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Allelopathic effect of aqueous leaf extract of *Parthenium hysterophorus* on germination and seedling growth of the *Gossypium hirsutum*

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ABSTRACT:

Weeds are sometimes considered as unwanted plants. Some weeds reduce human efficiency through physical discomfort caused by allergies and poisoning. Weeds such as parthenium (*Parthenium hysterophorus*) and rag weed (Limbrosia species) causes etching, hay fever and other debilitating allergies, which contribute markedly to chronic human illness and suffering. Cotton seeds were treated with different concentrations of leaf exudates of *parthenium hysterophorus* and the biochemical constituents of treated cotton plants were analysed. The protein and phenol were found to increase in treated plants due to the release of protease inhibitors. In addition to this, increase in the activity of Nitrate reductase and peroxidase were observed followed by dthe ecreased activity of catalase. The plants with higher concentration of phenol showed resistance to pest and they were healthy compound to that of controlled plants. The treated plants also showed some morphological variations. Increase in height was noted in 80% treated plants and there were no growth and decrease in 100% treated. This inhibitory effect was due to high concentration of first noted in 80% treated plants. Thus 80% treatment was found to have optimal activity.

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INTRODUCTION

Allelopathy refers to the beneficial or harmful effects of one plant on another, both crop and weed species, release chemicals from plant parts by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems. It is the science that studies any process involving secondary metabolites produced by plants, algae, bacteria, coral and fungi that influences the growth and development of agricultural and biological systems. (IAS, 1966). The biomolecules are called allelochemicals and are produced by some plants as secondary metabolites.

When the allelochemicals are released into the environment, they inhibit the development of neighbouring plants. Allelopathic plants release compounds into the environment through root exudation, leaching by dews and rains, and volatilization or decaying plant tissue (Rice, 1984). In most cases, the compounds inhibit, germination or growth of neighboring plants although sometimes the compounds stimulate their growth.

Allelopathy in crops may act as a biological weed control in the agroecosystem. The genetic improvement of the allelopathic effect in crops is a strategy for biological weed control in breeding programs. In the 1970s, germ plasm assessment was extensively undertaken to detect allelopathic accessions of crops. Accessions with an allelopathic effect have been found in crops such as beet (*Beta vulgaris* L.), lupine (*Lupinus lutens* L.), maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), oat (*Avena sativa* L.), pea (*Pisum sativum* L.), barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), and cucumber (*Cucumis sativus* L.) reviewed by Rice (1984). A total of 538 accessions of cultivated and wild cucumber were screened by the pot and field test, several accessions inhibited the growth of weeds (Putnam and Duke, 1974). Out of more than 3000 assessions of oat, several were found with a fluorescent microscope to exude a large amount of an allelochemical, scopoletin (Fay and Duke, 1977). One of the most studied aspects of allelopathy is the role of allelopathy in agriculture. Current research is focused on the effects of weeds on crops, crops on weeds, and crops on crops. This research furthers the possibility of using allelochemicals as growth regulators and natural herbicides, to promote sustainable agriculture. Weeds are always considered as unwanted plants (Oudhia, 1998). A number of such allelochemicals are commercially available or in the process of large-scale manufacture. For example, Leptospermone is a purported allelochemical in lemon bottlebrush (*Callistemon citrinus*). Although it was found to be too weak as a commercial herbicide, a chemical analog of it, mesotrione (tradename Callisto), was found to be effective.

The present study aims to investigate the seed germination and growth of cotton seeds which were treated with different concentrations of the leaf exudates of *Parthenium hysterophorus* at 20%, 40%, 60%,80%, and 100%. This investigation is proceeded for the future prospect of allelopathy. This may develop the legume crops with high biological N2 functionpotential and least inhibitory effects on components crops in intercropping systems and on succeeding crops in crop relations.
Green herbicides containing Green Allelochemicals are an integral part of eco or organic farming . Use of natural compounds as herbicides or as the chemical basis for the development of new herbicides offers several advantages.

