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Bioherbicidal Potential of Leaf-residue of *Hyptis suaveolens* on the Growth and Physiological Parameters of *Parthenium hysterophorus* L.

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Abstract: The effects of dry leaf-residues of *Hyptis suaveolens* L. on the growth and physiological parameters of *Parthenium hysterophorus* L. was studied in pot culture. Different growth parameters of *Parthenium* such as size and number of the leaves, height, branches, capitula and seeds/plant were inhibited by leaf residues of *H. suaveolens*. The amount of chlorophyll and protein was decreased with increased amount of residue. The inhibition in growth parameters of *Parthenium hysterophorus* was due to decrease in chlorophyll, sugar, protein and lipid contents while organic and amino acids were increased in treatments. The accumulation of organic acid reveals that respiration was hampered in test plant and increase in the amino acids might be the adaptation strategy of *Parthenium* to avoid environmental stress generated by the allelochemicals present in leaf residues of *Hyptis*. The decrease in the amount of lipids was proportional to the quantity of dry leaf residues used. The altered physiological parameters result in inhibited growth of *Parthenium* and the leaf residues of *Hyptis suaveolens* may be used as potent bioherbicide to control the spread of *Parthenium*.

Key words: Allelopathy, amino acid, chlorophyll, *Hyptis suaveolens* L., *Parthenium hysterophorus* L., sugar

INTRODUCTION

Invasion of exotic species is among the most important global scale problems experienced by natural ecosystems (Sharma *et al.*, 2005). Invasive alien species are such species whose introduction or spread threatens the environment, economy or society including human health. *Parthenium hysterophorus* is an annual herb of neotropical region now fairly distributed throughout the globe. *Parthenium hysterophorus* L. an obnoxious weed has been reported as a main source of nuisance and health hazard to mankind and animals, threat to biodiversity and danger to environment (Knox *et al.*, 2011). Today *Parthenium* has got a position among the list of top ten worst weeds of the world and has been listed in the global invasive species database and it has invaded almost all the states of India encroaching about thirty five million hectares of land. *Parthenium* is commonly seen lavishly growing in wasteland, roadsides, railway tracks, vacant sites, rock crevices, city waste dumped areas, orchards and construction sites. *Parthenium hysterophorus* is native of North-east Mexico, probably introduced in India along with wheat grains under the PL 480 scheme and spread alarmingly like a wild blaze to almost all the states in India and established as a naturalized weed. *Parthenium hysterophorus* L. popularly known as broom bush, carrot weed, congress weed, feverfew, star weed and whitetop. This plant belongs to the division: Magnoliophyta, class : Magnoliopsida, order : Asterales and family: Asteraceae (Kumar and Kumar, 2010). *Parthenium hysterophorus* L. has originated as a result of natural hybridization between *Parthenium confertum* and *Parthenium bipinnatifidum* (Nath, 1988). A successful establishment of *Parthenium* in any ecosystem is attributed to several reasons such as high germination ability throughout the year, easy dispersal of seeds, high reproductive potential and survival rate and extreme adaptability in a wide range of habitats (Kohli *et al*., 1997). Thus, *Parthenium* can be seen in all the stages of its growth round the year.

Plants release various compounds into their surroundings that have either deleterious or beneficial effects on other plants in the vicinity. This naturally occurring chemical - interaction between plants is known as allelopathy (Rice, 1984). The non-nutritional secondary metabolites produced by an organism of one species affect the growth and population biology of individuals of other species are known as allelochemicals (Minorsky, 2002; Callaway and Ridenour, 2004) and these allelochemicals impose environmental stress on other plants growing in their vicinity. Allelopathy as an ecological approach and allelochemicals as biological herbicides have been a challenge to current synthetic chemical approaches (Suma *et al*., 2002; El-Rokiek *et al*., 2006). The chemical analysis of *Parthenium hysterophorus* has indicated that all the plant parts including trichomes and pollen contain several secondary metabolites such as alkaloids, parthenin, kaempferol, p-coumaric acid and caffeic acid being high in leaves followed by inflorescence, fruit, root and stem (Kanchan, 1975; Patil and Hegde, 1988). The sesquiterpene lactones viz. parthenin and coronopilin

