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Reduction in Growth and Biological Pigments Present In *Parthenium hysterophorus* by the Allelopathic Leaf Extract of *Alstonia scholaris*

Nikita Banerjee[1], A.K. Pandey[2]

[1]Mycological Research Laboratory, Department of Biological Sciences, R.D. University, Jabalpur- 482001 (M.P), India
[2]M.P. Private University Regulatory Commission, Bhopal (M.P), India

***Abstract*: *Parthenium hysterophorus* is a well-known weed with numerous harmful impacts. During the present work, aqueous leaf extract of *Alstonia scholaris* was found allelopathic to the seeds and seedlings of the target weed in laboratory conditions. The allelopathic leaf extract was also found effective against the biological pigments like Chlorophyll a and b followed by protein. Various biochemical tests were performed by which the presence of alcohols, aldehydes and ketones was confirmed in the aqueous allelopathic leaf extract.**

***Keywords*: *Parthenium hysterophorus*, *Alstonia scholaris*, allelopathic leaf extract, biochemical tests.**

1. INTRODUCTION

P. hysterophorus is a noxious and pernicious weed of the family Asteraceae. In India, it is reported for the first time from Pune (Rao, 1956) and being invasive and aggressive weed has now naturalized itself from almost all parts of the country (Singh *et al.*, 2008; Dwivedi *et al.*, 2009). It is responsible for various health ailments to humans as well as livestock (Bajaj, 2001; Verma *et al.*, 2001). And also proved harmful to many economically important crop plants by secreting toxic allelochemicals that inhibits growth and reproduction of the neighbouring plants (Singh *et al.*, 2005; Belz *et al.*, 2007). Various manual and mechanical techniques tried for the management of this deadly weed had appeared with several harmful disadvantages. Chemicals may be hazardous in various direct or indirect ways namely toxic of residuals, injury to non-target crops and endangered species, development of resistance and resurgence at more serious levels.

Therefore, use of allelochemicals isolated from plants can be considered as a potential, cost effective and environmentally safe alternative for the management of target weed. It may be beneficial, harmful or with no effects to the associated plants (Inderjit, 1996; Maharjan *et al.*, 2007) and are found to be present in all parts of plant at different concentrations (Rizvi and Rizvi, 1986; Alam and Islam, 2002; Tinnin and Muller, 2006; Sarkar *et al.*, 2012). Factors like plant genetics and environmental conditions widely affect the production of allelochemicals (Yu *et al.*, 2003).

A. scholaris is a tree of tropical region. Allelopathic compound extracted from its leaves were found effective against the seedlings of the target weed during preliminary assessment, performed earlier by the authors (Banerjee *et al.*, 2013). Thus, the present work was done to determine the potential and mode of action of the phytotoxic allelopathic compound extracted from the leaves of *A. scholaris*.

2. MATERIALS AND METHOD

The aqueous extracts from the healthy leaves of *A. scholaris* was prepared as according to Banerjee *et al.*, 2013 and used for further assessment.

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1. Allelopathic potential of the test leaf extract on growth of the target weed was determined as follows-

a. Pot bioassay:

The healthy seeds of the target weed were surface sterilized by dipping in the solution of 0.1% $HgCl_2$ for 1 min. which was followed by thorough washing under running tap water, then were dipped in the resulted leaf extract and were sown in individual pots containing sterilized field soil, sand and peat in the ratio of 1:1:1 for 24, 48 and 72 hours at 27±2ºC with 100% humidity (Kadian and Suryanarayana, 1971; Nguyen *et al.*, 1973).

Inhibition in seed germination was calculated by using the following formula-

$$\text{Seed germination inhibition (\%)} = \frac{\text{No. of non-germinated seeds}}{\text{Total no. of seeds planted}} \times 100$$

The root length and shoot length was recorded in cm.

b. Seedling bioassay:

Plastic pots were used to raise the seedlings of the target weed filled with sterilized soil, sand and peat as above and sprayed with 30 ml. of the resulted allelopathic leaf extract. These pots were then incubated in growth chambers for 24, 48 and 72 hours at 27±2ºC with 100% humidity. On the basis of development of disease symptoms to the test seedlings, the results were recorded.

Distilled water served as control in each case.

