Impact of Selenium Nanoparticles on Growth, Biochemical Characteristics and Yield of Cluster Bean *Cyamopsis tetragonoloba*

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Abstract— *The present study deals with the impact of selenium nanoparticles on growth, biochemical characteristics and yield of Cluster bean Cyamopsis tetragonoloba grown for a period 60 days Sodium selenite and ascorbic acid was utilized for the synthesis of Selenium nanoparticles using precipitation method. Selenium nanoparticles were characterized by using SEM, EDAX, FTIR and XRD. Pot culture studies of cluster bean in different quantity of Selenium nanoparticles such as 0,100, 200, 300, 400 and 500mg for treatment T_0 (Control) T_1, T_2, T_3, T_4 and T_5 and growth biochemical and yield were estimated at the end of 60 days. SEM image of selenium nanoparticles was observed as spherical in shape. EDAX spectrum recorded on purity of selenium nanoparticles. The FTIR spectrum of selenium nanoparticles was analyzed in the range of 4000-400 cm^{-1} spectral bands were observed. The germination percentage in T_0, T_1, T_2, T3, T4 and T5 are 100,90,80,90,100 and 100 respectively. Among the treatments the shoot length is higher (21.8) in T_1 containing 100mg of selenium nanoparticles and lower in(12.01) T_5 containing 500mg of nanoparticles. Root length, fresh and dry weight and leaf area were higher in T_2. The vigor index is higher T_4.The chlorophyll a, b total Chlorophyll, carotenoids, anthocyanin, protein, L-proline, free amino acids and leaf nitrate were higher inT_4.Among the treatments yield of cluster bean is higher in T_4 and lower in T_0.*

Keywords*— *Impact, Selenium, Nanoparticles, Growth, Biochemical, Yield, Cluster bean.

I. INTRODUCTION

Nanotechnology is highly promising and rapidly progressing discipline in research and influencing every field of science and biology. Nanotechnology is creating many new materials and devices with a vast range of application such as medicine, biomaterials and energy production. Exploring comprehensive application profile nanoparticles may revolutionize research in crop science and transform agriculture in to industry (1). Application of nanotechnology in agriculture delivery to plant technology also holds the promise of controlled release of agro chemicals and its targeted delivery of various macromolecules needs for improved plant disease resistance, efficient nutrient utilization and enhanced plant growth. Recent research on nanoparticles in a number of crop like corn, wheat, soybean, tomato and cucumber have provided evidence of enhanced seedling growth, germination, nitrogen metabolism, photosynthetic activity and protein level indicating their potential use for crop improvement. Among nanoparticles, Selenium is proved to be an essential mineral required for proper health, immunity, and reproductive functions of animals. Plants are the main source of this element, it is important to increase its plant growth. A new approach to fertilization of plants is the use of selenium nanomaterials (2).The study related to the impact of selenium nanoparticles on growth, biochemical characteristics and yield of vegetable crop cluster bean is totally wanting. Hence the present study was carried out.

II. MATERIALS AND METHODS

2.1. Synthesis of Selenium Nanoparticles

Precipitation method is adopted for the synthesis of selenium nanoparticle. For the synthesis 0.7Mg of (700mg) sodium selenite were dissolved in 50ml of distilled water under stirring vigorously using magnetic stirrer for 20 minutes. After stirring, the precipitation was achieved by adding 50ml of ascorbic acid solution in drop wise under constant stirring. The initial pH was observed as 3 and it was increased to pH 14 .Then precipitating process was continued until the orange colour precipitate was obtained. Then this precipitate was centrifuged at 1500 rpm for 20 minutes. The centrifugal process was continued. The obtained precipitate was dried in room temperature. Finally selenium nanoparticles were obtained.

2.2. Characterization of selenium nanoparticles

The synthesized selenium (Se) nanoparticles were characterized by SEM, EDX,FTIR and XRD.