present in the trichomes of leaves and stems of *Parthenium* are responsible for causing various allergies like contact dermatitis, hay fever, asthma and bronchitis in human - beings (Navie *et al*., 1996; Wiesner *et al*., 2007). The adverse effects of *Parthenium* not only on human - beings but also on animal health have been well documented (Shukla and Pandey, 2008). *Hyptis suaveolens*, a potent medicinal herb, is commonly known as wilayati tulsi and it belongs to the family Lamiaceae. *H. suaveolens* contains several chemical constituents such as carbohydrate, phenol, tannin, saponin, alkaloid, steroid and flavonoid etc. which are responsible for its medicinal properties (Wealth of India, 1964). *H. suaveolens* is being used in traditional medicine to treat various diseases. The leaves of this plant have been used as stimulant, carminative, sudorific and as a cure for parasitic cutaneous diseases whereas the crude leaf extract is used as a relief to colic and stomachache. Leaves and twigs are considered to be antispasmodic and used in antirheumatic and antisuporific baths, an anti - inflammatory agent and also applied as an antiseptic in burns, wounds and other skin related problems (Shenoy *et al*., 2009). The decoction of the roots is highly valued as appetizer and is reported to contain urosolic acid, a natural HIV - integrase inhibitor (Chatterjee and Pakrashi, 1997). Fumes of dried leaves of *H. suaveolens* are also used to repel mosquitoes and control insect pests of stored grains (Mandal *et al*., 2007).

Parthenium has become resistant to an array of chemical pesticides like atrazine, 2, 4 - D, metribuzin, paraquat, trifluralin and diphenamid (Singh *et al*., 2004) and control of *Parthenium* through the pesticides does not last long enough as expected due to the large amount of seeds deposited in the seed bank. Senthil *et al*. (2004) reported that 100% concentration of aqueous extracts of *Hyptis suaveolens* completely inhibits the seed germination of *Parthenium hysterophorus*. It has been found that overuse of synthetic agro - chemicals often causes environmental hazards, imbalance of soil microbes, nutrient deficiency and change of physico-chemical properties of soil resulting in decrease of crop productivity. Furthermore, increasing public concern on environmental issues requires alternative weed management strategies which are less pesticide dependent or based on naturally occurring compounds (Singh *et al*., 2003). With increasing societal concern regarding the harmful effects of chemical or synthetic pesticides on humans as well as on environment have aroused substantial interest to evolve alternate eco-friendly approaches for weed management.

The most of the earlier reports deal with the allelopathic effects of *Parthenium hysterophorus* on the germination and growth of different crops but unfortunately no work has been done to study the effects of *H. suaveolens* on the physiological parameters i.e. sugar, organic acid, amino acid and lipid constituents of *Parthenium hysterophorus* L. Therefore, present investigation was carried out to study the bioherbicidal potential of leaf - residues of *Hyptis suaveolens* on the growth and physiological processes of *Parthenium* and for assessing their suitability as natural herbicide because these being biodegradable may be used for the weed control.

MATERIALS AND METHODS

The experiments were conducted at Noida (Uttar Pradesh, India) in April - June, 2010.

Geographical position of the study site: Noida is located in Gautam Budh Nagar district of Uttar Pradesh state, India. The latitude and longitude of Noida are 28º57′N and 77º32′E, respectively. It lies at an average elevation of 200 m above mean sea level and has a flat topography. Noida is about 20 kilometer south - east of New Delhi and it is bounded on the west and south-west by the Yamuna river, on the north by Delhi, on the north - east by Ghaziabad and on the north - east and south - east by the Hindon river. Noida occupies an area of about 203 km^2 and the total population of the region is approximately 2, 93, 908 as per the 2001 census.

Climate conditions of the study area: Noida experiences three seasons in a year i.e. summer (March - June), rainy (July - September) and winter (October - February). Sporadic winter rains are common in December and January months while dew fall continues up to February. The annual mean temperature is 25-33ºC, the mean maximum temperature is 42.5ºC recorded in May - June and the mean minimum temperature is 4.5ºC recorded in January - February.