2. Effect on chlorophyll (a and b) and protein contents:

Effected leaves were grounded (1.0 gm) and blended with 10 ml of ethanol using mortar and pestle and centrifuged for 10 min. at 3000 rpm. The collected supernatant was extracted with acetone (80%) and the above process was repeated until the residue become colourless. Thus, further subjected to chlorophyll estimation (Bradford, 1976).
Chlorophyll (a and b) content was determined on a pre-weighted basis employing the following formula-

$$\text{Chl a (mg g}^{-1}\text{ FW)} = 11.75 \times A663 - 2.35 \times A645$$

$$\text{Chl b (mg g}^{-1}\text{FW)} = 18.61 \times A645 - 3.96 \times A663$$

Where,

A = Length of light path in the cell (usually 1cm.).

FW= Fresh weight of the sample in gm.

The absorbance of extract (supernatant) was measured at 645 and 663 nm. respectively.

The amount of protein was calculated by following the method of Lowry *et al.*, (1951) with reference to the bovine serum albumin standard curve.

Healthy leaves served as control in each case.

3. Assessment of chemicals in the leaf extract:

Healthy leaves of *A. scholaris* were collected and oven dried at 28°C. for 72 hours. These dried leaves were finely crushed and the resulted powder thus obtained was used for the determination of presence of chemicals like alcohols, aldehydes and ketones.

For the presence of alcohols, acetyl chloride (2 ml.) was added in 1 ml. of the aqueous solution (1 gm of the resulted powder and 10 ml. of distilled water) and heated properly.

And for aldehydes, very little amount of the dried sample was added to 1 ml of the freshly prepared Tollen's reagent (2 ml of 0.2 M silver nitrate solution) was taken in a test tube which has been cleaned with 3M sodium hydroxide and a drop of 3M sodium hydroxide was added. Then, 2.8% ammonia solution was added drop wise with constant shaking, until the precipitate of silver oxide gets dissolved. Finally the entire solution was diluted with 10 ml of distilled water. Gentle heating resulted the observation of reaction.

To determine the presence of ketones, 5 ml. of ethanol and 1 gm of the resulted powder was taken in a test tube and 2,4-Dinitrophenylhydazine was added after shaking for 2 minutes.

3. RESULTS AND DISCUSSION

During pot bioassay, the shoot length 1.3 cm, root length 1.7 cm and maximum inhibition in seed germination 85.33% in comparison to control which was 2.2 cm shoot length, 2.8 cm root length and 0% inhibition in seed germination was recorded after 72 hours post treatment (hpt) respectively as in Fig. 1.

Also, seedling mortality was found maximum after 72 hours post treatment (hpt) (90.4%) followed by 48 hours post treatment (hpt) (85.13%) and 24 hours post treatment (hpt) (78.06) as in Fig. 2.

To the pigments present in leaves of the target weed, the allelopathic leaf extract was found potentially effective against chlorophyll a (0.63) and b (0.57) followed by protein (8.75) after 72 hours post treatment (hpt) (Fig. 3 and 4).

Also, presence of alcohols, aldehydes and ketones were recorded in the leaves of *A. scholaris* (Table: 1).

The aqueous extract obtained from the leaves of *A. scholaris* was found with remarkable seedling mortility against the target weed followed by seed germination inhibition, reduction in shoot and root length respectively.

Effect of allelopathic leaf extracts on growth of various weeds from plants like *Croton bonplandianum*, *Helianthus annuus* and *Lantana camara* was reporded earlier by many workers (Ashrafi *et al*., 2008; Sisodia and Siddiqui, 2010; Motwani *et al*., 2013). Germination inhibition and growth retardation in *Parthenium* seeds and seedlings due to allelopathic leaf extract was observed earlier by several workers (Javaid, 2010; Knox *et al*., 2010; Vitonde *et al*., 2014). However, the seedling mortality caused by the resulted leaf extract may be due to the phytotoxic activity of only one of the recorded chemicals (Einhelling and Rasmussen, 1979).

Reduction of pigments (chlorophyll a, b and protein) in leaves of the target weed is due to the allelopathic effects of the resulted leaf extract obtained from *A. scholaris*. Chlorophyll and protein plays a major role in photosynthesis and their reduction resulted in decreased photosynthesis rate. Depletion of chlorophyll (a and b) affects the photosystem (I and II) of photosynthesis, due to the rapid destruction of plasma membrane. Allelochemicals are responsible for reduction in chlorophyll contents of plants followed by cellular damage which causes retardation of photosynthesis, thus resulted in poor plant growth (Benyas *et al*., 2010).

The phytotoxic property of resulted leaf extract is due to the presence of certain chemicals in it. As a result of various biochemical tests, chemicals like alcohols, by acetyl chloride test with effervescence of HCl gas which changes the colour of litmus paper, aldehydes by using Tollen's reagent test resulted in formation of a black precipitate and ketones, using Brady's test with the formation of orange precipitate were found in the resulted leaf extract. Large number of chemicals are reported previously in the leaves of various plants (Ramamoorthy and Paliwal, 1993; Chaudhry *et al*., 2003; Salam and Kato-Noguchi, 2010; Sheela, *et al.*, 2011).

