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***J. Nat. Prod. Plant Resour.*, 2014, 4 (1):29-33**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Herbals and biofertilizer as a nutrient supplement for improving biochemical parameters of cluster bean (*Cyamopsis tetragonoloba* L. Var. PNB)

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ABSTRACT

In India, agriculture forms main occupation of majority of the population. Organic manures such as Farm Yard Manure, green manure in the form of leaf extracts, etc. when incorporated into the soil not only add the nutrients such as nitrogen etc. but the soil is enriched by the fixation of the atmospheric nitrogen. An experiment was carried out with to assess the influence of herbals and biofertilizer on the biochemical parameters of cluster bean (Cyamopsis tetragonoloba L. Var. PNB) . The experiment consisted of six treatments . A study was conducted to assess the effect of Herbals (Vitex negundo , Curcuma longa, Acorus calamus, Ribes uva-crispa, Acalypha indica) (Ocimum basilicum and Lantana camara) and bio fertilizer (Rhizobium) . The biochemical parameters like Protein , Chlorophyll, Carbohydrates and Ascorbic acid were tested on 30^{th} , 45^{th} , 60^{th} and 75^{th} day . The Herbals and biofertilizer were used to observe the change in biochemical parameters. The biochemical parameters of cluster bean was recorded on 30^{th} ,45^{th} ,60^{th} and 75^{th} day . After sowing Chlorophyll 'a', 'b' and 'total' chlorophyll were higher in biofertilizer treatment (T_6) on all the days. The maximum protein content was shown on 30^{th} day by T_3 on 45^{th} and 60^{th} it was higher in T_5 and On 75^{th} it was higher in T_6 .The highest carbohydrates was found in T_6 on 30 and 45 DAS (79.24 mg / g and 79.95 mg / g). on 60^{th} . The highest carbohydrates content had been observed in T_5 and on 75^{th} in T_6 . The highest ascorbic acid content had been observed in T_6 on 45^{th} and 60^{th} and 75^{th}. Application of biofertilizer with herbals favoured the biochemical's of cluster bean .

Key words: Biochemical parameters, *Cyamopsis tetragonoloba*, Herbals, Rhizobium

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INTRODUCTION

Agriculture is the core sector of Indian economy and is the backbone of more than 70 per cent of the Indian population. Agriculture has the power to change the economic and social condition of a nation. The agricultural development depends on a number of factors like natural, economical and social and sub factors like climate, soil, use of advanced technology, labour factor and fertility status. To increase the production per unit of land fertility of the soil and amount of nutritive elements in the soil need to be considered. Green manure is called a cover crop is a great way to add to the nutrients to the soil. Green manuring means planting a crop that is meant to be incorporated in to the soil to increase its fertility. Green manures can be planted in the fall after the herbs have been harvested. Green manuring is a method that successfully improves soil productivity as the nutrients used in plant growth are conserved and returned to the soil to enhance its fertility. Green manures can be used to interrupt pest and disease

cycles in much the same way as crop rotation. It is also used to control root knot nematodes and root rot fungal pathogens, reducing the need to use toxic chemical for soil.

Biofertilizers are the green manure and organics. Biofertilizers are carrier-based inoculants containing cells of efficient strains of specific microorganisms (namely bacteria) used by farmers for enhancing the productivity of the soil by fixing atmospheric nitrogen or by solubilizing soil phosphate or by stimulating plant growth for synthesis of growth promoting substances. Biofertilizers play a main key role for selective adsorption of immobile (P, Zn, Cu) and mobile (C, S, Ca, K, Mn, Cl, Br, and N) elements to plants (Tinker, 1984). The rhizosphere bacteria secrete growth substances and secondary metabolic, which contribute to seed germination and plant growth (Subba Rao, 1982, 2002; Dwivedi, 1989). In recent years, free living bacteria (*Azotobacter*), associate (*Azospirillum*) and symbiotic.

Cluster bean is one of the most important legume crops in the world belonging to the family Papilionaceae. The plant is drought resistant. Young pods are eaten like string beans, or may be dried, salted or fried in oil until crisp. Mature pods cooked as a vegetable. Leaves are eaten to cure night-blindness. Green pods contain protein, fat, fiber, carbohydrate, Cadmium (Ca), Phosphorus (P), Iron (Fe), Vitamin A and Vitamin C.

