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Evaluation of Allelopathic Effect of *Eclipta alba* (L.) Hassk of on Biochemical Activity *Amaranthus spinosus* L., *Cassia tora* L. and *Cassia sophera* L.

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Abstract: 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 showed that aqueous leachate and organic fractions reduced the level of biochemical activities. Carbohydrate content was increased in treated plants compared to control while chlorophyll content and protein content were reduced as compared to 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

Weeds are the major enemy to the crop plants and create harmful effects on agricultural crops due to several factors, such as competition for space, light and nutrients. Organic chemicals released as leaf leachates, root exudates affect the crop plants. Weeds species are considered as rich source of secondary metabolites (Allelochemicals) and these chemicals improve a certain kind of environmental system on other plants growing in their vicinity and the phenomenon known as allelopathy (Nandal *et al*., 1994). Allelopathy is a potential field of research all over the world. Few researchers consider only the deleterious interactions, as allelopathy while the latest thinking includes allelopathy to both harmful and beneficial interactions between the plants.

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 distant, greenish occasionally brownish.

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 *C. sophera*).

MATERIALS AND METHODS

Collection of material: Leaves of *Eclipta alba* were collected locally from AMU campus, Aligarh. Healthy and freshly collected leaves were surface cleaned, dried and powdered. Seeds of the weed species, *Amaranthus spinosus*, *Cassia tora* and *Cassia sophera* were collected from the road sides of the Aligarh Muslim University, Aligarh, UP India.

Preparation of leachable allelochemical: Based on the methods devised by Kumari *et al*. (1985), healthy and freshly collected leaves of *Eclipta 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, as such while the other part was chilled and subjected to acid hydrolysis using pre-chilled, 3N 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

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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 2 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 has been termed as the Water fraction.

Treatment to mature plants: For the estimation of various macro-molecular content (carbohydrate contents, protein content and chlorophyll content), 9 plants of each test plant (*Amaranthus spinosus*, *Cassia tora* and *C. sophera*) were sprayed with 100 mL of the treatment solution per plant daily for 5 days. On the 6th 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.

Water-soluble carbohydrates: To 5 mg dry powdered material was added 5 mL of pure water. It was kept in boiling water bath for 5 min and centrifuged, the supernatant was used as Water Soluble Fraction (WSF).

Acid soluble carbohydrates: To the residue left as above was added 5 mL of 6 N HCl. This was kept in a boiling water bath for 20 min and centrifuged. The supernatant was used as Acid-Soluble Fraction (ASF).

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 Israelstam (1979). Finely, cut uniform discs (100 mg fresh weight) were made 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 1 h (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 spectro-photometer 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 Israelstam (1979).

Estimation of total soluble proteins: The method as given by Lowry *et al.* (1951) was adopted for this purpose.

Statistical analysis: The data from the experiment samples were analyzed by one-way analysis of variance and mean were separated at $p<0.05$ by Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The plant Allelo-chemicals may interfere the growth, development and performance of a plant through their direct or indirect action on metabolism of plants. Many physiological and chemical activities altered by these chemical interference. Nevertheless, it is difficult to determine the primary mechanism involved for the actions of these chemicals. It is believed that a variety of mechanisms of action must exist for different allelo-chemicals or herbicides.

Even a specific compound may affect several metabolic functions and as a result, it is seldom possible to sort out primary effects from the secondary ones.

In addition, the uncertainty in interpreting the observed effects in isolated enzymes to other biochemical effects in intact plant system also exists. No doubt, llelo-chemicals or herbicides (natural or synthetic) act on plants though enzymatically controlled reactions.

One thing 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 weeds *A. spinosus*, *C. tora* and

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

	Amaranthus sinosus		*C. tora*		*C. sophera*	
Treatment solution	Acid soluble carbohydrates (mg g^{-1} dry wt.)	Water soluble carbohydrates (mg g^{-1} dry)	Acid soluble carbohydrates (mg g^{-1} dry wt.)	Water soluble carbohydrates (mg g^{-1} dry wt.)	Acid soluble carbohydrates (mg g^{-1} dry wt.)	Water soluble carbohydrates (mg g^{-1} 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^{-1} 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) (0.09 w/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	67.02±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	52.32±0.52	78.38±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	66.21±0.81	67.67±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

Table 2: 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.

	Total protein content		
Treatment solution	*Amaranthus spinosus*	*C. tora*	*C. sophera*
Control	58.17±0.17	58.78±2.21	55.94±0.99
Aqueous Leachates (AL) (1% g mL^{-1} 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

Table 3: 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.

	Total protein content		
Treatment solution	*Amaranthus spinosus*	*C. tora*	*C. sophera*
Control	2.85±0.13	3.46±0.09	3.20±0.11
Aqueous Leachates (AL) (1% g mL^{-1} fresh weight)	1.83±0.16	3.20±0.66	2.11±0.10
Petroleum ether Fraction (PF) (0.09 w/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

C. sophera. It is very well depicted by 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). Synthesis of carbohydrates takes place in the living tissue. 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 conditions of adversity.

Recently, there has been increased in research on the role of the demand for photo-assimilates in regulating photosynthesis through changes in carbohydrate partitioning and accumulation understress condition (Paul and Foyer, 2001; Paul and Driscoll, 1997; Nielsen *et al*., 1998; Osmond *et al*., 1986; Levitt, 1982).

The protein content and chlorophyll content was also reduced as compared to control (Table 2 and 3). 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 chlorophyll content in this experiment may be due to the reason that allelochemicals either inhibit the synthesis of chlorophyll or perhaps they breakdown the chlorophyll, molecule by acting on the pyrrolic ring and the phytol chain (Blum *et al*., 1985; Colton and Einhellig, 1980; Yang *et al*., 2002, 2006). Hence, the allelo-chemicals act by inhibiting the process of photosynthesis which ultimately can lead to the death of plant. Allelopathic effect of *Croton bonplandianum* cause reduction in chlorophyll content was reported by Sarkar and Chakraborty (2010) on *T. aestivam* and *Brassica campestris*. Liu *et al*. (2009) in *Lycopersicon esculentium* and Abu-Romman *et al*. (2010) in *Euphorbia hierosolymitana*.

CONCLUSION

The plant Allelo-chemicals may interfere the growth, development and performance of a plant through their direct or indirect action on metabolism of plants. Aqueous leachate and organic fractions reduce the level of biochemical activities. Carbohydrate content was increased in treated plants compared to control while chlorophyll content and protein content were reduced as compared to control. Aqueous leachate showed maximum toxicity on weeds than organic fractions. The reduction may be due to the allelochemicals present in *E. alba* leaves.

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