1. The wide array of phytotoxic compounds produced by plants provide many complex chemical structure that are unlikely to be discovered in the traditional synthetic strategies used by pesticides companies.
2. Degradation of natural compounds in the environment proceeds faster than that of synthetic compounds and thus reduces the environment pollution and ground water contamination etc.,
3. The halogenated hydrocarbon which constitute about 60% of the registered herbicides are of environmental concern, while vast majority of natural compounds from plant pose little hazards and therefore, are environmentally safe. The negative (stimulatory) allelopathic effects of weeds on agricultural crops can be used to develop “Green growth promoters”. Many studies conducted at department of Agronomy, Indira Gandhi Agricultural University, Raipur, India) have clearly revealeld that stimulatory allelopathic effects weeds on crops can be utilizied successfully for higher crop production.

Weeds reduces human efficiency through physical comfort caused by allergies and poisoning. Weeds such as Parthenium and rag weed (Ambrosia species) that cause etching, hay fever, and other debilitating allergies contribute markedly to chronic human illness and suffering. So objective of the present study is to investigate the effect of aqueous leaf extract of parthenium hysterophorous on

germination, seedling growth and development of cotton plant (*Gossypium hirsutum*).

MATERIALS AND METHODS

Materials:

***Gossypium hirsutum* (Cotton)**

Viable seeds of cotton were selected . The seeds were surface sterilized with 0.1% Mercuric Chloride for 2 minutes. It was then washed with tap water thrice and distilled water twice.

Parthenium hysterophorus

Pot Mixtures

Red soil, Black soil and dry manure in the ratio 1:2:1.

Aqueous leaf extract of *Parthenium hysterophorus*.

Methodology:

The sterilized seeds were sown in respective pots. The plant samples were taken at 6 ranges from all the treatments corresponding to germination stage. Control was maintained without adding the leaf extract of *parthenium hysterophorus*. Five Treatments were made in remaining five pots. The leaf extract was treated in the seed of *Gossypium hirsutum* in the concentration of 20%, 40%, 60%, 80% and 100%(Picture 2-5). The concentration was made by diluting with water.

Percentage of Germination

Healthy seeds were sown in land and treated with composted mycostraw. The seeds were considered to be germinated only when the radicle emergence was more than 1.0 cm. This was considered as the first day of germination percentage. The germination percentage was calculated using the formula:

$$\text{Germinating percentage} = \frac{\text{Total Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100.$$

The seed germination of Gossypium hirsutum was determined (Picture-1) in the concentration of (20%, 40%, 60%, 80% and 100%).

Parameters Analysis:

Assay of Nitrate Reductase:

5ml of 0.1 M Phosphate buffer (pH 7.5) to the freshly cut leaves, followed by the addition of 1ml of Potassium nitrite and 4 ml of Propanal and incubated in dark for 30 minutes. The constituents omitting plant tissues were kept as control. The 1 ml of sample from test and control solution was taken with different aliquots of potassium nitrite into a series of test tubes. The solution was made upto 1 ml with water. Then 1 ml of 1 % Sulphanilamide and 1 ml of NEDA was added to all the test tubes. After 10 minutes reading was noted at the absorbance of 540 nm.

Assay of Catalase:

100 ml of phosphate buffer from the conical flask was pipetted out and 0.4 ml of substrate into each flask were added. To one of the flask 0.5 ml of the enzyme extract was added and incubated for 15 minutes at room temperature. After 15 minutes 10 ml of 2N H_2SO_4 was added to both control and test flasks. Then the contents against 0.01N $Kmno_4$ one by one was titrated. Difference between these values give the volume of permanganate equivalent to enzymatic activity.

Assay of Peroxidase:

One minute fixed time assay is used to measure peroxidase activity. Two cuvettes were taken for blank and sample. 2.5 ml of aminoanmtipyrine-phenol solution and 2.5 ml of H_2O_2 was added and absorbance was readed. Then 0.1 ml of enzyme extract in the other cuvette was taken for the absorbance. The time was noted exactly for one minute to read the absorbance of the same cuvette.

The activity of the peroxidase can be calculated from the absorbance change.

$$\text{nit / mg} = \frac{\Delta A \text{ / min}}{6.58 \times \text{mg of the sample.}}$$

$$\Delta A = A_{1\text{ minute}} - A_{0\text{ minute}}$$

Where, A = Over all absorbance change

$A_{1\text{ minute}}$ = Absorbance at 510 nm after 1 minutes

$A_{0\text{ minute}}$ = Absorbane at 510 nm after 0 time.

