoxicity, 5 = Highest phytotoxicity

P₁= *Cassia occidentalis*

P₂= *Calotropis procera*

Table-2. Herbicidal potential of shoot leachates of *Cassia occidentalis* and *Calotropis procera* on bioactivity of *Parthenium hysterophorus*

Seedling Bioassay

Allelopathic plants	Incubation periods and concentration ± Phytotoxic damage rating			
	9 th day extract after 72 hours exposure time			
	25%	50%	75%	100%
P ₁	1.0±0.0	2.0±0.4	2.8±1.4	4.0±0.0
P ₂	0.3±0.0	1.6±0.1	1.8±0.2	3.2±0.0
Control	0.0±0.0			

n = 10; Mean± Standard Error; Shoot cut bioassay; 0 = No phytotoxicity, 4 = Highest

phytotoxicity

P₁= *Cassia occidentalis*

P₂= *Calotropis procera*

Table-3. Effect of *Cassia occidentalis* and *Calotropis procera* on chlorophyll, nitrogen and protein content of *Parthenium hysterophorus*

Allelopathic plants	Concentration	Chl 'a'	Chl 'b'	Total Chl	Nitrogen %	Protein Content
P ₁	25	11.32	3.01	14.33	4.33	27.06
	50	10.55	2.87	13.42	3.88	24.25
	75	9.78	2.56	12.34	3.48	21.75
	100	8.20	2.34	10.54	3.15	19.68
P ₂	25	15.33	3.98	19.31	7.89	49.31
	50	14.59	3.44	18.03	6.87	42.93
	75	13.28	3.21	16.49	6.00	37.50
	100	12.56	3.00	15.56	5.81	36.31
Control		21.24	3.42	24.66	5.85	36.56

P₁= *Cassia occidentalis*

P₂= *Calotropis procera*

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