ALLELOPATHIC INFLUENCE OF LEAF RESIDUE OF *AMARANTHUS SPINOSUS* ON GROWTH AND METABOLISM OF *PARTHENIUM HYSTEROPHORUS* L.

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ABSTRACT

The effect of leaf residue of *Amaranthus spinosus* L. on the growth and metabolism of *Parthenium hysterophorus* L. was studied in pot culture. The leaf residue of *Amaranthus spinosus* inhibited height of the plant, length of the leaves and number of branches, capitula and seeds per plant. The total sugar was decreased in different treatments with maximum reduction (25.37%) in leaf of *Parthenium* plants with T_2 treatment. The accumulation of organic acids reveals that respiration was hampered in tested plants. Increase in the amino acids might be adaptation of plant in environmental stress. The altered metabolism results in inhibited growth of *Parthenium*. The leaf residue of *Amaranthus spinosus* may be used as potent bioherbicide.

Key words: *Amaranthus spinosus*, amino acid, chlorophyll, lipid, organic acid, *Parthenium hysterophorus*, protein, sugar.

INTRODUCTION

Parthenium hysterophorus L. (family-Asteraceae) an exotic weed is believed to be native of north-east Mexico and was first reported in India from Pune but has spread alarmingly like a wild blaze to almost all the states in India and established as a naturalized weed. *Parthenium hysterophorus L.* has originated as a result of natural hybridization between *Parthenium confertum* and *Parthenium bipinnatifidum* (Nath 1988). It has wide physiological and ecological adaptations in varied climatic and soil conditions and grows round the year. *Parthenium* causes allergic contact dermatitis, asthma, hay fever in human-beings and is a menace to agricultural productivity due to its potent allelopathic effect (Kanchan 1975, Patil and Hegde 1988). *A. spinosus* contains several allelochemicals viz. phenolic acids, alkaloids and sesquiterpene lactones (Suma *et al.* 2002). Decoction of roots of *Amaranthus spinosus* is used in gonorrhoea. Leaves are used in curing piles, leprosy and in stomach troubles.

The overuse of synthetic agrochemicals often causes environmental hazards, an imbalance of soil microbes, nutrients deficiency and change of physico-chemical properties of soil resulting in decrease of crop productivity. The indiscriminate use of hazardous pesticides have eroded the ecological sustainability and deleterious effects on human health. Most of the reports deal with the allelopathic effects on the germination and growth of *Parthenium hysterophorus* but no work has been done on the effects of *A. spinosus* on the metabolic processes, i.e., sugar, organic acid, amino acid and lipid metabolism of the noxious weed. Therefore, this study aimed to investigate the allelopathic potential of leaf residue of *Amaranthus spinosus* on the metabolism of

Parthenium and for assessing their suitability as natural herbicide because these being bio-degradable may be used for the weed control.

MATERIALS AND METHODS

Study Site

The pot studies were conducted during summer season of 2004 in the Roxburgh Botanical Garden, Department of Botany, University of Allahabad, Allahabad (24^0 47' and 50^0 47' N latitude and 81^0 9' and 82^0 21' E longitude, 78 m above the sea level) India.

Cultural Technique

The healthy plants of *Amaranthus spinosus* L. were collected at vegetative stage. The air dried leaves were finely powdered with a grinder and placed in sealed polythene bags to prevent it from moisture and contamination. The fire clay pots of 30 cm deep and 30 cm in diameter were filled with 15 kg of sandy loam soil and 100 g of DAP (Diammonium phosphate). Powder of dry leaves of *Amaranthus spinosus* at the rate of 100 and 200 g/pot was mixed thoroughly in the soil according to the treatments. Ten viable seeds of *Parthenium* per pot were sown at equal distance and uniform watering (500 ml/pot) was continued upto 60 DAS (days after sowing). The leaves and stems were dried for about 48 h at 80°C in an electric oven. Sugars, organic and amino acids were extracted in 80% (v/v) ethanol. Fresh samples of leaves and stems were kept for lipid estimation.