Experimental design: The pot studies were conducted during April - June, 2010 (summer season) in the Botanical Garden, Amity Institute of Biotechnology, Amity University, Noida. The healthy plants of *Hyptis suaveolens* L. were collected at vegetative stage. Fresh leaves were removed, washed gently with tap water only for few seconds to avoid leaching losses of water soluble allelochemicals, followed by quick rinsing in distilled water and drying with clean absorbent paper. The air - dried *Hyptis* leaves were finely powdered with a grinder and placed in sealed polythene bags to prevent it from moisture and contamination. The earthern pots of 25 cm deep and 25 cm in diameter were filled with equal weights 12 kg of sandy loam soil and 100 g of DAP (Diammonium phosphate). Powder of dried leaves of *Hyptis suaveolens* at 100 g/pot (T_1) and 200 g/pot (T_2) was mixed thoroughly in the soil according to the treatments. Ten viable seeds of *Parthenium* per pot were

sown at equal distance on the surface and thin layer of soil was applied. The plants were thinned to five plants per pot at 15 DAS (Days after Sowing). Uniform watering (500 mL/pot) was continued for 60 days after sowing.

Growth parameters: The number of seedlings/ pot and other growth parameters viz. number of leaves, branches, capitula/plant, length of the leaves, height of the plant and seeds/five capitula of *Parthenium* were recorded at regular interval at 10, 30 and 60 DAS. The leaves and stems were sampled on 60 DAS for chromatographic assay and dried at 80ºC for 48 h in oven. Different biochemical parameters such as sugars, organic and amino acids were extracted in 80% (v/v) ethanol.

Estimation of 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 consisting of 4% aniline in n - butanol (5 vol.), 4% diphenylamine in n - butanol (5 vol.) and orthophosphoric acid (1 vol.). 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 (by dissolving 0.4 g anthrone in 100 mL of concentrated sulphuric acid). Quantitative estimation of sugars was done according to the method of Yemm and Willis (1954). After evaporation of alcohol, 3 mL of distilled water and 6 mL 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 (spectronic - 20 D) at 600 nm. Quantity of sugars was determined from the standard curve obtained from glucose.

Estimation of 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 dry at room temperature for two days to remove traces of formic acid. Lemon yellow spots of organic acids were detected against a blue background by spraying with 0.04% (w/v) bromophenol blue in 90% (v/v) ethanol. The area of different organic acid spots was 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 synthetic organic acids from pre-determined calibration curves.

Estimation of amino acid 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 and 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). After heating at 100ºC for 20 min the spots of proline appeared on the chromatograms as yellow spots. The amount of different amino acids and amides were obtained from the standard curves of authentic compounds. 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.

Estimation of lipid: Lipids were extracted and purified from fresh plant 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. The phospholipids were quantified by the method of Fiske and Subbarow (1925) in terms of phosphorus which was digested with 2.5 mL of 5 N H_2SO_4 and a drop of 20% H_2O_2.

Statistical analysis: The treatments in the experiment were laid out in randomized block design with three replicates and critical difference (CD at 5%) between means were determined.

RESULTS

Growth parameters: The dry leaf - residue of *Hyptis suaveolens* L. significantly reduced the germination and growth parameters (number of leaves, branches, length of the leaves, plant height, capitula/plant and seeds/five capitula) of *Parthenium* plant in pot culture. In control, *Parthenium* seeds begin to germinate after 5 DAS but in different treatments germination was delayed with lesser number of seedlings at 10 DAS. Number of *Parthenium* seedlings were reduced in different treatments with

Table 1: Effect of leaf residue of *Hyptis suaveolens* L. on growth parameters of *Parthenium hysterophorus* L.