4. CONCLUSION

The present work revealed the allelopathic potential of aqueous leaf extract of *A. scholaris* to the growth of target weed in laboratory conditions and possesses significant reduction in pigments like chlorophyll (a and b) and protein present in the seedlings which may be due to the presence of certain chemicals. Therefore, after assessment of its host specificity, accumulation and degradability, can be used further for the effective biological management of *P. hysterophorus*.

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APPENDIX- A

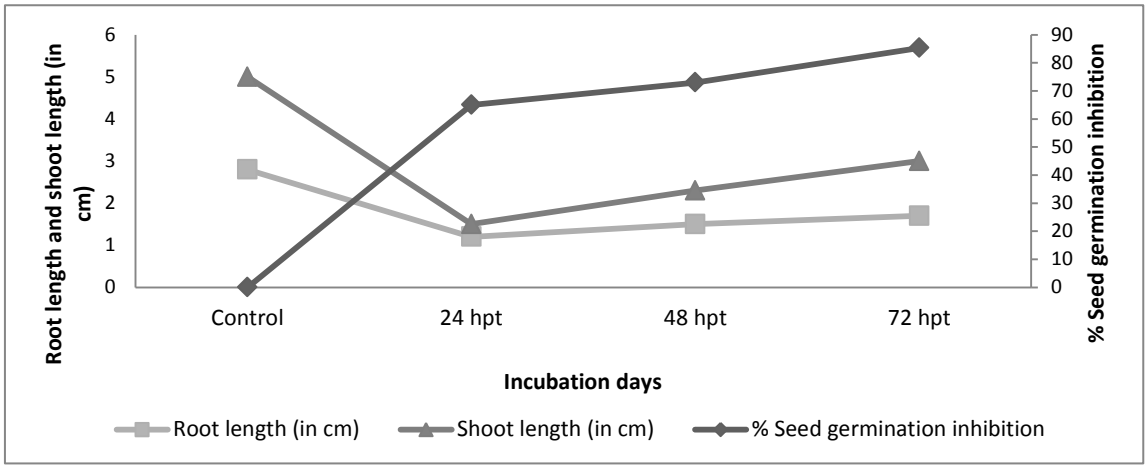

Fig.1: Allelopathic potential of the test leaf extract to the germination of seeds of *P. hysterophorus*

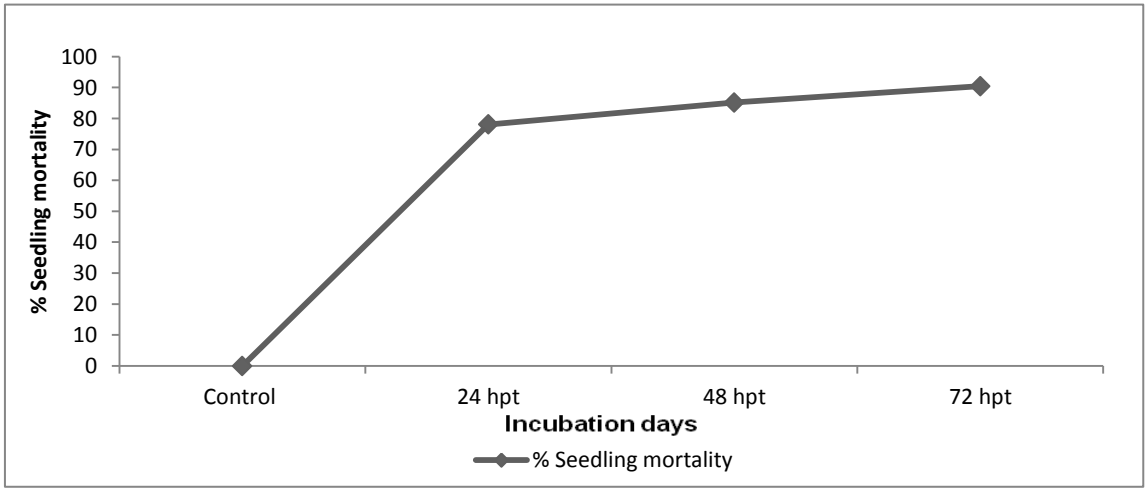

Fig.2: Allelopathic potential of the test leaf extract to the seedlings of *P. hysterophorus*