Analyses of Biochemical Constituents

Chlorophyll and Sugar: Chlorophyll from control and treated leaves were extracted with 80% acetone. The amount of chlorophyll was quantified by using the formulae of Arnon (1949). The qualitative analysis of sugars was done with paper chromatography. All samples were run three times in duplicate in solvent system, n-butanol: acetic acid: water (4:1:5). One paper was sprayed to detect the sugars and other was not sprayed. The sugars were detected by using Buchan and Savage's (1952) spraying reagent and unsprayed chromatograms were superimposed with sprayed chromatograms and area with spot was marked on the paper. Unsprayed paper with marked area was cut and eluted in 50% ethanol. The ethanol was evaporated and pure sugars were used for quantitative estimation using anthrone reagent (Yemm and Willis 1954) freshly prepared by dissolving 0.4 g anthrone in 100 ml of concentrated sulphuric acid. After evaporation of alcohol, 3 ml of distilled water and 6ml of anthrone reagent were added to each tube. All the tubes were placed in a boiling water bath for 3 min and then placed in ice cold water and allowed to cool. The intensity of the colour was measured by spectrophotometer at 600 nm. Quantity of sugars was determined from the standard curve obtained from glucose.

Organic Acids: Lugg and Overall's (1947) technique of one dimentional paper chromatography was employed for the detection of organic acids. After running in solvent n-butanol: formic acid: water (10:2:5) chromatograms were subjected to drying at room temperature for two days to remove traces of formic acid. Lemon yellow spots of organic acids were detected against blue background by spraying with 0.04% (w/v) bromophenol blue in 90% (v/v) ethanol. The areas of different organic acid spots were measured with the help of leaf area meter. The values thus obtained were expressed quantitatively in terms of area occupied by known amount of respective organic acids from predetermined calibration curves.

Amino Acids and Protein: Two dimensional ascending chromatographic technique of Consdon *et al.* (1944) was employed for the complete resolution of diverse amino acids. Partridge's (1948) solvent system, phenol: water: ammonia (80:20:3) was used for the first run. After the first

run the papers were dried for 48 h, rotated at the right angle to the first run and hung in other chromatographic chamber containing the solvent system, n-butanol: acetic acid: water (4:1:5) for the second run. Ninhydrin (0.1% w/v) in n-butanol was used as spraying reagent. The spots were cut and eluted in 50% ethanol and colour intensity was measured at 600 nm. Proline was detected by one dimensional ascending paper chromatographic technique using solvent system, n-butanol: acetic acid: water (4:1:5 v/v) and the spots were detected by spraying isatin reagent of Saifer and Oreskes (1954). Protein content was determined following the method of Lowry *et al.* (1951). The amount of protein was calculated with reference to standard curve of lysozyme.

Lipids: Lipids were extracted and purified from fresh samples following Bligh and Dyer (1959). The neutral lipids were separated from iodine developed spots on TLC plates and estimated by Amenta's (1964) procedure using acid dichromate reagent.

Statistical Analysis: The treatments in all experiments were laid out in randomized block design with three replicates and analysis of variance (ANOVA) and critical difference (CD at 5%) between means were determined.

RESULTS

Growth Parameters: Leaf residue of *Amaranthus spinosus* L. significantly inhibited the germination and growth parameters *viz.* number of leaves, branches and capitula/plant, length of the leaves and height of *Parthenium* plant. Maximum number of seedlings were observed in control pots. The number of seedlings/pot was reduced to 42.33 and 57.79% in T_1 and T_2 treatments, respectively. Maximum reduction was observed in plant height and branches/plant in T_2 treatment at 60 DAS. A significant decrease of 12 and 24% in the number of seeds/five capitula was recorded in T_1 and T_2 treatments, respectively (Table 1).

Table 1. Effect of leaf residue of *Amaranthus spinosus* L. on growth parameters of *Parthenium hysterophorus* L.