Weed treatments	Leaf residue of *Hyptis suaveolens*		
	C	T_1	T_2
At 10 DAS			
Number of seedlings/pot	7.32±0.21	5.91±0.02 (19.26)	4.16±0.27 (43.17)
At 30 DAS			
Number of leaves/plant	9.35±0.27	8.56±0.06 (8.45)	6.98±0.01 (25.35)
Length of the leaves (cm)	7.25 ± 0.45	6.98±0.21 (3.72)	4.11±0.25 (43.31)
Plant height (cm)	11.82±0.50	10.52±0.04 (10.99)	7.43±0.09 (37.14)
At 60 DAS			
Number of leaves/plant	28.12±0.74	21.64±0.82 (23.04)	17.59±0.07 (37.45)
Length of the leaves (cm)	13.60±0.02	11.48±0.16 (15.59)	9.66±0.18 (28.97)
Plant height (cm)	51.86±0.05	37.83±0.27 (27.05)	29.81±0.01 (42.52)
Branches/plant	7.22±0.27	5.16±0.20 (28.53)	3.85±0.04 (46.68)
Number of capitula/plant	282±0.92	242±0.07 (14.18)	180±0.50 (36.17)
Number of seeds/five capitula	25±0.00	21±0.05 (16)	19±0.06 (24)

C = Control, T_1 = Leaf residue of *Hyptis suaveolens* 100 g/pot, T_2 = Leaf residue of *Hyptis suaveolens* 200 g/pot; Values are mean of three replicates±sem, DAS = Days after sowing; Figures in parentheses indicate percent inhibition over control

maximum reduction 43.17% was observed in T_2 treatment of *Hyptis suaveolens* (Table 1). Growth of the plants can be determined best by taking plant height into consideration. A decrease in the height of the plant by 10.99 and 37.14% was observed at 30 DAS which decreased further to 27.05 and 42.52% at 60 DAS with 100 g and 200 g *Hyptis* leaf residues, respectively. At 30 DAS after the application of 100 and 200 g *Hyptis* leaf residues/pot drastically reduced the number of leaves/plant by 8.45 and 25.35% respectively over control. The decrease in the length of the leaves was 3.72 and 15.59% in 100 g and 43.31 and 28.97% in 200 g *Hyptis* leaf residues at 30 and 60 DAS, respectively. Dry leaf residues of *H. suaveolens* significantly reduced the number of capitula/plant and seeds/five capitula of *Parthenium* plant in T_1 and T_2 treatments. In control pots, number of seeds/five capitula were 25 which were significantly reduced to 16 and 24% in T_1 and T_2 treatments, respectively.

Chlorophyll content and sugar metabolism: Dry leaf residues of *Hyptis suaveolens* caused significant inhibition of total chlorophyll in leaves of potted *Parthenium.* In control total chlorophyll content was 2.73 mg/g which was significantly reduced to 1.96 and 1.24 mg/g in 100 and 200 g *Hyptis* leaf residues, respectively. Maximum inhibition (54.58%) in total chlorophyll content was observed in *Parthenium* leaves with T_2 treatment. The dry leaf residues of *Hyptis suaveolens* L. variously affected the sugar composition of leaf and stem of *Parthenium hysterophorus* L. The common sugars detected in leaf and stem of *Parthenium* were raffinose, sucrose, glucose, fructose, mannose and xylose. An unidentified sugar was also detected in leaf and stem of all the treatments and control. The high amount of total sugars observed in leaf and stem of control was decreased in treatments. Maximum inhibition of total sugars 24.88% was observed in *Parthenium* leaf of T_2 treatment over control (Table 2).

Organic acid metabolism: The applied leaf - residues of *Hyptis suaveolens* increased the citric, malic, malonic, succinic, fumaric and oxalo-succinic acids in the leaves and stems of *Parthenium*. However, succinic and oxalosuccinic acid contents were decreased in *Parthenium* leaf in different treatments. Leaves of T_1 and T_2 treatments contained higher amount of total organic acids as compared with the stem of respective treatments. Maximum accumulation of total organic acids (7.45%) was evident in leaf of T_2 treatment over respective control (Table 3).