2.3. Collection of Red Soil for Pot Culture Studies

Garden soil (red soil) was collected from the Nursery, Department of Biology, Gandhigram Rural Institute- Deemed University, Gandhigram. For the collection of red soil a trench of 25 cm depth was dug out and red soil was taken from the trench. The red soil was dried in the shade, powered using wooden mallet and sieved through a 2mm sieve before used for analysis. P^H

2.4 .Sources of Materials used in Pot Culture (Seeds and Cowdung)

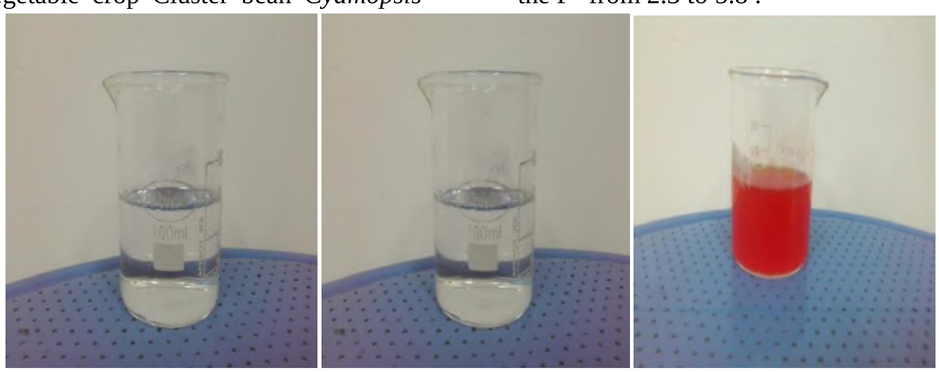
Seeds of Cluster bean were collected from Bavani store, Dindigul, Tamil Nadu, India, Cow dung was collected from School of Agriculture and Animal Science, Gandhigram Rural Institute- DeemedUniversity, Gandhigram, Tamil Nadu and India. Vegetable crop Cluster bean *Cyamopsis tetragonaloba* was selected for pot culture studies based on their easy availability, relative importance in daily diet of a common man, surviving capacity, growth capabilities and economic growth.

2.5. Pot Culture Studies:

For the pot culture studies, the seeds were soaked in ground water and kept as control. Both the control and experimental seeds were allowed to grow in plastic pots (25 cm diameter, 25 cm height) containing a mixture of red soil, cow dung manure in the ratio of 1:1 The experimental pots were supplied with different quantities of selenium nanoparticles such as 0,100,200,300,400 and 500 for treatment 1 (Control) 2, 3,4,5,6 respectively. Triplicates were maintained and grown in net house for a period of 60 days. Pots were irrigated with well water. After 60 days growth and biochemical characteristics were estimated.

III. RESULTS AND DISCUSSION

As $C_6H_8O_6$ was added to Na_2SeO_3, it is found to change colour from orange to red colour is shown in Fig.1 and this colour change indicates the synthesis of selenium nanoparticles (Se).Precipitation was observed by increasing the P^H from 2.3 to 5.8 .



a)SODIUM SELINITE ((Na_2SeO_3)   b) ASCORBIC ACID ($C_6H_8O_6$)   c) SELENIUM(Se)

Fig.1: Synthesis of Selenium Nanoparticles

The SEM image (Fig.2) showing the high density chemical by synthesized Se further confirmed the development of selenium nanostructures. Obtained nanoparticles showed spherical in nature. The microscopic image showed that the Se nanoparticles did not appear as discrete particles but form much larger dendritic flocks whose size could reached micron scale size range about 9.09mm (scale bar 5µm),9.07mm 27mm (scale bar 10µm),9.05mm (scale bar 20µm) for figure 1 a,b and c respectively. Scanning electron microscope (SEM) images were taken for the analysis of size and shape of SeNPs (Hitachi s-3400N) with resolution of 500 nm operated at 10 kV HV mode and detectors contain secondary electron; semiconductor BSE (Quad type)(3). The SEM images of selenium nanoparticles synthesized by different combinations were oval in shape with smooth surface. The particle size was found to be around 50–150 nm. Sonam Malhotra et al.,(2014)(4) suggested that properties forming a spherical shape nanoparticle having a size range of 20 to 30 nm as measured using particle size analyser, purity of the Nano selenium were further measured by the (SEM) Scanning Electron Microscope. Selenium nanoparticles were highly using

