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Magnitude of suppressing ability of *Eclipta alba (L.) Hassk.* on selected weeds of Aligarh

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A study was conducted to assess the magnitude of suppressing ability of *Eclipta alba* (L.) Hassk. on some selected weeds of Aligarh namely, *Amaranthus spinosus* L., *Cassia tora* L. and *Cassia sophera* L. The research finding was conducted to evaluate the effect of aqueous leaf leachate and organic fractions of donor plant on biochemical activities (carbohydrate content, chlorophyll content and protein content). The result shows that aqueous leachate and organic fractions reduced the level of biochemical activities. Carbohydrate content was increased in treated plants as compared to control while chlorophyll content and protein content were reduced as compared to the control. Aqueous leachate showed maximum toxicity on weeds than organic fractions. The reduction may be due to the allelochemicals present in *E. alba* leaves.

Key words: Allelochemicals, aqueous leachate, organic fractions, chlorophyll content, carbohydrate content, *Eclipta alba*.

INTRODUCTION

Weed infestation is one of the major causes of yield reduction in crops. The incidence of allelopathic effect of weeds on growth of crops has become increasingly widespread. When the two plant species grow together, they interact with each other by either inhibiting or stimulating their growth or yield through direct or indirect allelopathic interaction (Rice, 1984; Nandal et al., 1994; Abendroth and Elmore, 2007; Kumari et al., 2006). Several reports have documented the deleterious effect of decaying weed residues on the growth and yield of subsequent crops in the field (Guenzi and McCalla, 1966; Shaukat et al., 1985, 2003; Burhan and Shaukat, 1999). The effects of decaying weed residues depend upon the release of allelochemicals from them into the soil. These chemicals may be washed directly from the residues, or may result from microbial activity during decomposition (Putnum and Duke, 1978; Lynch and Cannell, 1980; Kumar et al., 2006). The effect of allelopathic chemicals tends to be highly species-specific (Stowe, 1979; Melkania, 1983). Normally, the effect is harmful, but beneficial effect is also possible (Newman, 1978).

Eclipta alba is a small branched annual herbaceous plant, occasionally rooting at nodes, cylindrical or flat, rough due to the presence of white hairs, nodes are distant, greenish but occasionally brownish. The allelopathic potential of *E. alba* has been revealed by many researchers (Yonli et al., 2010; Nisar and Hussain, 1992; Pawinde et al., 2008).

The present study was to evaluate the aqueous leaf leachate and organic fractions effect on biochemical activity of some weed plants (*Amaranthus spinosus*, *Cassia tora* and *Cassia sophera*).

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MATERIALS AND METHODS

Collection of material

Leaves of *E. alba* were collected locally from AMU campus, Aligarh. Healthy and freshly collected leaves were surface cleaned, dried and powdered. Seeds of the weed species, *A. spinosus*, *C. tora* and *C. sophera* were collected from the road sides of the Aligarh Muslim University, Aligarh, U.P., India.

Preparation of leachable allelochemical

Based on the methods devised by Kumari et al. (1985), healthy and freshly collected leaves of *E. alba* were cut roughly into pieces after clearing their surface and their dry weight per unit fresh weight were determined by desiccating the tissue in the oven. The weighed amount of fresh leaf pieces of the plant was soaked in requisite amount of pure water for a period of 20 h at room temperature. It was filtered completely through double layer of muslin cloth followed by Whatman No. 1 filter paper and the requisite concentration was made with distilled water. One half of this filtrate referred to as the aqueous leachates was used, while the other part was chilled and subjected to acid hydrolysis using pre-chilled, 3 N HCl. The precipitates so formed were recovered through centrifugation (2000 rpm). These were washed 5-6 times with pure water. Every time the recovery was made through mild centrifugation. For experimental purpose, requisite amount of the precipitate was dissolved in a few drop of ethyl alcohol and the final volume was made with pure water. A drop of Tween 20 was added to it, to serve as surfactant. This is referred to as aglycone or aglyconic or organic component of aqueous leachates.