Weed	Leaf residue of *Amaranthus spinosus*		
Treatments	C	T_1	T_2
At 10 DAS			
Number of seedlings/pot	8.67 ± 0.27	5 ± 0.00 (42.33)	3.66 ± 0.27 (57.79)
At 30 DAS			
Number of leaves/plant	12.66 ± 0.27	8.66 ± 0.72 (31.59)	7.7 ± 0.27 (39.18)
Length of the leaves (cm)	6 ± 0.47	5.87 ± 0.23 (2.17)	4.73 ± 0.21 (21.17)
Plant height (cm)	11.59 ± 0.59	9.8 ± 0.38 (15.44)	8.2 ± 0.84 (29.25)
At 60 DAS			
Number of leaves/plant	28 ± 0.88	26.67 ± 0.68 (4.75)	22.69 ± 0.73 (18.96)
Length of the leaves (cm)	14.67 ± 0.33	12.73 ± 0.64 (13.22)	9.37 ± 0.35 (36.13)
Plant height (cm)	51.50 ± 0.68	45.3 ± 0.73 (12.04)	29.35 ± 0.85 (43.01)
Branches/plant	8.70 ± 0.27	7.66 ± 0.27 (11.95)	5 ± 0.47 (42.53)
Number of capitula/plant	290 ± 0.94	275 ± 0.00 (5.17)	171 ± 0.50 (41.03)
Number of seeds/five capitula	25 ± 0.00	22 ± 0.47 (12)	19 ± 0.50 (24)

C = Control, T_1 = Leaf residue of *A. spinosus* 100 g/pot, T_2 = Leaf residue of *A. spinosus* 200 g/pot.
Values are mean of three replicates ± SE.
Figures in parenthesis indicate percent inhibition over control.

Chlorophyll and Sugar Contents: In control total chlorophyll was 2.73 mg/g which significantly reduced in treatments. Maximum inhibition (35.16%) in total chlorophyll was observed in *Parthenium* leaves with T_2 treatment. Glucose, sucrose and fructose sugars exhibited decreasing trends in treatments. The total sugars were, decreased in leaf and stem with maximum reduction (25.37%) in leaf of T_2 treatment (Table 2).

Organic Acid: Citric acid was present in all the treatments except in leaf of T_1 treatment. Minimum amount of malonic acid in leaf was increased in treatments with maximum (0.39%) in T_2. Fumaric acid was not detected in leaf of control but increased to highest level (0.39%) in leaf of T_1 treatment. A significant accumulation of total organic acids in leaf and stem of all the treatments was observed. Maximum stimulation 15.07% of total organic acid was recorded in leaf of T_2 treatment (Table 3).

Table 2. Effect of leaf residue of *Amaranthus spinosus* L. on sugar composition of leaf and stem of *Parthenium hysterophorus* L. (mg/100 mg dry weight).

Sugars	Leaf residue of *Amaranthus spinosus*					
	Leaf			Stem		
	C	T_1	T_2	C	T_1	T_2
Raffinose	1.65 ± 0.01	1.23 ± 0.04	1.50 ± 0.02	0.78 ± 0.09	0.60 ± 0.02	0.37 ± 0.12
Sucrose	1.80 ± 0.04	1.15 ± 0.01	0.97 ± 0.04	0.95 ± 0.03	0.90 ± 0.01	0.79 ± 0.02
Glucose	1.20 ± 0.10	0.97 ± 0.08	0.70 ± 0.16	0.95 ± 0.05	0.65 ± 0.03	0.58 ± 0.01
Fructose	1.15 ± 0.01	0.95 ± 0.10	0.60 ± 0.03	0.83 ± 0.14	0.50 ± 0.28	0.45 ± 0.07
Mannose	1.42 ± 0.03	1.45 ± 0.22	1.65 ± 0.48	0.48 ± 0.02	0.55 ± 0.01	0.60 ± 0.02
Xylose	1.45 ± 0.02	1.85 ± 0.14	1.30 ± 0.01	0.65 ± 0.32	0.60 ± 0.02	0.55 ± 0.02
Unidentified (U)	1.50 ± 0.11	0.92 ± 0.01	0.87 ± 0.05	0.80 ± 0.05	0.71 ± 0.04	0.65 ± 0.01
Total	10.17	8.52	7.59	5.24	4.51	3.99
C.D. at 5%		0.07			0.02	

C = Control, T_1 = Leaf residue of *A. spinosus* 100 g/pot, T_2 = Leaf residue of *A. spinosus* 200 g/pot.
Values are mean of three replicates ± SE. Effect of treatments and sugars on leaf and stem are significantly different (P>0.05).