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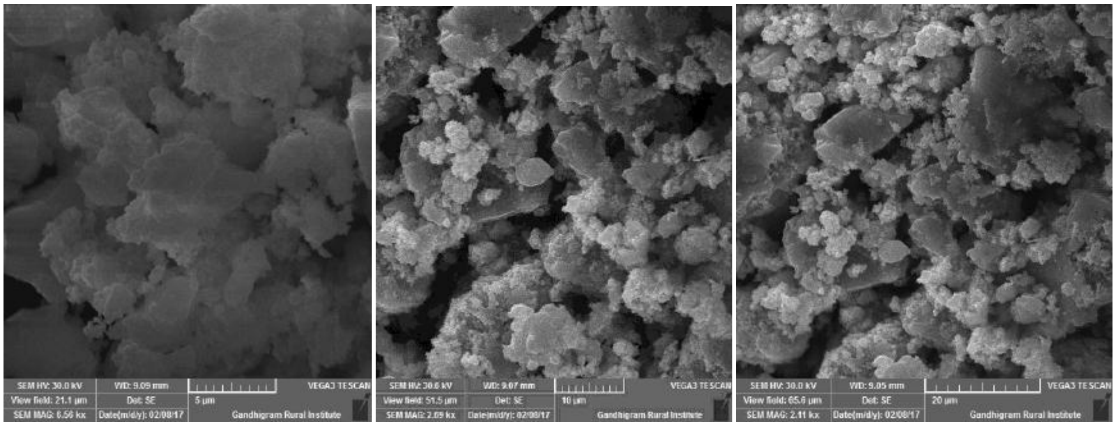
Dextrin obtained from Maize starch. Selenium nanoparticles coated varied from 5% - 20%.



a) 5µm of selenium nanoparticles *b)10µm of selenium nanoparticles* *c) 20µm of selenium nanoparticles*

Fig.2: Scanning Electron Microscopic (SEM) Image

EDAX spectrum recorded on the selenium nanoparticles is shown as two peaks located between 1.6Kev and 10.8Kev (Fig. 3),those maxima are directly related to the selenium characterized lines.The maximum peak located on the spectrum at 10.9Kev clearly coming from selenium. The second maximum peak located on the spectrum at 1.6Kev. JonneRodenburg et al.,(2014)(5) suggested that EDX profile shows a strong selenium signal along with weak sulfur group peaks. The result indicated that 92.76% (wt.) of the sample had the presence of selenium nanoparticles. The detection of the presence of sulfur 7.24% (wt.) in the EDX spectra, confirms the presence of sulfur containing protein/peptide molecules bound to the surface of the nanoparticles.