Extraction of organic fraction

The healthy leaves of the donor plant were freshly collected, surface cleaned, then dried under shade and powdered. The powder was immersed in petroleum ether (60-80°C) for 20 h. The liquid was separated residue (marc), through mild centrifugation (500 rpm for 2 min). From the liquid portion the solvent (petroleum ether) was recovered on a hot water bath. Requisite amount of the residue so obtained was weighed and a few drops of xylene, a part from a drop of Tween-20 (to act as surfactant) were added to it. Final volume was made with pure water. This was termed petroleum ether fraction. The marc (residue from petroleum ether suspension) was suspended in methanol for 20 h and filtered, from one half of the filtrate, methanol was covered on a hot water bath. The residue, so obtained was dissolved in a drop of methanol and the final volume was made with pure water. It has been called methanol fraction. From another half of methanol filtrate, the solvent was removed and the residue was partitioned between chloroform and water (1:1 v/v). The two layers so formed were separated in a separating funnel. The chloroform was recovered over a hot water bath. To the requisite amount of residue, a few drops of methanol were added and the final volume was made with pure water. This has been termed as the chloroform fraction. The water from the aqueous layer after separating chloroform fraction was dried under low pressure on a rotary flash evaporator. The solution made with water was termed the water fraction.

Treatment for mature plants

For the estimation of various macro-molecular content (carbohydrate contents, protein content and chlorophyll content), nine plants of each test plant (*A. spinosus*, *C. tora* and *C. sophera*) were sprayed with 100 ml of the treatment solution per plant daily for five for five days. On the sixth day, the estimation of the carbohydrate content, protein content and chlorophyll content was done from the freshly plucked leaves.

Determination of carbohydrate content

The methodology employed by Loweus (1952) was followed for this purpose.

Estimation of chlorophyll content

The total chlorophyll content from leaves of treated or control plants were extracted in Di-methyl sulphoxide (DMSO) following the method of Hiscox and Israelstan (1979). Finely cut uniform discs (100 mg fresh weight) were made from fully expanded leaves of test plants. Dry weight equivalents of each of the treated samples were determined by keeping 100 mg fresh weight discs in an oven. The weighted material (100 mg fresh weight leaf disc) was suspended in 10 ml of di-methyl sulphoxide (DMSO) incubated at 65°C for one hour (the period of incubation was found sufficient for the complete extraction of chlorophyll). The DMSO was recovered by thorough decantation. The final volume was corrected to 10 ml with fresh DMSO.

The extinction of chlorophyll thus recovered in DMSO was measured at dual wave-length of 645 and 663 nm on spectrophotometer against DMSO as blank. The extinction values were read and the amount of chlorophyll was calculated according to the equation given by Arnon (1949), with modification by Hiscox and Israelstan (1979).

Estimation of total soluble proteins

The method as given by Lowry et al. (1951) was adopted for this purpose.

Statistical analysis

Each experiment was performed in completely random design block and results were mean of three replicates. All results were statistically analyzed through LSD.

RESULTS AND DISCUSSION

It is very clear from the result of this experiment that *E. alba* leaves exert a very negative influence on the acid soluble and water soluble carbohydrates of the weeds *A. spinosus*, *C. tora* and *C. sophera*. It is very well depicted that an increased amount of carbohydrates content exerts its influence mainly through its aqueous leachates. Effect of different treatments on carbohydrate content is in the following order AL>CF>PF>WF>MF (Table 1).

An increased amount of carbohydrates points out to the fact that the plant is under stress and it is gathering up its energy reserves to meet any condition of adversity. The results are in line with radish where appreciable increase in the increased concentration of soluble sugars in response to leaf extracts of heliotrope (*Heliotropium foertherianum*) was reported (Abdulghader et al., 2008). Similar increase in soluble sugars of maize in response to leaf extracts of *Acacia* and *Eucalyptus* has been reported

Table 1. Effect of aqueous leachates and organic extract fractions derived from the leaves of *E. alba* on total carbohydrate content of the leaves of *Amaranthus spinosus* L., *Cassia tora* L. and *Cassia sophera* L.

Treatment solution	*Amaranthus sinosus*		*C. tora*		*C. sophera*	
	Acid soluble carbohydrates (mg/gdry wt.)	**Water soluble carbohydrate (mg/g dry)**	**Acid soluble carbohydrate (mg/g dry wt.)**	**Water soluble carbohydrate (mg/g dry wt.)**	**Acid soluble carbohydrate (mg/g dry wt.)**	**Water soluble Carbohydrate (mg/g dry wt.)**
Control	49.45±0.65	52.12±0.31	58.47±0.36	56.79±0.49	52.83±0.57	64.83±0.33
Aqueous leachates AL (1% g/ml fresh weight)	76.45±0.12	79.63±0.37	65.34±0.15	69.11±0.15	81.04±0.67	80.37±0.52
Petroleum ether fraction PF (0.09w/v)	67.34±0.15	70.11±0.15	65.34±0.15	69.11±0.15	75.31±2.57	77.84±2.65
Methanolic fraction MF (0.09% w/v)	52.63±0.92	64.52±0.39	51.63±0.92	64.52±0.39	69.02±1.53	81.05±1.14
Chloroform fraction CF (0.09% w/v)	74.12±0.29	82.02±.0.61	72.15±0.13	70.19±0.92	79.32±0.52	70.21±0.43
Water fracrtion WF (0.09% w/v)	64.71±0.54	59.45±0.37	47.36±0.20	61.29±0.38	52.21±0.81	69.67±2.32
LSD at 5%	6.28	6.59	6.55	7.39	5.96	7.10
LSD at 1%	8.94	9.38	9.32	10.51	8.48	10.10

± Represents standard deviation.

