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RESEARCH ARTICLE

Allelopathic influence of *Azadirachta indica* leaf, stem and bark extracts on physiological responses of *Phaseolus vulgaris* L.

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Abstract

In the present investigation an attempt has been made to assess the allelopathic efficacy of neem leaf, stem and bark extracts on physiological parameters of bean seedlings. The aqueous leaf, stem and bark extract of neem showed inhibitory and stimulatory effects on protein, carbohydrate, chlorophyll and phenol content. The chl. a, b, total chlorophyll and carotenoid content were found to be decreased as the concentrations of the extract increased when compared to control except in 5% concentration. The total carbohydrate content in root shoot axis decreased as the concentration of the extract increased except in 5% concentration on the other hand in cotyledon increased compared to control. Total phenol and protein content in root shoot axis decreased as the concentration increased except in 5% concentration when compared to control on the other hand in endosperm it was found to be increased as the concentration of the extracts increased. The results of current study showed that both negative and positive effect of neem aqueous leaf, stem and bark extract may be due to the presence of allelochemicals.

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INTRODUCTION

Agriculture is the backbone of Indian Economy. About 65% of Indian population depends directly on agriculture. Agriculture derives its importance from the fact that it has vital supply and demand links with the manufacturing sector. During the past ten years agriculture sector has witnessed spectacular advances in the production and productivity of food grains, oilseeds, commercial crops, fruits, vegetables, food grains, poultry and dairy. Soil is the medium which supports the growth of plants which provides mechanical support, the water and oxygen supply to plant roots as well as the plant nutrients. The fertility of soil is an important factor determining fertilizer requirements as well as the level of crop production that can be obtained (Batish, 2001). Therefore in a scenario of decreasing availability of productive land, decreasing soil and water resources, rising population and environmental pollution, the sustainable land management and adaptation of agro-forestry practices on all kinds of land is necessary. Agro-forestry is a suitable land management system which increases the yield of a particular piece of land by combining agricultural crops and forest plant and animal production system, simultaneously or sequentially on the same unit of land including management practices that are compatible with the local cultural practices (King and Chandler, 1978). The main idea of agro-forestry is to optimize production and economic development per unit area without affecting ecology or environment (Bandyopadyay, 1997).

Chemical fertilizers play an important role in high productivity, pests control and weed control (Jeffrey, 2007). Fertilization increases efficiency and obtains better quality of product recovery in agricultural activities. However, in recent years, fertilizer consumption increased exponentially throughout the world, causes serious environmental problems (Sonmez, 2007). Allelopathy is a phenomenon where a plant species chemically interfere with the

germination, growth and development of other plant species and has been known for over 2000 years. A variety of crop and weed species have been reported to possess allelopathic activity on the growth of other plant species (Rice, 1974). Compounds with allelopathic activity are present in many plants and in many plant organs including leaves, stems, fruits and buds (Mahall and Callaway, 1991; Indrajit, 1996 and Ashrafi *et al.,* 2007). Al-Charchafchi *et al.*, (2007) suggested about the allelopathic effect of Neem (*A. Indica*) plant on many other plants especially during their germination and seedling growth. In the present study an attempt was been made to evaluate the effect of neem plant parts on biochemical and yield parameters of *Phaseolous vulgaris*.

MATERIALS AND METHODS

Collection of plant materials and seed samples

Fresh and healthy plant materials like leaves, stem and bark of neem tree were collected from the various places of Manasagangotri, campus. The certified seed sample of Common beans Selection -9 variety was procured from Annadaatha agro kendra, Mysore.

Preparation of aqueous extracts:- The leaves, stem and bark were shade dried for about two-three weeks and made into a fine powder using grinder. 5, 10, 15, 20 and 25 grams of the powder was taken separately and add 100ml of water was added to each of the conical flask. The conical flasks were kept in a rotatory shaker for about 24 hours. The contents were filtered through muslin cloth. The extract was stored at 4^0C in dark bottles or in a refrigerator to reduce the allelochemicals degradation. Seeds were sown in triplicates in plastic cups and filled with soil. Two to three seeds per cup were sown. To each of the plastic cups aqueous extract of 5ml of concentrations ranging from 5%, 10%, 15%, 20% and 25% were added and distilled water was taken as control. Then plastic cups were kept in light and after the completion of 9 days of seed germination, morphological and biochemical experiments were conducted. The same procedure was followed for remaining trials. The final obtained results was calculated and statistically represented.