Table 3. Effect of leaf residue of *Amaranthus spinosus* L. on organic acid composition of leaf and stem of *Parthenium hysterophorus* L. (mg/100 mg dry weight).

Organic acids	Leaf residue of *Amaranthus spinosus*					
	Leaf			Stem		
	C	T_1	T_2	C	T_1	T_2
Citric acid	0.13 ± 0.01	-	0.15 ± 0.02	0.12 ± 0.01	0.18 ± 0.01	0.12 ± 0.05
Unknown (U)	-	-	-	-	0.16 ± 0.04	0.29 ± 0.03
Malic acid	0.22 ± 0.14	0.24 ± 0.04	0.29 ± 0.06	0.19 ± 0.02	0.21 ± 0.10	0.21 ± 0.09
Malonic acid	0.25 ± 0.03	0.27 ± 0.13	0.39 ± 0.07	0.22 ± 0.05	-	0.19 ± 0.02
Succinic acid	0.44 ± 0.22	0.38 ± 0.27	0.47 ± 0.05	0.29 ± 0.09	-	0.28 ± 0.16
Fumaric acid	-	0.39 ± 0.08	0.38 ± 0.03	0.27 ± 0.11	0.38 ± 0.14	-
Oxalosuccinic acid	0.42 ± 0.28	0.37 ± 0.12	-	0.33 ± 0.23	0.51 ± 0.18	0.38 ± 0.09
Total	1.46	1.65	1.68	1.42	1.44	1.47
C.D. at 5%		0.02			0.02	

C = Control, T_1 = Leaf residue of *A. spinosus* 100 g/pot, T_2 = Leaf residue of *A. spinosus* 200 g/pot.
Values are mean of three replicates ± SE. Effect of treatments and organic acids on leaf and stem are significantly different (P>0.05).

Amino Acid: The amino acid composition of *Parthenium* was variously affected under the influence of leaf residue of *Amaranthus spinosus*. Valine, tyrosine, proline, α-alanine, threonine, cysteine and unknown amino acids were successively increased to maximum in T_2 treatment. Transport amino acids, i.e., glutamic acid, aspartic acid and asparagine were increased in treatments. Total amino acids of free pool were increased in treatments with maximum in leaf and stem of T_2 in comparison to respective control (Table 4). The highest amount 98 µg/ml protein in leaf of *Parthenium* in control was reduced to 94 µg/ml and 82 µg/ml in T_1 and T_2 treatments, respectively with maximum inhibition 16.33% in T_2 treatment.

Table 4. Effect of leaf residue of *Amaranthus spinosus* L. on amino acid composition of leaf and stem of *Parthenium hysterophorus* L. (mg/100 mg dry weight).