The main objective of the present study was to assess the impact of biofertilizer and Herbals on the biochemical parameters of clusterbean .

MATERIALS AND METHODS

An experiment was conducted to assess the manurial value of the following botanicals (*Vitex negundo* , *Curcuma longa*, *Acorus calamus*, *Ribes uva-crispa*, *Acalypha indica*) leaves and biofertilizer (Rhizobium). The impact of soil treatment on the quality parameters of cluster bean (*Cyamopsis tetragonoloba* L. Var. PNB) was studied.

Seeds of cluster bean (*Cyamopsis tetragonoloba* L. Var. PNB) were bought from Department of Pulses, Tamil Nadu Agricultural University, Coimbatore, Tamilnadu , India. Red sandy loam soil was collected from Saravanampaty, Coimbatore, Tamilnadu , India. The experiments were conducted at the Laboratory of Botany, Avinashilingam University, Coimbatore, Tamilnadu , India.

The soil was cleaned absolutely by removing stones and other unwanted materials and was homogenized properly by mixing red soil and sand at 1 : 1 ratio and filled in pots having 7 kg capacity. Each treatment consisting of three replications was used for the experiment. The experiment was set up in completely randomized design. Pots were watered regularly. There were six treatments as given below which were evaluated against the control.

Treatment Details

T_0 - Control – Red loamy soil (7 kg)
T_1 - Red loamy soil + 35 g *Vitex negundo* leaf powder / pot
T_2 - Red loamy soil + 35 g *Curcuma longa* rhizome powder / pot
T_3 - Red loamy soil + 35 g *Acorus calamus* rhizome powder / pot
T_4 - Red loamy soil + 35 g *Ribes uva-crispa* leaf powder / pot
T_5 - Red loamy soil + 35 g *Acalypha indica* leaf powder / pot
T_6 - Red loamy soil + 10 g *Rhizobium* / pot

Biochemical Parameters assessed on 30th , 45th , 60th and 75th Days after Sowing

The plant samples were collected on 30th, 45th, 60th and 75th day after treatment and analyzed for total protein, carbohydrates, reducing sugar and chlorophylls content. The total protein was estimated by the method of Lowry *et al*. (1951), total carbohydrates by Hedge and Hofreiter (1962and Ascorbic acid by Sadasivam and Theymoli (1987) method.

Statistical Analysis

The study was in completely randomized design with three replications. The data obtained from various biochemical parameters were statistically analyzed by one way ANOVA method. Based on the results inferences were drawn. For the significant treatment differences critical differences were worked out.

RESULTS AND DISCUSSION

The experiments were conducted in Cluster bean(*Cyamopsis tetragonoloba* L. Var. PNB) with leaves (*Vitex negundo* , *Curcuma longa*, *Acorus calamus*, *Ribes uva-crispa*, *Acalypha indica*) leaves and biofertilizer (Rhizobium) on the biochemical parameters. All the parameters have been statistically analysed.

Chlorophyll Content

The maximum Chlorophyll 'a' content was higher in T_6 (biofertilizer) on 30th DAS (0.138 mg / g), 45th DAS (0.160 mg / g), 60th DAS (0.162 mg / g and 75th DAS (0.170 mg / g). The chlorophyll 'b' was also higher in T_6 (biofertilizer) on 30th DAS (0.073 mg / g), 45th DAS (0.087 mg / g), 60th DAS (0.095 mg / g) and 75th DAS (0.106 mg / g). Total chlorophyll was found to be higher on biofertilizer (T_6) on 30, 45, 60 and 75 DAS (0.182 mg / g, 0.248 mg / g, 0.256 mg / g and 0.270 mg / g respectively). (Table – I).

The minimum Content of Chlorophyll 'a', 'b' and 'total' chlorophyll were recorded in control plants (T_0). Chlorophyll 'a' on 30, 45, 60 and 75 DAS (0.032 mg / g, 0.084 mg / g, 0.089 mg /g and 0.112 mg / g respectively), chlorophyll 'b' on 30, 45, 60 and 75 DAS (0.046 mg / g, 0.073 mg / g, 0.077 mg / g and 0.089 mg /g respectively) and total chlorophyll on 30, 45, 60 and 75 DAS (0.124 mg / g, 0.160 mg / g, 0.168 mg / g and 0.204 mg / g respectively).

