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Phytotoxic Effects of Parthenin on *Ageratum conyzoides* L.

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Parthenin was extracted from *Parthenium hysterophorus* L. leaves growing in northern part of India, and its effect was tested on the seed germination parameters and other related characteristics of *Ageratum conyzoides* L. weed. Parthenin proved phytotoxic to *A. conyzoides* as most of the studied parameters were inhibited. It may lead to a possible biological eradication of the *A. conyzoides* weed.

The allelopathic response of sesquiterpene lactones, in general, has been studied by a few workers through bioassay techniques (Kanchan, 1975; Stevens and Merryll, 1985). Parthenin comes from one such group of sesquiterpene lactones (Picman and Picman, 1984; Kumari and Kohli,1987). The occurrence of parthenin has been indicated in all parts of *P. hysterophorus* except the roots. Parthenin has also been reported to be phytotoxic to some plants and to its parent plant also (Kumari, 1989, Daizy and Kohli, 1991).

Weeds constitute an integral part of any ecosystem. They are found in almost all places where vegetation can be seen (Lee et al., 1999). Though unwanted they still play an important role in ecosystem balancing. In agricultural practices, however, they assume the role of the most unwanted plants(Cho and Kong, 1998). Every year, millions of dollars are spent in their eradication with huge wastage of manpower.

Ageratum conyzoides L., an obnoxious weed, belongs to the family Asteraceae and is commonly known as "Buckwheat", "Goat Weed", "Bill Goat Weed", "White Weed" or "Neela Phulnu" in various parts of the world. It can be seen at an altitudinal range of 800-1800 meters and is a medium sized shrub. It bears blue or white flowers and is a shade - and moisture - loving plant. Thus, it grows abundantly around some water sources. Of late, it has been posing some serious threat to the nearby vegetation by invading fast into the native vegetation. Many chemicals from *Parthenium hysterophorus*, are reported which may provide phytotoxicity to some weeds (Eyini et al., 1999).

Many synthetic chemicals known as herbicides or weedicides are known, which are available for the eradication of the weeds. But these being synthetic in nature may pose serious health problems apart from threatening the environment. Moreover, their accumulation may further add on to the sterility of the land of their application. In the present studies, an attempt has been made to utilize the natural chemical (parthenin) from another weed *Parthenium hysterophorus* L. to check the germination and other parameters of *A. conyzoides*.

Materials and Methods

Extraction of parthenin

Freshly plucked and shade-dried leaves of *P. hysterophorus* were ground to powder and repeatedly washed (100 gm) with petroleum ether (40-60℃). The residue after freeing of washing was suspended in ethyl alcohol for 24 h before concentration in a flash rotary evaporator. Dried extract was dissolved in chloroform. From one half of this chloroform, the solvent was evaporated over hot water bath. The resulting residue was subjected to silica gel column chromatography through elusion with chloroform + methanol (9:1) to get parthenin. Requisite amount of parthenin was first dissolved in a few drops of ethanol and the final volume was made with pure water for further experimentation. Different concentrations of parthenin were made ranging from 0.02 mg/ml to 0.1 mg/ml.

Bioefficacy studies

Healthy and viable seeds (viability checked through TTC test) of *A. conyzoides* were collected from locally growing plants of *A. conyzoides*. These were divided into six groups of 400 seeds each. One group was subdivided into 4 to 100 seeds each.

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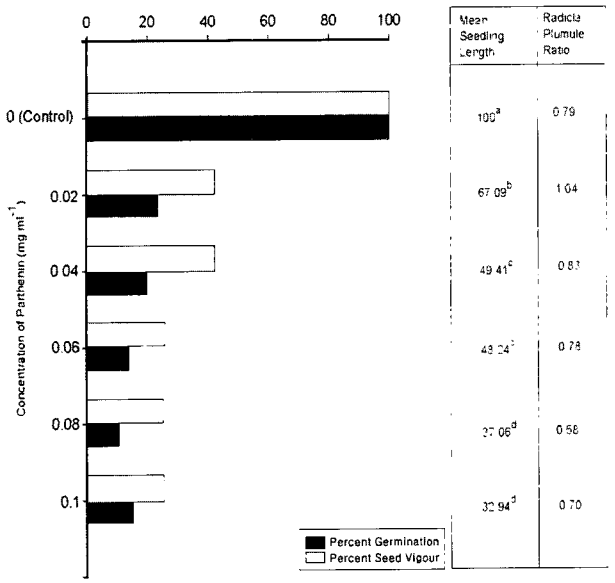


