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EVALUATION OF ALLELOPATHIC INFLUENCE OF *PARTHENIUM HYSTEROPHORUS* L. PLANT PARTS ON BIOCHEMICAL RESPONSE OF *PHASEOLUS VULGARIS* L

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ABSTRACT

In the present study an attempt has been made to evaluate the allelopathic effect of parthenium leaf, stem and flower extracts on biochemical parameters of bean seedlings. The aqueous leaf, stem and flower extract of parthenium showed both stimulatory and inhibitory effects on chlorophyll, carbohydrate, protein and phenol content. The chl. a, b, total chlorophyll and carotenoid content were found to be decreased significantly as the concentrations of the extract increased when compared to control. The total carbohydrate content in both root shoot axis and cotyledon increased as the concentration of the extract increased when compared to control. Total protein content in root shoot axis decreased as the concentration of the extract increased when compared to control on the other hand in cotyledon it was found to be increased as the concentration of the extracts increased. The total phenol content in both root shoot axis and cotyledon decreased significantly as the concentration of the extract increased when compared to control. The results of present study clearly showed that both stimulatory and inhibitory effect of parthenium aqueous leaf, stem and flower extract may be due to the presence of allelochemicals like terpenoids, tannins, phenolic acids so on.

INTRODUCTION

Ever increasing human population has made the food scarcity, a challenge for scientists and farming community. Agriculture zone particularly crop fabrication is under gigantic pressure. The biotic and abiotic stresses are major threats to crop production (Semenov and Halford, 2009). About 65% of Indian population honestly depends on agriculture. In the past ten years agriculture sector has witnessed impressive advances in the fabrication and productivity of food grains, commercial crops, fruits, vegetables, food grains and dairy. Soil is the medium which supports the growth and development of plants. The fertility of soil is an significant factor determining fertilizer requirements as well as the crop production (Batish *et al*., 2001).

Chemical fertilizers play an vital role in high productivity, pests and weed control (Jeffrey, 2007). Fertilization increases efficiency and obtains improved quality product in agricultural activities. However, in recent years, fertilizer utilization increased exponentially throughout the world, causing severe environmental evils (Sonmez *et al*., 2007). The term "allelopathy" signifies the interactions between plants, might lead to either stimulation or inhibition of growth. Different groups of plants like; algae, lichens, crops, annual and perennial weeds have wide known allelopathic interactions. Allelopathy is a phenomenon where plants chemically obstruct with the growth and development of other plants and has been known for over 2000 years. A variety of crops and weeds have been reported to acquire allelopathic activity on the growth of other plants (Rice, 1974).

Allelopathy, may also play an eminent role in the intraspecific and interspecific competition and may determine the type of interspecific association. The plant may exhibit inhibitory or rarely stimulatory effect on the germination and growth of other plants in the immediate vicinity. Due to the action of allelochemicals, a large number of physiological functions and biochemical reactions are affected, such as seed germination, cell division, cell elongation, membrane permeability and ion uptake (Setia *et al*., 2007).

Secondary metabolites in plants have been investigated by phytochemists. Originally classified as waste products, but these compounds have recently been investigated extensively by ecologists and pharmacologists and many complex biological functions have been discovered. Various secondary metabolites produced by plants and micro-organisms have been considered as potential allelochemicals that play an important

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role in shaping interactions and communities. Many allelochemicals have been identified since experiments began to isolate and determine allelopathic potentials of plant compounds. Compounds that have been identified thus far include a variety of chemical classes such as phenolic acids, coumarins, benzoquinones, terpenoids, glucosinolates, and tannins (Putnam and Duke 1978). The compounds which are responsible for allelopathic activity are present in many plants and plant organs like leaves, stems, fruits and buds (Mahall and Callaway, 1991; Indrajit, 1996 and Ashrafi *et al*., 2007). The current worldwide demand for cheaper, more environmentally-friendly weed management technologies has motivated a number of studies on the allelopathic interaction between crops and weeds (Om *et al*., 2002).