Amino acid and protein metabolism: The amino acid composition of *Parthenium* was variously affected by different amount of dry leaf residues of *Hyptis suaveolens* used in T_1 and T_2 treatments. Among the free amino acids (leucine+isoleucine, valine, γ-aminobutyric acid, tyrosine, proline, α-alanine and β- alanine, threonine, arginine, glycine, serine, cysteine, lysine+histidine) showed increasing trend in treated plants. The transport amino

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

	Leaf residue of *Hyptis suaveolens*					
		Leaf			Stem	
Sugars	C	T_1	T_2	C	T_1	T_2
Raffinose	1.76±0.01	1.33±0.04	1.41±0.02	0.83±0.09	0.63±0.02	0.38±0.12
Sucrose	1.82±0.04	1.28±0.01	0.83±0.04	0.96±0.03	0.94±0.01	0.71±0.02
Glucose	1.24±0.10	0.91±0.08	0.94±0.16	0.74±0.05	0.62±0.03	0.69±0.01
Fructose	1.30±0.01	1.02±0.10	0.82±0.03	0.80±0.14	0.73±0.28	0.55±0.07
Mannose	1.52±0.03	1.21±0.22	1.67±0.48	0.45±0.02	0.61±0.01	0.75±0.02
Xylose	1.45±0.02	1.71±0.14	1.45±0.01	0.72±0.32	0.69±0.02	0.67±0.02
Unidentified (U)	1.60±0.11	0.85±0.01	0.91±0.05	0.91±0.05	0.83±0.04	0.81±0.01
Total	10.69	8.31 (22.26)	8.03 (24.88)	5.41	5.05 (6.65)	4.56 (15.71)
CD at 5%			0.12			0.03

C = Control, T_1 = Leaf residue of *Hyptis suaveolens* 100 g/pot, T_2 = Leaf residue of *Hyptis suaveolens* 200 g/pot; Values are mean of three replicates±sem; Figures in parentheses indicate percent inhibition over control

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

	Leaf residue of *Hyptis suaveolens*					
		Leaf			Stem	
Organic acids	C	T_1	T_2	C	T_1	T_2
Citric acid	0.21±0.01	-	0.23±0.02	0.12±0.01	0.17±0.01	0.13±0.05
Unknown (U)	-	-	-	-	0.18±0.04	0.27±0.03
Malic acid	0.26±0.14	0.28±0.04	0.31±0.06	0.20±0.02	0.22±0.10	0.18±0.09
Malonic acid	0.34±0.03	0.37±0.13	0.44±0.07	0.23±0.05	-	0.22±0.02
Succinic acid	0.41±0.22	0.34±0.27	0.39±0.05	0.21±0.09	-	0.23±0.16
Fumaric acid	-	0.39±0.08	0.36±0.03	0.24±0.11	0.34±0.14	-
Oxalosuccinic acid	0.39±0.28	0.31±0.12	-	0.28±0.23	0.40±0.18	0.30±0.09
Total	1.61	1.69 (4.97)	1.73 (7.45)	1.28	1.31 (2.34)	1.33 (3.91)
CD at 5%			0.09			0.05

C = Control, T_1 = Leaf residue of *Hyptis suaveolens* 100 g/pot, T_2 = Leaf residue of *Hyptis suaveolens* 200 g/pot; Values are mean of three replicates±sem; Figures in parentheses indicate percent stimulation over control