Free- Amino acids	Leaf residue of *Amaranthus spinosus*					
	Leaf			Stem		
	C	T_1	T_2	C	T_1	T_2
Leucine + Isoleucine	0.02 ± 0.01	0.03 ± 0.01	0.11 ± 0.01	0.02 ± 0.01	0.07 ± 0.04	0.03 ± 0.01
Valine	0.13 ± 0.10	0.20 ± 0.18	0.25 ± 0.05	0.07 ± 0.06	0.19 ± 0.14	0.13 ± 0.10
γ-Aminobutyric acid	0.11 ± 0.09	0.16 ± 0.02	0.22 ± 0.10	0.06 ± 0.05	0.07 ± 0.02	0.09 ± 0.02
Tyrosine	0.09 ± 0.05	0.10 ± 0.09	0.20 ± 0.06	0.05 ± 0.03	0.09 ± 0.07	0.12 ± 0.06
Proline	0.07 ± 0.03	0.25 ± 0.11	0.37 ± 0.08	0.04 ± 0.03	0.12 ± 0.10	0.13 ± 0.10
α-alanine	0.02 ± 0.01	0.09 ± 0.05	0.12 ± 0.01	0.02 ± 0.01	0.09 ± 0.05	0.09 ± 0.05
β-alanine	0.05 ± 0.04	0.13 ± 0.10	0.19 ± 0.02	0.02 ± 0.01	0.13 ± 0.11	0.16 ± 0.08
Threonine	0.12 ± 0.11	0.35 ± 0.23	0.49 ± 0.20	0.06 ± 0.02	0.11 ± 0.02	0.15 ± 0.08
Arginine	0.10 ± 0.08	0.09 ± 0.08	0.14 ± 0.08	0.02 ± 0.01	0.04 ± 0.01	0.12 ± 0.09
Glutamine	0.29 ± 0.19	0.20 ± 0.09	0.16 ± 0.05	0.10 ± 0.09	0.07 ± 0.04	0.05 ± 0.02
Asparagine	0.10 ± 0.09	0.17 ± 0.01	0.11 ± 0.09	0.04 ± 0.02	0.05 ± 0.012	0.07 ± 0.05
Glycine + Serine	0.07 ± 0.05	0.07 ± 0.03	0.10 ± 0.05	0.05 ± 0.04	0.04 ± 0.03	0.12 ± 0.06
Glutamic acid	0.05 ± 0.03	0.10 ± 0.08	0.15 ± 0.10	0.03 ± 0.01	0.05 ± 0.01	0.07 ± 0.02
Aspartic acid	0.11 ± 0.08	0.15 ± 0.06	-	0.01 ± 0.01	0.08 ± 0.05	0.13 ± 0.04
U	0.01 ± 0.01	0.15 ± 0.12	-	0.25 ± 0.06	0.11 ± 0.09	0.19 ± 0.09
Cysteine	0.07 ± 0.06	0.31 ± 0.23	-	0.01 ± 0.01	0.25 ± 0.05	0.30 ± 0.11
Lysine + Histidine	0.04 ± 0.02	0.10 ± 0.09	0.16 ± 0.11	0.02 ± 0.02	-	0.12 ± 0.07
U_0	0.06 ± 0.04	0.12 ± 0.06	0.14 ± 0.09	0.03 ± 0.01	-	0.10 ± 0.05
U_1	0.13 ± 0.10	-	0.19 ± 0.13	0.03 ± 0.02	-	-
U_2	0.06 ± 0.03	-	-	0.05 ± 0.04	-	-
U_3	0.13 ± 0.11	0.12 ± 0.03	-	0.01 ± 0.01	-	-
U_4	0.06 ± 0.04	-	-	0.01 ± 0.01	-	0.14 ± 0.05
U_5	0.05 ± 0.02	-	0.12 ± 0.07	0.02 ± 0.02	0.20 ± 0.11	-
U_6	0.29 ± 0.17	-	0.13 ± 0.10	0.06 ± 0.05	-	-
Total	2.23	2.89	3.35	1.08	1.76	2.31
C.D. at 5%		0.01			0.002	

C = Control, T_1 = Leaf residue of *A. spinosus* 100 g/pot, T_2 = Leaf residue of *A. spinosus* 200 g/pot.
Values are mean of three replicates ± SE. Effect of treatments and amino acids on leaf and stem are significantly different ($P<0.05$) but on stem are significantly different ($P>0.05$).

Table 5. Effect of leaf residue of *Amaranthus spinosus* L. on lipid composition of leaf and stem of *Parthenium hysterophorus* L. (mg/100 mg dry weight).

Lipids	Leaf residue of *Amaranthus spinosus*					
	Leaf			Stem		
	C	T_1	T_2	C	T_1	T_2
Phospholipids	3.40 ± 0.09	1.98 ± 0.08	1.27 ± 0.46	2.96 ± 0.16	1.57 ± 0.49	1.19 ± 0.16
Monoglycerides	1.21 ± 0.82	1.84 ± 0.19	2.69 ± 0.07	1.08 ± 0.28	1.73 ± 0.15	2.13 ± 0.02
Sterol	2.42 ± 0.24	1.39 ± 0.21	0.98 ± 0.58	1.71 ± 0.64	1.25 ± 0.01	0.86 ± 0.05
Diglycerides	2.91 ± 0.46	2.80 ± 0.09	1.92 ± 0.02	1.82 ± 0.32	1.47 ± 0.46	0.88 ± 0.03
Fatty acids	2.59 ± 0.95	2.13 ± 0.27	1.94 ± 0.38	1.94 ± 0.06	1.23 ± 0.28	0.83 ± 0.24
Triglycerides	2.80 ± 0.01	2.31 ± 0.06	1.09 ± 0.47	1.75 ± 0.45	1.28 ± 0.02	0.94 ± 0.32
Methyl ester	0.89 ± 0.05	1.36 ± 0.12	2.10 ± 0.92	0.78 ± 0.02	1.05 ± 0.19	1.23 ± 0.04
Hydrocarbons	2.50 ± 0.17	2.90 ± 0.27	3.43 ± 0.01	1.10 ± 0.92	1.40 ± 0.05	1.96 ± 0.01
Total	18.75	16.71	15.42	13.14	10.98	10.02
C.D. at 5%		0.58			0.04	