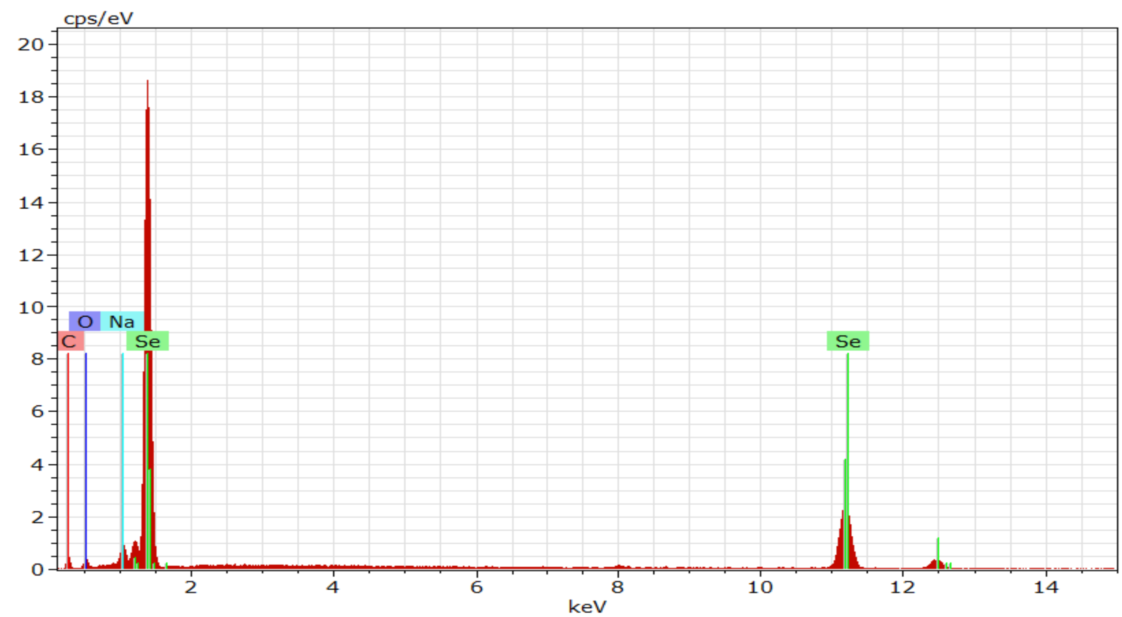


Fig.3: Energy Dispersive X-Ray Spectroscopy (EDX) Image

The XRD diffraction Peaks indexed as 22.01583,6.957353, 2.733253,0.9307381,0.3921799, 0.2205399(Fig. 4).All diffraction peaks indexed according to the hexagonal phase of selenium characteristic peaks of impurity phase except selenium are found which revealed that good crystalline in nature of the sample. The broading of the peaks in the above XRD pattern can be attributed to the small particles size of the synthesized selenium. This proves that pure selenium nanoparticles were synthesized.Similar X-ray diffraction (XRD) patterns of selenium nanoparticles was also reported(3).

http://dx.doi.org/10.22161/ijeab/2.6.19 ISSN: 2456-1878

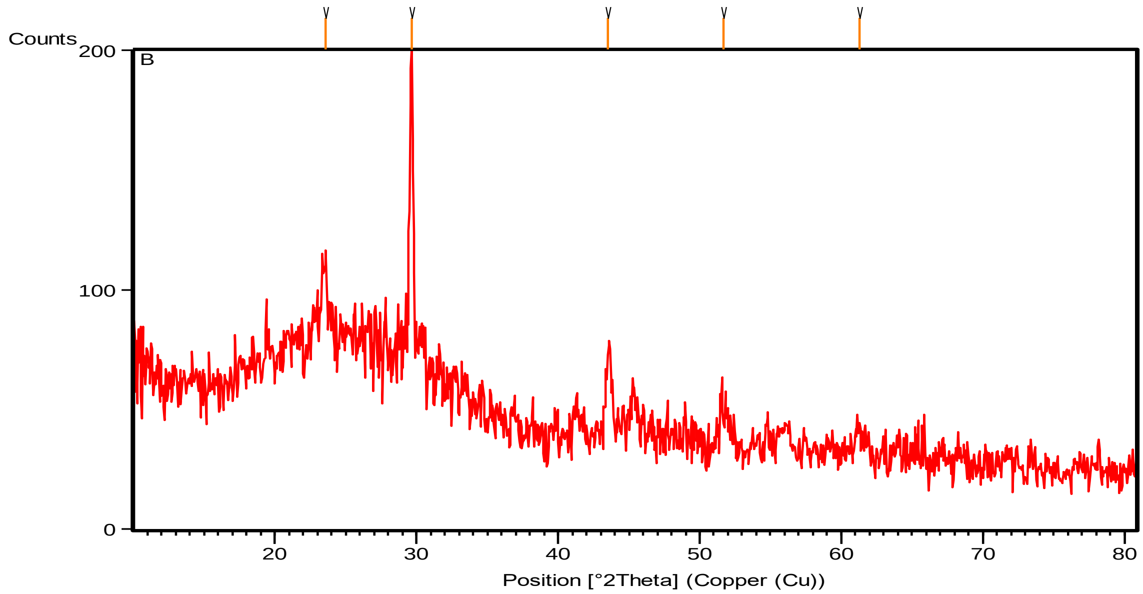


Fig.4: Analysis of Selenium Nanoparticles (XRD) Image

Fourier Transform Infrared Spectroscopy measurements were carried out to identify the possible functional group responsible for the reduction of the selenite in chemical synthesized selenium nanoparticle. The FTIR spectrum of the selenium was analyzed in the range 4000 – 400cm (Fig. 5) and bands observed at 3441, 2920, 2858, 1625, 1537, 1324,1025, 1032. Which are associated with O-H stretch, free hydroxyl-C-H stretch, H-C-H stretch=C-H asymmetric stretch=O bend, C-O stretch (Table 1). The peaks obtained were plotted as % transmit- trance in X axis and wave number (cm – 1) in Y axis. Salwa and Abbas.,(2012)(6)suggested that FTIR study was carried out to confirm the coating. In dextrin coated nanoparticles, shift in peak 1417 per cm in FTIR spectrum was observed indicating H-C-OH bond. As the concentration of Dextrin increases the shift in the peak from 1417 cm-1 to 1384 cm-1 was observed.) The FTIR analysis was performed to characterize the surface chemistry of selenium nanoparticles produced by BSA and analysis of FTIR indicated protein mediated synthesis of selenium nanoparticles, the strong absorption bands at 1649 and at 1551/ cm are characteristic of amide I and C-H vibrations of CH_2 groups of protein moiety respectively, with albumin as the stabilizing and capping agent surrounding the selenium nanoparticles.