Correlation Coefficient (r) between

Concentration and Germination	-0 8148	Conc and Seed Vigour	-0 7551
Concentration and Mean Seedling Lenght	-0 9226	Conc and RP Ratio	-0 6588

Values in Control

Per cent Germination	*90 + 5*	*Per cent Seed Vigour*	*78 75 + 3 2*
Mean Seedling Length	*1 70 + 0 11*	*Radicle Plumule Ratio*	*0 79 + 0 10*

Means have been presented in the table and graph. Similar superscripts on the bars and vaules (a,b,c ...) represent the insignificant differences at 5 per cent level of significance applying DMRT (Duncan, 1955).

Fig. 1. Effect of parthenin extracted from *P. hysterophorus* on germination parameters of *A. conyzoides*. Values presented are per cent w.r.t. control.

The 4 seeds were soaked in pure water for 16 h, which served as control. The remaining 100 seeds each were soaked in comparable period of time in the respective treatment solutions (0.02, 0.04, 0.06, 0.08 & 0.1 mg/ml parthenin). Parthenin was purchased from Sigma. The imbibed seeds from both control as well as treatment groups were arranged on Whatman no. 1 filter paper underlined with a thin wad of absorbent cotton in Petri dishes of 6 cm in diameter. Each Petri dish contained 100 seeds each and four such replica were maintained for each treatment. The whole setup was maintained in a seed germinator at 25±3℃ temperature. The number of the seeds that germinated were daily observed till no more seeds germinated for eight consecutive days. The rate of germination was measured using the formula given by Agarwal (1980) based on ISTA rules. The lengths of the seedlings were directly measured using a scale at the time of termination of the experiment.

Treatment of the plants and estimations : Healthy, uniform *A. conyzoides* (about 50±10 days old) were grouped into six sets of ten plants each. They were spray-treated with respective concentrations of parthenin, while treatment with pure water served as control. For each treatment, 20 ml of the solution was sprayed with a hand held spray pump, for three consecutive evenings. Uniform punches of the leaves of control as well as treated plants were made in the morning following the last spray. From a weighed sample of the leaf punches chlorophyll was extracted in dimethyl sulphoxide (DMSO) following the method of Hiscox and Israelstam (1979) and improved by Daizy and Kohli (1991). The values of cellular respiration (a measure of the cell survival) was determined using the method of Steponkus and Lanphear (1967), while the water content was measured using the Dean and Stark apparatus. Leaf temperature and relative humidity were measured by the LI-COR made Steady State Porometer.

Results and Discussion

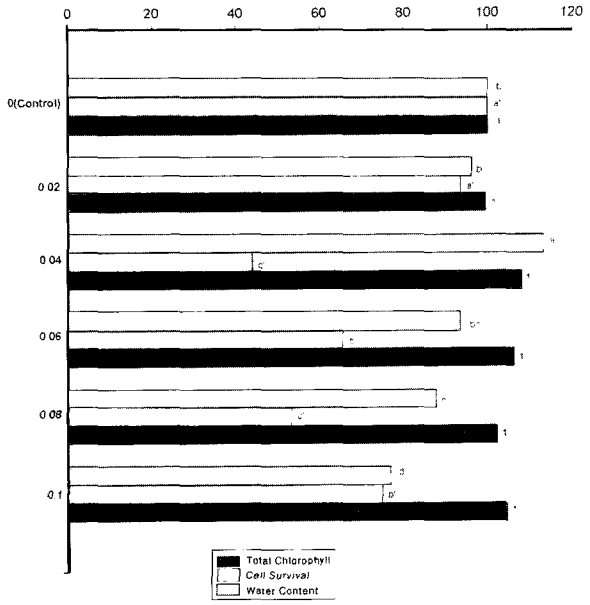
Bioefficacy studies

The results of the present experiment on germination parameters clearly reveal that parthenin is toxic to *A. conyzoides* plants. It also reduces the cellular respiration and chlorophyll content of leaves thus obviously affecting the photosynthesis. The near unity (negative) values of the correlation coefficient between concentrations and mean seedling length and germination showed that the increase in concentration linearly decreased the values of these parameters. Visual observations after one month of the last spray revealed that the treated plants were wilting. The degree of wilting was directly proportional to the concentration applied. Biomass was recorded which clearly demonstrated the toxic effect of parthenin towards *A. conyzoides*.