C = Control, T_1 = Leaf residue of *A. spinosus* 100 g/pot, T_2 = Leaf residue of *A. spinosus* 200 g/pot.
Values are mean of three replicates ± SE. Effect of treatments and lipids on leaf and stem are significantly different ($P<0.05$).

Lipid: A significant increasing trend of monoglycerides, methyl esters and hydrocarbons was observed in leaf and stem of treatments. The higher amount of total lipids in leaf and stem of control was successively decreased in treatments. Maximum inhibition 23.74% in total lipids was observed in stem of T_2 treatment (Table 5).

DISCUSSION

Allelochemicals present in *Amaranthus spinosus* reduced the growth parameters and other metabolic processes of *Parthenium* (Epstein *et al.* 1967, Colton and Einhellig 1980). Germination is the resumption of metabolic activity and growth of seed tissues which starts with the imbibition of water and ends with the protrusion of embryonic roots. Allelochemicals present in leaf residue stimulated lignin biosynthesis which increases the rigidity of the cell wall to limit the cell growth. Carbohydrates are the cellular source of energy and are the starting materials for the synthesis of protein, lipid and other plant products. Glucose and fructose are hexoses which are precursors of transport sugars, i.e., sucrose and raffinose showed decreasing trends in leaf and stem of treatments under the influence of allelochemicals. The sugars synthesized in photosynthesis are transported from source to sink, i.e., growing plant parts or metabolic active organ or storage organ. Inhibition of photosynthesis leading to decreased amount of photosynthates might be due to decreased biosynthesis of chlorophyll or degradation of photosynthetic pigments (Pandey 1994) or inhibition of photosynthesis by allelochemicals resulting in decreased dry matter, i.e., photosynthates. Allelochemicals are known to impede the absorption of water (Rice 1984) and ions (Bhowmik and Doll 1984) from the soil which may cause the loss of turgidity of cell and affect the metabolic activity of cells. Citric acid cycle is amphibolic pathway, i.e., it plays role in both anabolic and catabolic processes. The total organic acids showed successive accumulation in leaf and stem of treatments. The deamination of amino

acids or inhibition of some respiratory reactions might have resulted in accumulation of total organic acids (Demos *et al.* 1975). According, to Moreland and Novitzky (1987) allelochemicals inhibit electron transport in mitochondria and impaired enzyme activity as a primary target of allelopathic activity which may result in reduced ability to metabolize reserve materials. Intermediates of citric acid cycle particularly α-ketoglutarate and oxaloacetate can be removed from the cycle and serve as precursors of amino acids. The resulting decrease in concentration of these intermediates would be expected to slow the flux of citric acid cycle however these intermediates can be replenished by anaplerotic reaction. Proline acts as osmolyte and is induced in stress. Allelochemicals are known to alter the rate of absorption of water resulting in stress which might have induced proline synthesis. Increased synthesis of asparagine may be a device to detoxify the effects of excess of ammonia produced in deamination process (Lam *et al.* 1995). Proteins play a pivotal role in biological processes. Protein synthesis regulates growth, development and reproduction of plant. The protein may serve as respiratory substrate if the supply of carbohydrates are inadequate because of decreased photosynthetic rate (Salisbury and Ross 1991). The lipid composition of *Parthenium* was modified by leaf residue of *Amaranthus spinosus.* Phospholipids are important component of plasmalemma. The reduction in the amount of total lipids was due to decrease in the storage lipids, i.e., triglycerides and building blocks, i.e., fatty acids or due to lipid peroxidation (Politycka 2002).

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