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

	Leaf residue of *Hyptis suaveolens*					
		Leaf			Stem	
Free- amino acids	C	T_1	T_2	C	T_1	T_2
Leucine+Isoleucine	0.03±0.01	0.02±0.01	0.13±0.01	0.04±0.01	0.07±0.04	0.05±0.01
Valine	0.10±0.10	0.18±0.18	0.24±0.05	0.07±0.06	0.15±0.14	0.13±0.10
γ-Aminobutyric acid	0.15±0.09	0.20±0.02	0.22±0.10	0.06±0.05	0.07±0.02	0.08±0.02
Tyrosine	0.08±0.05	0.15±0.09	0.20±0.06	0.07±0.03	0.09±0.07	0.12±0.06
Proline	0.05±0.03	0.23±0.11	0.35±0.08	0.04±0.03	0.11±0.10	0.16±0.10
α-alanine	0.02±0.01	0.07±0.05	0.12±0.01	0.02±0.01	0.09±0.05	0.09±0.05
β-alanine	0.07±0.04	0.12±0.10	0.19±0.02	0.03±0.01	0.12±0.11	0.16±0.08
Threonine	0.13±0.11	0.30±0.23	0.30±0.20	0.06±0.02	0.10±0.02	0.11±0.08
Arginine	0.10±0.08	0.10±0.08	0.13±0.08	0.05±0.01	0.04±0.01	0.12±0.09
Glutamine	0.25±0.19	0.17±0.09	0.16±0.05	0.19±0.09	0.09±0.04	0.05±0.02
Asparagine	0.13±0.09	0.15±0.01	0.11±0.09	0.04±0.02	0.05±0.01	0.07±0.05
Glycine + Serine	0.06±0.05	0.07±0.03	0.14±0.05	0.05±0.04	0.04±0.03	0.10±0.06
Glutamic acid	0.03±0.03	0.10±0.08	0.15±0.10	0.03±0.01	0.05±0.01	0.08±0.02
Aspartic acid	0.14±0.08	0.14±0.06	-	0.02±0.01	0.06±0.05	0.13±0.04
U	0.02±0.01	0.15±0.12	-	0.18±0.06	0.11±0.09	0.12±0.09
Cysteine	0.08±0.06	0.25±0.23	-	0.04±0.01	0.22±0.05	0.25±0.11
Lysine + Histidine	0.02±0.02	0.10±0.09	0.18±0.11	0.02±0.02	-	0.12±0.07
U_0	0.05±0.04	0.12±0.06	0.14±0.09	0.05±0.01	-	0.10±0.05
Methionine	-	-	-	-	-	-
U	0.14±0.10	-	0.25±0.13	0.03±0.02	-	-
U_1	0.05±0.03	-	-	0.05±0.04	-	-

Table 4: continued

	Leaf residue of *Hyptis suaveolens*					
		Leaf			Stem	
Free- amino acids	C	T_1	T_2	C	T_1	T_2
U_2	0.16±0.11	0.13±0.03	-	0.11±0.01	-	-
U_3	0.04±0.04	-	-	0.01±0.01	-	0.12±0.05
U_4	0.03±0.02	-	0.28±0.07	0.10±0.02	0.25±0.11	-
U_5	0.23±0.17	-	0.13±0.10	0.06±0.05	-	-
Total	2.16	2.75 (27.31)	3.42 (58.33)	1.42	1.71 (20.42)	2.16 (52.11)
CD at 5%			0.16			0.05

C = Control, T_1 = Leaf residue of *Hyptis suaveolens* 100 g/pot, T_2 = Leaf residue of *Hyptis suaveolens* 200 g/pot; Values are mean of three replicates± sem; Figures in parentheses indicate percent stimulation over control

Table 5: Effect of leaf residue of *Hyptis suaveolens* L. on lipid composition of leaf and stem of *Parthenium hysterophorus* L. (mg/100gm fresh weight)

Lipids	Leaf residue of *Hyptis suaveolens*					
		Leaf			Stem	
	C	T_1	T_2	C	T_1	T_2
Phospholipids	3.21±0.09	2.10±0.08	1.38±0.46	2.82±0.16	1.47±0.49	1.21±0.16
Monoglycerides	1.15±0.82	1.92±0.19	2.53±0.07	1.19±0.28	1.24±0.15	2.16±0.02
Sterol	2.63±0.24	1.86±0.21	0.91±0.58	1.68±0.64	1.52±0.01	0.91±0.05
Diglycerides	3.10±0.46	2.51±0.09	1.64±0.02	1.59±0.32	1.36±0.46	0.85±0.03
Fatty acids	2.93±0.95	2.10±0.27	1.82±0.38	1.81±0.06	1.70±0.28	0.87±0.24
Triglycerides	2.56±0.01	2.14±0.06	0.93 ±0.47	1.62±0.45	1.41±0.02	0.96±0.32
Methyl ester	0.92±0.05	1.29±0.12	1.98±0.92	0.69±0.02	1.17±0.19	1.32±0.04
Hydrocarbons	2.38±0.17	2.48±0.27	3.14±0.01	1.07±0.92	1.33±0.05	1.84±0.01
Total	18.88	16.4 (13.14)	14.33 (24.09)	12.47	11.2 (10.18)	10.12 (18.85)
CD at 5%			0.64			0.32