Parthenium is an aggressive ubiquitous invasive annual herbaceous weed with no economic importance unravelled till now and has made wide distribution globally affecting the growth of native plants species (Bhnsal *et al*., 1997). Pulse crops play an important role in Indian agriculture and India is the largest producer and consumer of pulses in the world. Pulses contain a high percentage of quality protein nearly three times as much as cereals. Thus, they are cheaper source to overcome protein malnutrition among human beings. Pulses constitute the main source of protein and essential amino acids for the predominantly vegetarian population and lower income groups of the country. *Phaseolus vulgaris* L. is commonly known as French bean belongs to the family leguminosae or Fabaceae (legume or bean family) that originated in Central and South America and grown worldwide for its edible fruit, either the dry seed or the unripe fruit, both of which are referred to as beans. In the present study an attempt was been made to evaluate the efficacy of parthenium plant parts on biochemical parameters of *Phaseolous vulgaris*.

MATERIALS AND METHODS

Collection of seed sample and plant material

The seed sample of French bean (*Phaseolus vulgaris* L.) (cv. selection-9) was obtained from Arjun agro agencies, Mysore. The plant *P. hysterophorus* was collected from in and around the Mysore University Campus, Manasagangotri. The seed samples were tested for their sensitivity to *P. hysterophorus* extracts by conducting preliminary germination experiments.

Preparation of aqueous extract

P. hysterophorus was collected and instantly separated into shoot (stem and branch), leaf and flower. The shoot and leaf material was cut into 1-2 cm pieces and shade dried for a week. The dried leaf, shoot and flower were ground in to a fine powder separately using a mixer grinder. 2, 4, 6, 8 and 10 g powder of each plant part was weighed separately and soaked in 100 ml of distilled water and mixed thoroughly by keeping in rotatory shaker keep over night at the room temperature to dissolve allelochemicals contents in to the solution. After 24 hours of soaking, extracts were collected by sieving through muslin cloth and designated as 2, 4, 6, 8 and 10% respectively.

Biochemical assay

Seeds were sown in triplicates in poly cups and filled with soil. Two to three seeds per poly cup were sown. To each of the poly cups 5ml of aqueous extract concentrations ranging from 2%, 4%, 6%, 8% and 10% were added and distilled water served as control. The poly cups were kept in light and after the completion of 9 days of seed germination, seedlings were used for biochemical experiments for the estimation of Chlorophyll a, b; total chlorophyll (Arnon, 1949), Carotenoids (Krick and Allen, 1965), carbohydrates (Hedge and Hofreiter, 1962), phenols (Malick and Singh, 1980) and proteins (Lowry's *et al*., 1951). The experiments were conducted in triplicates. The obtained results was calculated and statistically represented.

RESULTS AND DISCUSSION

Effect of parthenium leaf, stem and flower extract on pigment content of bean is presented in table- 1. The mean value of pigment content in leaves differed significantly when treated with different concentration of aqueous extracts when compared to control. The maximum and minimum content of chlorophyll a, b, total chlorophyll and carotenoid were observed in control and 10 % concentrations in all the extracts.

The seedlings showed decrease in chlorophyll a from 35.9 to 85.4 %, 40.7 to 69.9 % and 40.6 to 90.2 % from 2 to 10 % concentration respectively, chlorophyll b decreased from 25.6 to 71.52 %, 31.2 to 77.3 % and 21.4 to 81.7 % from 2 to 10 % respectively in leaf, stem and flower extracts. Total chlorophyll and carotenoid decreased from 23.1 to 72.6 %, 30.6 to 72.7 %, 33.3 to 78.5 % and 32.3 to 86.7 %, 32.2 to 88.7 %, 11.1 to 85.1 % from 2 to 10% respectively in leaf, stem and flower extracts. Chlorophylls are important molecules which act as core component of pigment protein complexes surrounded the photosynthetic membranes and play a foremost role in photosynthesis (Siddiqui and Zaman, 2005).