Biochemical analysis:

The seedlings were selected for the biochemical analysis viz, 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).

Statistical analysis

Statistical analyzed data were subjected to analysis of variance, using SPSS package version 14.0 according to Tukey's mean range test at 5% level significance.

Results and discussion

Effect of neem leaf, stem and bark extract on pigment content of bean is represented in table- 1. The mean value of pigment content differed significantly when treated with different concentration of aqueous extracts when compared to control. The seedlings showed decrease in chlorophyll a from 1.09 to 0.40, 1.08 to 0.28 and 1.05 to 0.26 mg/g F.Wt. from 10 to 25% concentration respectively and chlorophyll b decreased from 3.05 to 0.99, 3.04 to 0.88 and 2.25 to 0.62 mg/g F.Wt. from 10 to 25% respectively in leaf, stem and bark extracts. Total chlorophyll and carotenoid decreased from 2.70 to 0.69, 2.44 to 0.51, 1.85 to 0.07 and 0.69 to 0.375, 0.56 to 0.06, 0.055 to 0.05 mg/g F.Wt. from 10 to 25% respectively in leaf, stem and bark extracts. Chlorophylls are biomolecules which act as component of pigment protein complexes embedded in the photosynthetic membranes and play a major role in photosynthesis process (Siddiqui and Zaman, 2005). Several researchers have mentioned that chlorophyll content and ion uptake reduced significantly by allelochemicals (Al-saadawi *et al.* 1986). Romman (2011) investigated that effect of different concentration of aqueous leachate of *Achillea bibersteinii* on germination characteristics, seedling growth, photosynthetic pigments and protein contents of pepper and found that stem diameter was slightly affected and leaf number was significantly unaffected. Significant reduction in the amount of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and protein were recorded in response to allelochemicals stress. Hussain *et al*, 2011 reported that allelochemicals significantly inhibits chlorophyll synthesis in target plant and suppress photosynthesis.

It has been reported that the allelochemicals formed by invasive species affect the photosynthesis and plant growth by destroying the chlorophyll (Peng *et al.* 2004). The action of allelochemicals affects large number of biochemical reactions of target species resulting in alteration of different physiological functions (Gniazdowska and Bogatek, 2007). Allelochemicals leaching from plants with phenolic property may partially block the biosynthetic pathway of chlorophyll or stimulate the degrading pathway of chlorophyll and reduce photosynthesis process (Siddiqui and Zaman, 2005). The present findings corroborate the earliest report by Oyerinde *et al*. (2009) who revealed the decrease in chlorophyll-a, chlorophyll-b and total chlorophyll accumulation in young plants of maize after being treated with fresh shoot aqueous extract of *Tithonia diversifolia* which possess allelopathic characteristics. Our

results are also in agreement with the findings of Stupnicka-Rodzynkiewicz *et al.* (2006) who reported similar results regarding the effects of allelochemicals on chlorophyll content and photosynthesis process in plants.

Total carbohydrate content present in the root-shoot axis and cotyledon treated with different concentrations of neem leaf, stem and bark extract showed significant variations (Table 2). The total carbohydrate content showed increase in cotyledon and decrease in root shoot axis as the concentration of the extract increased when compared to control. However in root shoot axis at 5% concentration a significant increase in total carbohydrate content was observed compared to all other treatments including control. In aqueous extract of leaf, stem and bark, in root shoot axis the total carbohydrate content decreased from 52.23 to 16.33, 51.33 to 25.22, 52.33 to 30.33 however in the cotyledon it increased from 33.66 to 184.5, 27.0 to 169.9, 45.0 to 251.6 mg/g F.Wt. from 5 to 25% concentration 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). 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 Abdulghader *et al.* (2008) where appreciable increase in the increased concentration of soluble sugars in response to leaf extracts of heliotrope (*Heliotropium foertherianum*) in raddish. Similarly increase insoluble sugars of maize in response to leaf extracts of *Acacia* and *Eucalyptus* has been reported (Sahar *et al.* 2005). On the other hand Total carbohydrates in the sunflower seeds increased significantly with spraying or soil applied leaf extracts of *E. citriodora* leaf extracts, when used as spraying treatments, were more effective. The increase in total carbohydrate content was noticeable in the seeds of the fresh leaf sprayed plants as compared to the infected untreated unweeded plants.