Compared to 90±5 percent germination in the water treated control, there was a significant decrease in the treated samples (Fig. 1). The decrease was directly proportional to the increase in the concentration applied. The lowest percent germination (25 percent) was recorded in the seeds treated with the highest concentration of parthenin (0.1 mg/ml). A statistically significant decrease (as compared to the control) was found with all treatments with parthenin. Germination or seed vigour was less than half the value of the control. The lengths of the seedlings were significantly shorter in the treated. (Fig. 1). The value was less than half of the control especially at higher concentrations.

Chlorophyll content, cellular respiration and water content

Total chlorophyll content showed a linear decrease in those treated with increasing concentration (Fig. 2) of parthenin except at 0.04 mg/ml (where an increase was found compared to control). The cellular respiration (measured in terms of cell survival values) was significantly low in response to each, except the lowest concentration of 0.02 mg/ ml^{-1}(Fig. 2). The maximum



Correlation Coefficient (r) between

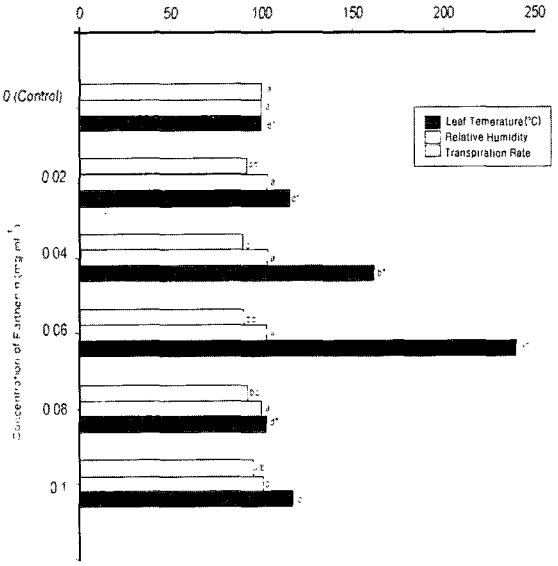
Concentration and Chlorophyll Content	0 8148
Conc and Cell Survival	-0 7551
Concentration and Water Content	-0 9226

Values in Control

Total Chlorophyll Content	*18 76 + 0 31 µg mg^{-1} d w*
Optical Density at 530 nm	*1 22 + 0 29*
Per cent Water Content	*75 0 + 2 5*

Means have been presented in the table and graph Similar superscripts on the bars and vaules (a,b,c .) represent the insignificant differences at 5 per cent level of significance applying DMRT (Duncan, 1955)

Fig. 2. Effect of parthenin extracted from *P. hysterophorus* on chlorophyll content, cell survival and water content of *A. conyzoides.* Values presented are cent w.r.t. control.



Correlation Coefficient (r) between

Concentration and Leaf Temperature	-0 2758
Concentration and Relative Humidity	-0 3550
Concentration and Transpiration Rate	-0 1125

Values in Control

Leaf Temperature	24 8 + 0 76 C
Relative Humidity	15 87 + 0 13 per cent
Transpiration Rate	2 65 + 0 13

Means have been presented in the table and graph Similar superscripts on the bars and vaules (a,b,c) represent the insignificant differences at 5 per cent level of significance applying DMRT (Duncan 1955)

Fig. 3. Effect of parthenin extracted from *P. hysterophorus* on various leaf parameters of *A. conyzoides.* Values presented are per cent w.r.t. control.

decrease was up to more than half at 0.04 mg/ml^{-1}. The water content was relatively high in almost all the treatments, but the increase was statistically non-significant (Fig. 2).

Leaf parameters

The mean leaf temperature of the water-treated control was 24.8±0.76℃. Compared to this, there was a non-significant decrease in the leaf temperature when any of the concentrations of parthenin were sprayed on *A. conyzoides* plants. The relative humidity of the leaves treated with parthenin was either exactly the same or slightly higher, as compared to the control. The transpiration rate of leaves was relatively high with all concentrations of parthenin as compared to the control (Fig. 3), the maximum increase being about 2.5 fold (with 0.06 mg/ml^{-1}). The treated plants depicted increased rate of transpiration. However, like other parameters of leaf, the effect was not consistent with the concentration as seen from the weak values of correlation coefficient.

Acknowledgement

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