Estimation of Chlorophyll:

1 gram of finely cut and well mixed representative sample of leaf was weighed and grinded to a fine pulp with the addition of 20 ml of 80 % acetone. It was centrifuged and the supernatant was transferred to 100 ml volumetric flask. The residue was grinded with 20 ml of 80 % acetone. Again centrifuged and the supernatant was transferred to the same volumetric flask. This procedure was repeated until this residue becomes colourless. The absorbance was recorded at 645 nm against the solvent (80% acetone) blank.

$$\text{mg chlorophyll a / g tissue} = 12.7\,(A663) - (A645) \times \frac{V}{1000 \times w}$$

$$\text{mg chlorophyll b / g tissue} = 22.9\,(A645) - 4.68\,(A663) \times \frac{V}{1000 \times w}$$

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$$\text{mg chlorophyll / g tissue} = 20.2\,(A\,645) + 8.02\,(A663) \times \frac{V}{1000 \times w}$$

Where,
A = Absorbance of specific wavelength
V = Final volume of chlorophyll extract on 80 % acetone.

Table-1. Percentage of Seed Germination

Treatment	Percentage of Germination (%)
Control	60
20%	70
40%	80
60%	60
80%	90

Estimation of Protein:

The different aliquots of working standard and sample extract was pipetted out to make upto 4 ml with distilled water. A tube with 4 ml distilled water serve as blank. 5.5 ml of alkaline copper solution was added in all the test tubes and mixed well. It was incubated at room temperature in the dark for 30 minutes. The blue color was developed. Then the color development was readed colorimetrically at 660 nm.

Estimation of Phenol

The different aliquots were pipetted out in the series of test tubes. The volume was made up to 3 ml with distilled water. Then 0.5 ml of Folin – Ciocalteau reagent was added. After 3 minutes 20 % Sodium Carbonate solution was added and mixed thoroughly and kept in the boiling water for one minute. The absorbance was noted at 650 nm.

RESULT

The present study was made to investigate the effect of aqueous leaf extract of *Parthenium hysterophorus* on the biochemical characteristics of Cotton (*Gossypium hirsutum*). The plumules arising from seeds during germination and the leaves during 15th,30th and 45th days taken for the analysis of various parameters (Picture 2-5).

A notable increase in activity was observed in 80% treated plants on all days especially in 30th day. The increase in nitrate in nitrate reductase activity may be due to the nitrate or nitrite released from the root nodules of plants.

The catalase activity was found to be decrease in the treated plants compared to that of control. The catalase activity was decreased on 80% treated plants during the germination and slightly in 20% and 60% treated plants. The activity decreased from 3.5 to 7.1 in 80% treated plants in control. The activity decreased with increased concentration of aqueous extract with some fluctuations.

Table- 2. Effect of Aqueous leaf extract of Parthenium Hysterophorus on Nitrate Reductase (ug of Nitrate formed / 30 minutes)

Treatment	Seed Germination	15th day	30th day	45th day
Control	87.5	92.0	102.5	69.5
20%	89.4	95.0	105.6	104.5
40%	89.5	93.0	106.0	103.5
60%	86.9	93.0	104.5	102.5
80%	98.8	99.5	107.5	105.5

Table – 3. Effect of Aqueous leaf extract of *Parthenium Hysterophorus* on the Catalase Activity (ml of 0.01 N Kmno 4 Consumed / mt / ml / of enzyme).