C = Control, T_1 = Leaf residue of *Hyptis suaveolens* 100 g/pot, T_2 = Leaf residue of *Hyptis suaveolens* 200 g/pot; Values are mean of three replicates±sem; Figures in parentheses indicate percent inhibition over control

acids (asparagine, glutamic and aspartic acid) were also increased in the treated plants. Proline, a cyclized derivative of glutamic acid was present in highest amount (0.35 mg) in leaf of T_2 treatment. Maximum number of unknown amino acids detected in control were absent in T_1 and T_2 treatments. The total free amino acids were increased in treated plants with maximum accumulation (58.33%) in the leaves treated with 200 g *Hyptis* leaf residues (Table 4). 98 µg/mL protein was recorded in control leaves which was reduced to 89 and 64 µg/mL in T_1 and T_2 treatments respectively. Maximum reduction (34.69%) of protein content was observed in *Parthenium* leaves in T_2 treatment.

Lipid metabolism: Common lipids detected in leaf and stem of *Parthenium* were phospholipids, monoglycerides, sterol, diglycerides, fatty acids, triglycerides, methyl esters and hydrocarbons. Leaf of control and treatments showed higher amount of hydrocarbons as compared with stem of control and treatments. The amount of total lipids exhibited decreasing trend in leaves and stems of treated plants. Maximum inhibition 24.09% of total lipids was observed in leaf of T_2 treatment (Table 5).

DISCUSSION

The dry leaf - residue of *Hyptis suaveolens* significantly affected the growth and physiological parameters of *Parthenium hysterophorus* L. The allelochemicals present in leaf - residues have been found to affect the various metabolic processes in higher plants (Einhellig, 1984). Germination is the resumption of metabolic activity and growth by the seed tissues which starts with the imbibition of water and ends with the protrusion of embryonic roots. Inhibition of growth parameters might be due to inhibition in synthesis of gibberellin, auxin and other growth hormones under the influence of allelochemicals (Beffa *et al*., 1990). The growth inhibition caused by allelochemicals from leaf - residues of *Hyptis* could be due to interference with different plant growth processes like cell division and cell enlargement (Shettel and Balke, 1983), inhibition in nutrient uptake (Harper and Balke, 1980), reduction in dry matter production due to inhibition of metabolic processes such as photosynthesis (Colton and Einhellig, 1980) and respiration (Demos *et al*., 1975). 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. Reduction in height of *Parthenium* plant might be due to inhibition of CO_2 - fixing efficiency or delaying of germination coupled with low efficiency in dry matter production (Uniyal and Nautiyal, 1996). Blum *et al.* (1985) observed that phenolic acids reduce the expansion of the leaves. Allelochemicals present in *Hyptis suaveolens* reduced the chlorophyll content in the leaves

of *Parthenium* and altered the photosynthetic rate, respiration and other metabolic processes (Epstein *et al*., 1967; Colton and Einhellig, 1980). The reduction in the amount of chlorophyll might be due to inhibition of synthesis of enzymes, proteins and cofactors required for synthesis of chlorophyll (Kohli, 1992) or excessive breakdown of chlorophyll under the influence of allelochemicals (Thapar and Singh, 2006). Carbohydrates are the cellular source of energy and starting materials for the synthesis of protein, lipid and other plant products. Sucrose is principal disaccharide synthesized and to be transported in higher plants. The lower amount of sucrose in treatments might be due to inhibition of sucrose synthesis from fructose and glucose in a reaction catalyzed by sucrose synthetase. Inhibition of photosynthesis leading to decreased amount of photosynthates might be due to decreased biosynthesis of chlorophyll or degradation of photosynthetic pigments (Pandey, 1994; Chetti *et al*., 1997; Kohli *et al*., 1997; Bajaj *et al*., 2004) or inhibition of photosynthesis by allelochemicals resulting in decreased dry matter i.e., photosynthates (Overland, 1966; Rice, 1984). Colton and Einhellig (1980) reported that the allelochemicals inhibit the rate of photosynthesis due to interference with water balance and chlorophyll content.