The observations of the present work agrees with the previous findings obtained in vegetable crop (Singh and Singh, 2005 also reported that Total chlorophyll content per plant at harvest increased significantly with increasing levels of rhizobium in cluster bean Biofertilizers influenced chlorophyll, chlorophyll 'a' and 'b' significantly and the rhizobium culture produced significantly higher values of chlorophyll 'a' and 'b'. The beneficial effects of bacteria inoculation on increased chlorophyll content might have been due to the supply of higher amounts of nitrogen to the growing tissues and organs (Singh *et al*., 1983). The comparative effect of bacterial biofertilizers such as *Rhizobium*, Phosphobacteria and *Azozpirillum* on growth and yield of green gram (*Phaseolus radiata* L.) and cowpea (*Vigna siensis* Edhl.) was studied. After 65 days of plant growth, the morphological and bio-chemical parameters of cowpea were increased in combined inoculation of *Rhizobium*, Phosphobacteria and *Azospirillum* than green gram plants (Sivakumar *et al* ., 2013)

TABLE – I influence of herbals and biofertilizer on the chlorophyll contents of cluster bean

Treatments	Chlorophyll 'a' (mg / g)				Chlorophyll 'b' (mg / g)				'Total' chlorophyll (mg / g)			
	30 DAS	45 DAS	60 DAS	75 DAS	30 DAS	45 DAS	60 DAS	75 DAS	30 DAS	45 DAS	60 DAS	75 DAS
T_0	0.032	0.084	0.089	0.112	0.046	0.073	0.077	0.089	0.124	0.160	0.168	0.204
T_1	0.123	0.146	0.148	0.154	0.053	0.085	0.092	0.097	0.173	0.231	0.242	0.253
T_2	0.107	0.124	0.133	0.143	0.061	0.082	0.092	0.102	0.148	0.208	0.227	0.247
T_3	0.041	0.085	0.153	0.160	0.065	0.075	0.094	0.104	0.139	0.161	0.249	0.265
T_4	0.130	0.147	0.151	0.159	0.071	0.084	0.093	0.102	0.177	0.232	0.245	0.264
T_5	0.118	0.120	0.129	0.140	0.069	0.083	0.092	0.100	0.179	0.204	0.223	0.243
T_6	0.138	0.160	0.162	0.170	0.073	0.087	0.095	0.106	0.182	0.248	0.256	0.270
SEd	0.00134				0.00854				0.00240			
CD (0.05)	0.00269				0.01711				0.00481			
CD (0.01)	0.00358				0.02278				0.00640			

SEd – Standard Error Deviation ; CD – Critical Difference ; DAS – Days After Sowing
The values indicate average of three replications

Protein

The results on the effect of various plant extracts and the bio fertilizer on biochemical studies of Clusterbean are presented in Table 3. The highest protein content are recorded on 30th day by T_3 as 31.47 mg / g (*Acorus calamus* rhizome powder) and on 45th and 60th DAS it was higher in T_5 (*Ribes uva-crispa*) (33.80 mg / g and 35.86 mg /g). On 75 DAS it was higher in T_6 as biofertilizer (38.52 mg / g). The control plants registered lower content of protein 20.92 mg / g (30 DAS), 24.33 mg / g (45 DAS), 27.90 mg / g (60th DAS) and 29.51 mg / g (75th DAS) (Table – II). The higher proportion of biochemical constituents due to biofertilizer inoculation might be attributed to higher foliar nutrition, uptake of N, P, K and growth of seedlings. The above results are in corroboration with the findings of Jain and Trivedi (2005) who recorded that application of 19.65 kg P ha^{-1} with rhizobium and PSB registered higher seed yield, oil yield and protein content in green gram.

Significantly more protein content was recorded in mulberry plants grown in the presence of green manure. According to Ravignanam and Gunathilagaraj (1996) the higher nutritional levels of mulberry is attributed to the increased root growth resulting in greater uptake of nutrients from soil. Sankar *et al*. (2000) observed significantly higher crude protein content in mulberry leaves by application of green manure.