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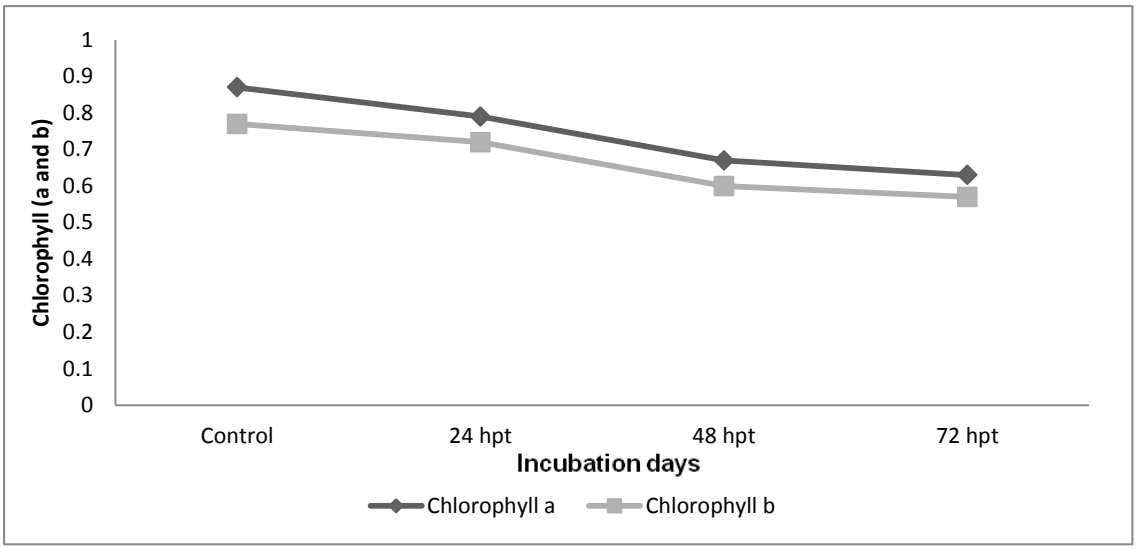

Fig.3: Effect of the allelopathic leaf extract to chlorophyll contents of leaves of *P. hysterophorus*

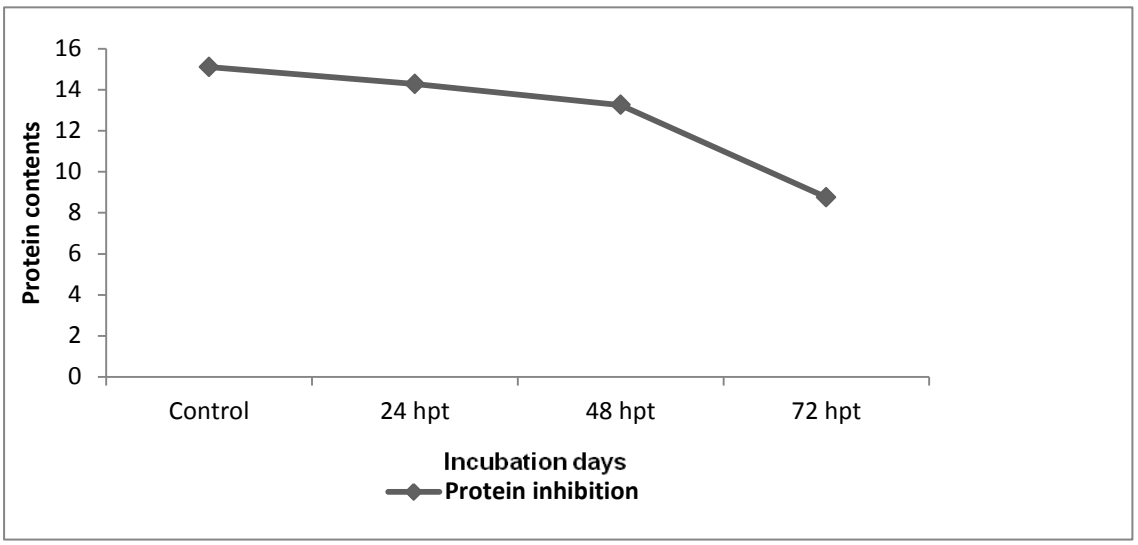

Fig.4: Effect of the allelopathic leaf extract to protein contents of leaves of *P. hysterophorus*

Table.1: Determination of various chemical contents present in the allelopathic leaf extract of *A. scholaris*

S. no.	Chemicals	Appearance
1.	Alcohols	+
2.	Aldehydes	+
3.	Ketones	+