Table 2. Effect of aqueous leachates and organic extract fractions derived from the leaves of *E. alba* on total chlorophyll content of the leaves of *Amaranthus spinosus* L., *Cassia tora* L. and *Cassia sophera* L.

Treatment solution	Total chlorophyll content (µg/mg fresh wt.)		
	A. spinosus	*C. tora*	*C. sophera*
Control	2.85±0.13	3.46±0.09	3.20±0.11
Aqueous leachates AL (1%g/ml fresh weight)	1.83±0.16	3.20±0.66	2.11±0.10
Petroleum ether fraction PF (0.09w/v)	1.63±0.42	2.74±0.04	2.01±.0.37
Methanolic fraction MF (0.09%w/v)	1.64±0.19	2.34±0.65	2.26±1.75
Chloroform fraction CF (0.09%w/v)	1.39±0.73	1.69±0.03	2.61±0.33
LSD at 5%	0.270	0.321	0.339
LSD at 1%	0.384	0.456	0.482

± Represents standard deviation.

(Sahar et al., 2005).

The chlorophyll content was also reduced as compared to the control (Table 2). Aqueous leachate and organic fraction shows different level of inhibition on different weeds. In *A. spinosus,* maximum inhibition was seen in aqueous leachate treatment. The reduction in the chloro-phyll content in this experiment may be due to the fact that allelochemicals either inhibit the synthesis of chloro-phyll or perhaps they breakdown the chlorophyll molecule by acting on the pyrrolic ring and the phytol chain (Blum et al., 1985; Colton and Einhelling, 1980; Yang et al., 2002, 2006). Hence the allelo-chemicals act by inhibiting the process of photosynthesis which ulti-mately can lead to the death of plant. Allelopathic effect of *Croton bonplandianum* cause reduction in chlo-rophyll content as reported by Sarkar and Chakraborty (2010) on *Triticum uestivam* and *Brassica campestris*; Liu et al. (2009) on *Lycopersicon esculentium* and Abu-Romman et al. (2010) in *Euphorbia hierosolymitana*. Recently, there has been increase in research on the role of the demand for photo-assimilates in regulating photo-synthesis through changes in carbohydrate partitioning and accumulation under stress condition (Paul and Foyer, 2001; Paul and Driscoll, 1997; Nielsen et al., 1998; Osmond et al., 1987; Levitt, 1982).

The decrease in protein content is shown in Table 3.

Table 3. Effect of aqueous leachates and organic extract fractions derived from the leaves of *E. alba* on total protein content of the leaves of *Amaranthus spinosus* L., *Cassia tora* L. and *Cassia sophera* L.

Treatment solution	Total protein content (mg/g dry wt.)		
	A. spinosus	***C. tora***	***C. sophera***
Control	58.17±0.17	58.78±2.21	55.94±0.99
Aqueous leachates AL (1% g/ml fresh weight)	32.44±0-73	45.52±1.14	44.69±0.17
Petroleum ether fraction PF (0.09% w/v)	34.17±0.76	42.62±0.65	47.21±0.65
Methanolic fraction MF (0.09% w/v)	46.24±0.23	33.81±0.82	35.27±0.19
Chloroform fraction CF (0.09% w/v)	47.16±0.31	48.4±0.84	36.27±0.12
Water fracrtion WF (0.09% w/v)	32.17±0.12	39.02±0.32	33.93±0.32
LSD at 5%	3.99	4.38	4.24
LSD at 1%	5.68	6.22	6.03

± Represents standard deviation.

Our findings are supported by the results of Duhan et al. (1995) who demonstrated significant decrease in the level of soluble proteins in legume crops in response to *Acaccia nilotica extracts*. Baziramakenga et al. (1997) demonstrated that phenolic acids reduced the incorporation of certain amino acids into proteins and thus reduce the rate of protein synthesis. Maize has been reported to contain 3 phenolic acids (Iman et al., 2006), which might have resulted in decreasing the protein content of soybean leaves. The phenolic acids have been shown to be toxic to activities of many enzymes (Hopkins, 1999).

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