Effect of different concentration of neem leaf, stem and bark extracts on total protein content of root shoot axis and cotyledon is represented in the table 3. The total protein showed significant increase in cotyledon and significant decrease in root shoot axis as the concentration of the extract increased when compared to control. However in root shoot axis at 5% concentration showed significant increase (25.13 mg/g F.wt.) in total protein compared to all other treatments including control. A significant increase in total protein content (30.143 mg/g F. wt.) in cotyledon was observed at 25% concentration. In aqueous extract of leaf, stem and bark the root shoot axis it is decreased from 24.07 to 9.88, 25.53 to 3.13 while the cotyledon showed an increase from 17.92 to 30.14, 12.53 to 25.13, 6.66 to 20.13 mg/g F.Wt. from 10 to 25% concentration respectively when compared to control. Effect of different concentration of neem leaf, stem and bark extract on total phenol content of root shoot axis and cotyledon were represented in the (Table-4). The total phenol content showed increase in cotyledon and decrease in root shoot axis as the concentration of the extract increased when compared to control. In aqueous extract of leaf, stem and bark in root shoot axis phenol content decreased from 4.41 to 0.56, 4.13 to 0.53, 3.63 to 0.12 and increased from 4.89 to 8.63, 1.86 to 5.55, 1.43 to 4.06 mg/g F.Wt. in the cotyledon from 5 to 25% concentration respectively when compared to control.

TABLE 1: Leaf pigments of bean seedlings treated with different concentrations of leaf, stem and bark extract of neem.

Concentration	Chlorophyll a (mg/g F.Wt)	Chlorophyll b (mg/g F.Wt)	Total Chlorophyll (mg/g F.Wt)	Carotenoid (mg/g F.Wt)
Different concentrations of neem leaf extract				
Control	1.05±0.0012[b]	2.69±0.0012[b]	2.19±0.0008[b]	0.543±0.0008[b]
5%	1.09±0.0008[a]	3.05±0.0016[a]	2.70±0.0124[a]	0.696±0.0026 [a]
10%	0.89±0.0004[c]	2.24±0.0020[c]	1.81±0.0020[c]	0.464±0.0021[c]
15%	0.68±0.0030[d]	2.06±0.0017[d]	1.67±0.0021[d]	0.222±0.0017[e]
20%	0.52±0.0020[e]	1.93±0.0044[e]	1.45±0.0017[e]	0.186±0.0012[f]
25%	0.40 ±0.0017[f]	0.99± 0.020[f]	0.69±0.0016[f]	0.375±0.3924[d]
Different concentrations of neem stem extract				
Control	0.93±0.0008[b]	2.11±0.0041[b]	1.70±0.0012[b]	0.44±0.0017[b]
5%	1.08±0.0012[a]	3.04±0.0021[a]	2.44±0.0026[a]	0.56±0.0021[a]
10%	0.69±0.0020[c]	1.88±0.0026[c]	1.44±0.0016[c]	0.33±0.0008[c]
15%	0.46± 0.0026[d]	1.52±0.0028[d]	1.32±0.0017[d]	0.28±0.0012[d]
20%	0.33±0.0017[e]	1.49±0.0032[e]	1.24±0.0032[e]	0.15±0.0024[e]

25%	0.28± 0.0033[f]	0.88±0.0026[f]	0.51±0.0004[f]	0.06±0.0262[f]
	Different concentrations of neem bark extract			
Control	0.88±0.0081[b]	1.98±0.0016[b]	1.57±0.0008[b]	0.40±0.0008[b]
5%	1.05±0.0016[a]	2.25±0.0030[a]	1.85±0.0129[a]	0.55±0.0004[a]
10%	0.65±0.0021[c]	1.67±0.0017[c]	1.31±0.0020[c]	0.36±0.0012[c]
15%	0.42±0.0020[d]	1.36±0.0085[d]	1.08±0.0012[d]	0.29±0.0017[d]
20%	0.38±0.0017[e]	0.98±0.0012[e]	0.77±0.0017[e]	0.12±0.0020[e]
25%	0.26±0.0020[f]	0.62±0.0058[f]	0.07±0.0021[f]	0.05±0.0021[f]

Mean ±SD followed by the same superscript are not statistically significant between the concentrations when subjected to SPSS package ver.14.00 according to Tukey's mean range test at 5% level.

TABLE 2: Total carbohydrate content of bean seedlings treated with different concentrations of leaf, stem and bark extract of neem.