Chlorophyll a and b and carotenoids concentrations correlate to the photosynthetic potential of a plant and give indication of the physiological status of the plant (Young and Britton, 1990). Numerous researchers have reported that chlorophyll content and ion uptake was reduced significantly by allelochemicals (Alsaadawi *et al*., 1986). Romman (2011) reported the effect of different concentrations of aqueous extracts of *Achillea bibersteinii* on germination, seedling growth, photosynthetic pigments and protein contents of pepper and observed that stem diameter of the plant was slightly affected and leaf number was significantly unaffected. (Peng *et al*., 2004) reported that allelochemicals affect the photosynthetic activity in plant by destroying chlorophyll molecules. Allelochemicals alters the physiological functions adversely in target plants by acting upon the necessary enzymes which are required for biosynthesis of certain molecules (Gniazdowska and Bogatek, 2007). The reduction in chlorophyll content may be due to the fact 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*., 1993).

Effect of different concentrations of leaf, stem and flower extracts of parthenium on bean root shoot axis and cotyledon is presented in the table 2. The total carbohydrate content increased in both root shoot axis and cotyledon as the concentrations of the leaf, stem and flower extracts of parthenium increased when compared to control. In both root shoot axis and cotyledon the maximum and minimum values were observed in 2 and 10 % in the extracts when compared to control.

Table-1 Effect of leaf, stem and flower extracts of *Parthenium* on Chl. a, Chl. b, Total Chl. and carotenoid content of French bean seedlings.

Concentration	Chlorophyll a (mg/g F.Wt.)	Chlorophyll b (mg/g F.Wt.)	Total Chlorophyll (mg/g F.Wt.)	Carotenoid (mg/g F.Wt.)
		Parthenium leaf extract		
Control	1.03±0.0081^{a}	1.44±0.0008^{a}	0.95±0.0012^{a}	0.68±0.0012^{a}
2%	0.66±0.0012^{b}	1.07±0.0021^{b}	0.73±0.0017^{b}	0.46±0.0016^{b}
4%	0.63±0.0094^{b}	0.95±0.0008^{c}	0.64±0.0016^{c}	0.41±0.0008^{b}
6%	0.59±0.0081^{c}	0.87±0.0017^{d}	0.56±0.0012^{d}	0.38±0.0004^{c}
8%	0.31±0.0020^{d}	0.61±0.0012^{e}	0.36±0.0008^{e}	0.02±0.0012^{e}
10%	0.15 ±0.0073^{e}	0.41± 0.0012^{f}	0.26±0.0017^{f}	0.09±0.0008^{d}
		Parthenium stem extract		
Control	1.03±0.0008^{a}	1.41±0.0008^{a}	0.88±0.0008^{a}	0.62±0.0012^{a}
2%	0.61±0.0047^{b}	0.97±0.0017^{b}	0.61±0.0012^{b}	0.42±0.0017^{b}
4%	0.56±0.0004^{c}	0.89±0.0008^{c}	0.59±0.0008^{c}	0.38±0.0008^{c}
6%	0.35± 0.0008^{d}	0.63±0.0012^{d}	0.41±0.0014^{d}	0.35±0.0017^{c}
8%	0.28±0.0049^{e}	0.58±0.0012^{e}	0.34±0.0017^{e}	0.22±0.0021^{d}
10%	0.13± 0.0061^{f}	0.32±0.0016^{f}	0.24±0.0012^{f}	0.07±0.0020^{e}
		Parthenium flower extract		
Control	0.96±0.0009^{a}	1.37±0.0008^{a}	0.84±0.0008^{a}	0.54±0.0008^{a}
2%	0.57±0.575^{b}	0.95±0.0024^{b}	0.56±0.0004^{b}	0.48±0.0017^{b}
4%	047±0.0012^{c}	0.82±0.0009^{c}	0.54±0.0016^{b}	0.31±0.0016^{c}
6%	0.37±0.0021^{d}	0.51±0.0008^{d}	0.33±0.0012^{c}	0.20±0.0012^{d}
8%	0.18±0.0012^{e}	0.45±0.0012^{e}	0.30±0.0008^{c}	0.16±0.0021^{e}
10%	0.09±0.0017^{f}	0.25±0.0016^{f}	0.18±0.0017^{d}	0.05±0.0012^{f}

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level.