According to Mohammad Hosein Bijeh keshavarzi and Seyed Mohsen Mousavi Nik (2011) the protein content, is recorded that applying biological fertilizersspecially Vermicompost has better effect than chemical fertilizers, also Vermicompost + N80P80 had better impact on protein content in comparison with other treatments.

Carbohydrates

The highest carbohydrates was found in T_6 (biofertilizer) on 30th and 45th DAS (79.24 mg / g and 79.95 mg / g). On 60th day the highest carbohydrates content had been observed in T_5 (Gooseberry) 86.36 mg / g. On 75th day it was higher in T_6 (94.93 mg / g). The decreased content was in T_0, control on 30th , 45th , 60th and 75th DAS (47.74 mg / g, 62.73 mg / g, 78.10 mg / g and 85.41 mg / g). (Table – II). Application of biofertilizers in combination with organic manure favoured higher accumulation of total carbohydrates in maize (Kamalakumari and Singaram, 1996). Increase in carbohydrate content by the application of biofertilizer may be due to availability of nutrients in higher amounts from the fertilizer. Total carbohydrates were highest in bio-dynamic compost treatment of Soy bean (Bindhu *et al* ., 2013).

TABLE – II Influence of green leaf manure and biofertilizer on the protein, carbohydrates and ascorbic acid of cluster bean

Treatments	Protein (mg / g)				Carbohydrates (mg / g)				Ascorbic Acid (mg / g)			
	30 DAS	45 DAS	60 DAS	75 DAS	30 DAS	45 DAS	60 DAS	75 DAS	30 DAS	45 DAS	60 DAS	75 DAS
T_0	20.92	24.33	27.90	29.51	47.74	62.73	78.10	85.41	26.66	40.00	173.3	213.3
T_1	26.28	29.92	31.28	31.90	57.41	67.06	80.72	87.95	38.33	53.33	186.66	220.00
T_2	29.38	30.86	32.05	34.48	52.74	65.26	81.45	88.57	27.77	46.6	226.66	246.66
T_3	31.47	32.48	33.44	34.60	51.29	68.22	81.53	86.91	33.33	46.6	220.00	246.6
T_4	23.22	25.86	29.03	30.62	62.92	63.87	82.70	86.31	28.88	53.33	186.6	233.33
T_5	25.99	33.80	35.86	37.03	66.12	74.03	86.36	90.90	33.33	60.00	206.66	246.66
T_6	28.61	31.34	35.78	38.52	79.24	79.95	87.47	94.93	40.00	80.00	246.66	266.66
SEd	3.33059				5.92479				17.27463			
CD (0.05)	6.67216				11.86911				34.60619			
CD (0.01)	8.88266				15.80137				46.07129			

SEd – Standard Error Deviation ; CD – Critical Difference ; DAS – Days After Sowing
The values indicate average of three replication

Ascorbic Acid Content

Ascorbic acid content was analysed in leaves (Table – II). The highest ascorbic acid content had been observed in T_6 (biofertilizer) on 30 DAS (40.00 mg / g), 45 DAS (80.00 mg / g), 60 DAS (246.66 mg / g) and 75 DAS (266.66 mg / g). The lowest ascorbic acid content was observed in control (T_0) on 30 DAS (26.66 mg / g), 45 DAS (40.00 mg / g), 60 DAS (173.3 mg / g) and 75 DAS (213.33 mg / g). Biofertilizer application to maize resulted in an increased ascorbic acid, crude protein, starch, carbohydrates and phenol (Kamalakumari and Singaram, 1996). Vijayakumari *et al* . (2012) also reported that the ascorbic acid was maximum in Pachagavya + humic acid + micro herbal fertilizer treated.

CONCLUSION

Organic manures such as Farm Yard Manure, green manure in the form of leaf extracts, etc. when incorporated into the soil not only add the nutrients such as nitrogen etc. but the soil is enriche by the fixation of the atmospheric nitrogen. From this investigation it is concluded that the treatment containing biofertilizer (rhizobium), *Curcuma longo* rhizome powder, *Acorus calamus* rhizome powder and *Acalypha* leaf powder could be an ideal and suitable potting mixture for better seedling and crop production in cluster bean (*Cyamopsis tetragonoloba* (L.) PNB).

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