Concentration	Root-shoot axis (mg/g F.Wt)	cotyledons (mg/g F.Wt)
	Different concentrations of neem leaf extract	
Control	41.66±0.566[b]	12.00±0.811[f]
5%	52.33±0.783[a]	33.66±0.662[e]
10%	31.00±0.054[c]	72.33±0.841[d]
15%	22.33±0.531[d]	82.66±0.677[c]
20%	20.33±0.051[e]	156.6±0.588[b]
25%	16.33±0.024[f]	184.5±0.654[a]
	Different concentrations of neem stem extract	
Control	46.00±0.884[b]	14.00±0.289[e]
5%	51.33±0.588[a]	27.00±0.153[d]
10%	42.00±0.456[c]	30.33±0.345[c]
15%	35.33±0.412[d]	76.00±0.565[b]
20%	31.98±0.388[e]	104.66±0.69[a]
25%	25.22±0.465[f]	0.699±0.893[f]
	Different concentrations of neem bark extract	
Control	43.00±0.579[b]	27.00±0.277[f]
5%	52.33±0.886[a]	45.00±0.898[e]
10%	42.00±0.573[c]	75.33±0.654[d]
15%	37.33±0.581[d]	86.00±0.554[c]
20%	36.33±0.513[e]	181.33±0.476[b]
25%	30.33±0.488[f]	251.66±0.227[a]

Mean ±SD followed by the same superscript are not statistically significant between the concentrations when subjected to SPSS package ver.14.00 according to Tukey's mean range test at 5% level.

Table 3: Total protein content of bean seedlings treated with different concentrations of leaf, stem and bark extract of neem

Concentration	Root-shoot axis (mg/g F.Wt)	cotyledons (mg/g F.Wt)
	Different concentrations of neem leaf extract	
Control	23.133±0.03[b]	33.36±0.016[a]
5%	25.496±0.009[a]	17.92±0.009[f]
10%	20.433±0.015[c]	19.54±0.008[e]
15%	18.722±0.059[d]	21.22±0.006[d]
20%	16.666±0.017[e]	25.31±0.011[c]
25%	12.253±0.014[f]	30.14±0.008[b]

Different concentrations of neem stem extract		
Control	20.066±0.013[b]	15.686±0.015[e]
5%	24.076±0.009[a]	12.536±0.166[f]
10%	18.433±0.008[c]	18.311±0.008[d]
15%	16.888±0.008[d]	20.243±0.009[c]
20%	13.344±0.017[e]	23.146±0.013[b]
25%	9.888±0.0015[f]	25.133±0.026[a]
Different concentrations of Neem bark extract		
Control	17.166±0.009[b]	5.643±0.006[f]
5%	25.533±0.125[a]	6.663±0.013[e]
10%	14.876±0.008[c]	8.322±0.015[d]
15%	11.133±0.015[d]	15.87±0.008[c]
20%	06.446±0.019[e]	19.76±0.028[b]
25%	03.133±0.05[f]	20.13±0.005[a]

Mean ±SD followed by the same superscript are not statistically significant between the concentrations when subjected to SPSS package ver.14.00 according to Tukey's mean range test at 5% level.

Table. 4: Total phenol content of bean seedlings treated with different concentrations of leaf, stem and bark extract of neem.

Concentration	Root-shoot axis (mg/g F.Wt)	cotyledons (mg/g F.Wt)
	Different concentrations of neem leaf extract	
Control	3.23±0.255[b]	4.33±0.074[f]
5%	4.41±0.345[a]	4.89±0.066[e]
10%	2.93±0.189[c]	5.11±0.167[d]
15%	1.89±0.146[d]	5.76±0.878[c]
20%	1.03±0.777[e]	6.33±0.588[b]
25%	0.56±0.127[f]	8.63±0.438[a]
	Different concentrations of neem stem extract	
Control	2.99±0.058[b]	1.56±0.121[f]
5%	4.13±0.134[a]	1.86±0.099[e]
10%	2.22±0.417[c]	2.63±0.089[d]
15%	1.73±0.813[d]	2.96±0.033[c]
20%	1.44±0.044[e]	4.66±0.065[b]
25%	0.53±0.881[f]	5.55±0.034[a]
	Different concentrations of neem bark extract	
Control	2.33±0.057[b]	1.55±0.12[e]
5%	3.63±0.122[a]	1.43±0.846[f]
10%	1.86±0.111[c]	2.33±0.098[d]
15%	1.33±0.042[d]	2.76±0.888[c]
20%	1.13±0.885[e]	3.22±0.012[b]
25%	0.12±0.123[f]	4.06±0.058[a]

Mean ±SD followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver.14.00 according to Tukey's mean range test at 5% level.

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