Table-2 Effect of leaf, stem and flower extracts of *Parthenium* on total carbohydrate content of French bean seedlings.

Concentration	Root-shoot axis (mg/g F.Wt)	cotyledons (mg/g F.Wt)
	Parthenium leaf extract	
Control	21.45±0.085^{a}	24.47±0.226^{f}
2%	37.57±0.010^{b}	52.21±0.198^{e}
4%	43.39±0.094^{c}	76.66±0.129^{d}
6%	46.32±0.050^{d}	108.41±0.231^{c}
8%	48.44±0.164^{e}	166.39±0.158^{b}
10%	51.31±0.137^{f}	254.37±0.215^{a}
	Parthenium stem extract	
Control	16.47±0.168^{a}	19.67±0.155^{f}
2%	22.40±0.415^{b}	49.45±0.235^{e}
4%	30.48±0.385^{c}	65.24±0.151^{d}
6%	38.34±0.224^{d}	92.56±0.171^{c}
8%	42.44±0.297^{e}	152.09±0.057^{b}
10%	49.37±0.198^{f}	189.29±0.255^{a}
	Parthenium flower extract	
Control	11.76±0.156^{a}	14.72±0.195^{f}
2%	19.61±0.170^{b}	34.54±0.307^{e}
4%	24.26±0.135^{c}	56.49±0.287^{d}
6%	29.67±0.155^{d}	83.72±0.193^{c}
8%	35.76±0.217^{e}	129.50±0.363^{b}
10%	42.43±0.390^{f}	152.69±0.220^{a}

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level.

In root shoot axis and cotyledon the total carbohydrate content increased from 75.5 to 139.9 %, 36 to 199 %, 66.7 to 265 % and 11.33 to 939 %, 51.3 to 862.3 %, 134 to 937.2 % from 2 to 10% concentration in leaf, stem and flower extracts respectively when compared to control. It is very clearly depicted that an increased amount of carbohydrates content exerts its influence mainly through its aqueous leachates (Gulzar and Siddiqui, 2014). Increased amount of carbohydrates indicates to the fact that the plant is under stress and it is gathering up its energy reserves to meet any condition of adversity.

The present results are in line with Abdulghader *et al* (2008) who reported that significant increase in the soluble sugars in response to leaf extracts of heliotrope (*Heliotropium foertherianum*) in raddish. Similarly increase in soluble sugars of maize in response to leaf extracts of *Acacia* and *Eucalyptus* has been reported (Sahar *et al*., 2005).

The effect of different concentrations of parthenium leaf, stem and flowers extracts on total protein content of bean root shoot axis and cotyledon is represented in the table 3. The total protein content showed significant decrease in root shoot axis and significant increase in cotyledon as the concentrations of the extract increased when compared to control. In root shoot axis the maximum and minimum value was observed in control and 10 % while in the cotyledon the maximum and minimum values were observed in 10% and control respectively in all the extracts. In aqueous extract of leaf, stem and flower, protein content in root shoot axis decreased from 2.99 to 68.3 %, 28.8 to 83.3 % and 30.3 to 84.2 % respectively when compared to control.

However in cotyledon the protein content increased from 55.5 to 149 %, 57.8 to 318.2 % and 72.3 to 387 % respectively when compared to control. Impairment of various metabolic activities under the influence of leachate inhibited the protein synthesis or stimulated the degradation (Mersie and Singh, 1993). Normal ways of protein synthesis is inhibited in lettuce seedlings (*Lactuca sativa*) when treated with cinnamic acid (Einhellig, 1996). Padhy (2000) have reported that the leaf

litter leachate of *Eucalyptus globulus* decreased the protein content in both root and shoot of finger millet. These findings strongly support the present observation. Kolesnichenko and Aleikina (1976) showed that the chemical compounds secreted from the roots of *Fraxinus excelsior* inhibited the protein synthesis in the root of *Quercus robur*. The aqueous extract of *Ranunculus arvensis* plant materials inhibited the germination of wheat varieties and also it caused a decrease in the protein content (Bansal, 1997).

Table-3 Effect of leaf, stem and flower extracts of *Parthenium* on total protein content of French bean seedlings.