Organic acids are present in catalytic amount in all the living cells where they participate in citric acid cycle. The accumulation of organic acids in *Parthenium* might be due to stimulation of some organic acids, deamination of amino acids or inhibition of some respiratory processes under the influence of allelochemicals present in the leaf - residues of *Hyptis* (Demos *et al*., 1975). Accumulation of amino acids inhibited its own biosynthesis leading to the accumulation of organic acids (Coruzzi and Last, 2000). 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. The increase in the total amino acids might be an adaptation of plants to the stress generated by the allelochemicals present in *Hyptis* leaves. Proline which is produced from glutamate, showed significant increase in treatments and it acts as osmolyte. The synthesis of asparagine was increased when the rate of photosynthesis was decreased under the influence of allelochemicals (Lam *et al*., 1995). Increased synthesis of asparagine may be a device to detoxify the effects of excess of ammonia produced in deamination process. The total amino acids increased due to degradation or inhibition of biosynthesis of proteins or more biosynthesis of some amino acids under the influence of allelochemicals. Proteins play a pivotal role in biological processes and it regulates growth, development and reproduction of plant (Pushpangadan *et al*., 1979). The inhibition of protein synthesis might have resulted in inhibition of biosynthesis of chlorophyll molecules, photosystem I, II, ATPase and other enzymes required for photosynthesis. The protein may serve as respiratory substrate if the supply of carbohydrates are inadequate because of decreased photosynthetic rate (Salisbury and Ross, 1991). It has been observed that protein degradation due to the allelochemicals increases the amount of free amino acids (Singh and Thapar, 2003).

The lipid composition of *Parthenium* was modified by leaf residues of *Hyptis suaveolens.* Phospholipids are important component of plasmalemma and these were present in higher amount in leaf and stem of control in comparison to the treatments. Monoglycerides were increased in different treatments due to stimulated biosynthesis or breakdown of storage neutral triglycerides. Sterols are important constituent of plasma-membrane family and as such may be capable of controlling membrane permeability (Simon, 1974). Sterols provide the stability to membrane as these link molecules of protein and phospholipid were also decreased. Decrease in di and triglycerides might be due to breakdown of the two lipid classes under the influence of allelochemicals. The decrease in building blocks of lipid, fatty acids might be due to oxidation to yield acetyl coenzyme, a key metabolite in citric acid cycle. Methyl esters and hydrocarbons showed increasing trend in treatments. Hydrocarbons, a bulk constituent of epicuticular lipids (Hadley, 1980) and plasmalemma were increased due to influence of allelochemicals. 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). Reduced rate of photosynthesis caused decreased synthesis of carbohydrates, the precursors of amino acids, protein and this might have resulted in reduction of lipids. The breakdown of lipid classes caused recycling of building blocks i.e. glycerol and fatty acids to be utilized in glycolysis and Krebs cycle when the supply of carbohydrates are inadequate under the influence of allelochemicals.

CONCLUSION

In conclusion, it can be said that the change in physiological processes inhibited and delayed the seed germination and growth of *Parthenium* under the influence of allelochemicals present in leaf residues of *Hyptis suaveolens.* The inhibition of growth parameters (plant height, branching and photosynthetic area) decreased the number of seeds per plant and this may restrict the spread of obnoxious weed. Sowing of seeds of *Hyptis suaveolens* among the population of *Parthenium* may help in controlling the spread and growth of *Parthenium.* Therefore, leaf - residues of *Hyptis suaveolens* may be used as potential bioherbicide to control *Parthenium hysterophorus*.

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