Treatment	Seed Germination	15th day	30th day	45th day
Control	3.5	6.3	7.2	6.8
20%	5.2	8.5	9.0	9.2
40%	5.5	8.3	9.1	9.0
60%	5.4	8.8	9.5	9.4
80%	7.1	10.2	12.8	13.5

Table -4. Effect of Aqueous leaf extract of *Parthenium Hysterophorus* on Peroxidase activity (units / mg)

Treatment	Seed Germination	15th day	30th day	45th day
Control	0.15	3.5	5.6	2.5
20%	0.30	3.9	7.0	4.1
40%	0.75	4.2	6.8	3.4
60%	0.60	3.7	7.2	3.3
80%	0.91	4.5	8.1	4.5

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Table – 5. Effect of Aqueous leaf extract of Parthenium Hysterophorus on Chlorophyll

Treatment	Chlorophyll	Seed Germination	15th day	30th day	45th day
Control	a	0.0002	0.0009	0.0004	0.0004
	b	0.0004	0.0007	0.0007	0.0003
	Total	0.0006	0.0016	0.0011	0.0007
20%	a	0.0003	0.0008	0.0005	0.0004
	b	0.0005	0.0007	0.0001	0.0003
	Total	0.0018	0.0015	0.0006	0.0007
40%	a	0.0003	0.0005	0.0005	0.0004
	b	0.0003	0.0003	0.0001	0.0002
	Total	0.0006	0.0008	0.0006	0.0006
60%	a	0.0005	0.0005	0.0003	0.0004
	b	0.0002	0.0003	0.0005	0.0001
	Total	0.0007	0.0008	0.0008	0.0005
80%	a	0.0002	0.0004	0.0002	0.0003
	b	0.0003	0.0005	0.0004	0.0001
	Total	0.0005	0.0009	0.0006	0.0004

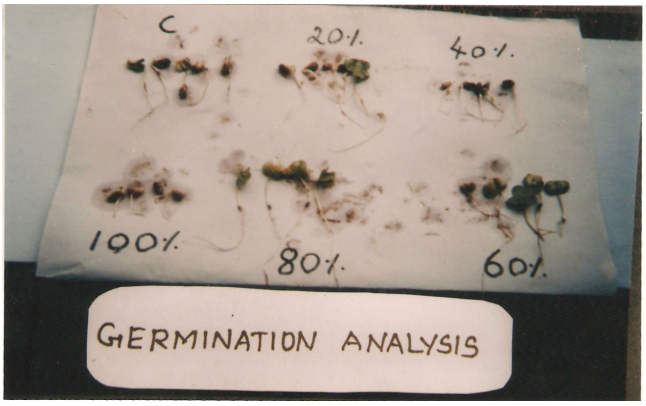
The peroxidase activity was increased in all the treated plants. Votable increase was found in 80% during 30th day analysis. The activity increased from 0.15 to 0.91 in 80% treated plants during seed germination.

The Chlorophyll concentration was increased from 20% - 80% treated plants compared to that of control during the germination and 15th day analysis. Chlorophyll concentration was decreased from 0.0011 in control and finally to 0.0006 in treated plants, and 0.0007 in control and finally to 0.0004 in treated plants in during 30th day and 45th day.

SUMMARY AND CONCLUSION:

In the present study cotton seeds were treated with different concentrations of leaf exudates of *Parthenium hysterophorus* and the biochemical constituents of the obtained cotton plants were analysed. The present study was conducted to investigate the allelopathic effects of *Parthenium hysterophorus* weed on seed germination and seedling growth of *Gossypium hirsutum*. The aqueous leaf extracts of *Parthenium* at 20%, 40%, 60%, 80%, and 100% of concentrations were applied to determine their effect on the seed germination and seedling growth under laboratory conditions. The protein, phenol were found to be increased in the treated plants due to the release of protease inhibitors and other non determined allelochemicals from the root exudates. In addition to this, increased activity of Nitrate reductase activity and peroxidase were observed followed by decreased activity of Catalase.

Inspite of this, the plants with higher concentration of phenol showed resistance to pest and they were healthy compared to that of control



Picture – 1. SEED GERMINATION AT DIFFERENT CONCENTRATION

2 plant growth at 15th day at different concentration

plant. The treated plants also showed some morphological variations. Increase in height noted in 80 % treated plants, and there is no growth and decrease in 100% treated. The inhibitory effect was due to high concentration of first noted in 80% treated plants. Thus 80% treatment was found to have optimal activity. Thus it can be concluded that efficient elimination of some toxic compound, the application of leaf extract of *Parthenium hysterophorus* can be used for the growth and yield of the cotton plants, though the many of works implies. Leaf extracts at the high concentration (80%) greatly promoted root length.

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