Concentration	Root-shoot axis (mg/g F.Wt)	Cotyledons (mg/g F.Wt)
Parthenium leaf extract		
Control	25.34±0.001^{a}	10.05±0.020^{f}
2%	24.58±0.002^{b}	15.63±0.008^{e}
4%	20.36±0.016^{c}	19.34±0.014^{d}
6%	19.32±0.001^{d}	25.45±0.008^{c}
8%	14.25±0.008^{e}	26.33±0.012^{b}
10%	8.03±0.009^{f}	28.03±0.008^{a}
Parthenium stem extract		
Control	24.14±0.004^{a}	6.02±0.012^{f}
2%	17.17±0.008^{b}	12.54±0.009^{e}
4%	15.03±0.021^{c}	15.04±0.008^{d}
6%	13.25±0.009^{d}	19.45±0.004^{c}
8%	11.02±0.008^{e}	22.02±0.008^{b}
10%	4.03±0.012^{f}	25.18±0.004^{a}
Parthenium flower extract		
Control	20.14±0.008^{a}	4.13±0.012^{f}
2%	14.03±0.016^{b}	7.12±0.004^{e}
4%	10.14±0.004^{c}	8.86±0.016^{d}
6%	9.03±0.012^{d}	13.27±0.012^{c}
8%	8.46±0.004^{e}	18.02±0.008^{b}
10%	3.17±0.016^{f}	20.15±0.004^{a}

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level.

Table-4 Effect of leaf, stem and flower extracts of *Parthenium* on total phenol content of French bean seedlings.

Concentration	Root-shoot axis (mg/g F.Wt)	cotyledons (mg/g F.Wt)
Parthenium leaf extract		
Control	3.69±0.061^{a}	5.20±0.055^{a}
2%	2.79±0.099^{b}	4.20±0.056^{b}
4%	2.58±0.046^{b}	3.52±0.050^{c}
6%	2.11±0.014^{c}	3.24±0.085^{c}
8%	1.51±0.045^{d}	2.66±0.014^{d}
10%	1.38±0.079^{d}	2.15±0.011^{e}
Parthenium stem extract		
Control	3.56±0.032^{a}	4.13±0.014^{a}
2%	2.65±0.029^{b}	3.24±0.085^{b}
4%	2.52±0.048^{b}	3.13±0.013^{b}
6%	1.51±0.038^{c}	2.71±0.099^{c}
8%	1.42±0.049^{c}	2.60±0.049^{c}
10%	0.46±0.019^{d}	2.04±0.015^{d}
Parthenium flower extract		
Control	3.33±0.087^{a}	3.07±0.039^{a}
2%	2.48±0.040^{b}	2.57±0.083^{b}
4%	2.04±0.014^{c}	1.57±0.045^{c}
6%	1.34±0.033^{d}	0.90±0.084^{d}
8%	0.97±0.010^{e}	0.84±0.046^{d}
10%	0.38±0.049^{f}	0.59±0.052^{e}

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver. 14.0 according to Tukey's mean range test at 5% level.

Effect of different concentration of parthenium leaf, stem and flowers extracts on total phenol content of root shoot axis and cotyledon is represented in the Table-4. The total phenol content decreased in both root shoot axis and cotyledon as the concentration of the extract increased when compared to control. In aqueous extract of leaf, stem and flower in root shoot axis and cotyledon phenol content decreased from 24.3 to 62.6 %, 25.5 to 87.1 %, 25.5 to 88.5 % and 19.2 to 58.6 %, 21.5 to 50.6 %, 16.2 to 80.78 % respectively when compared to control. Different secondary metabolites like phenolics, terpenoids, alkaloids, polyacetylenes, fatty acids, and steroids can act as allelochemicals (Inderjit and Dakshini 1994). These chemicals are present in various plant parts; however, their mere presence does not established allelopathy (Putnam and Tang, 1986). Chum *et al* (2012) reported that 2-benzoxazolinone increased the phenolic content in some vegetable crops over the control. Accumulation of phenols is often a characteristic of stress condition (Ahmed and Rashad, 1996).

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