http://dx.doi.org/10.22161/ijeab/2.6.19 ISSN: 2456-1878

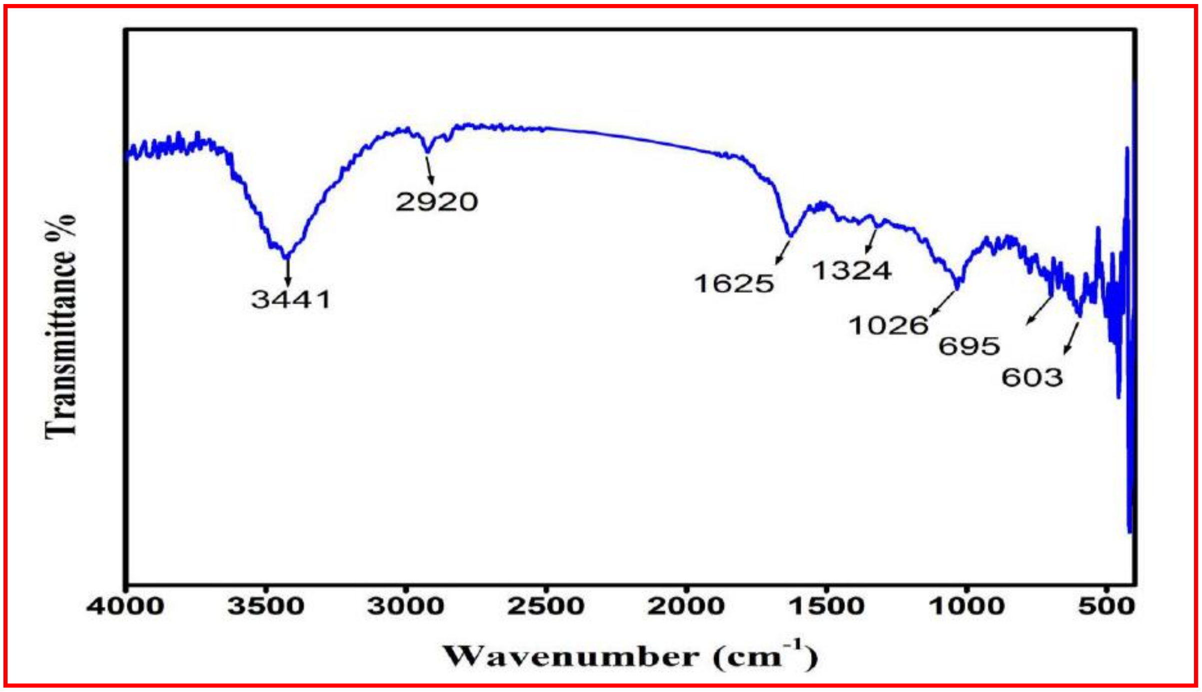


Fig.5: Fourier Transform Infrared Spectroscopy(FTIR) of Selenium Nanoparticles (XRD)Image

Table.1: FTIR Functional group representation

S.NO	WAVE NUMBER	BONDS	FUNCTIONAL GROUP
1	3441	O-H	Phenol and Alcohols
2	2920	H-C-H	Alkanes
3	2858	H-C-H	Alkanes
4	1625	C-C-=C Stretch	Aromatic rings
5	1537	N=O	Nitro group
6	1324	C-O	Esters
7	1025	C-O	Esters
8	1032	C-O	Esters

Effect of different quantities of selenium nanoparticles on growth characteristics of cluster bean is presented in table 2.The germination efficiency of cluster bean T_0,T_1, T_2T_3,T_4 and T_5 are 100,90,80,90,100 and 100 respectively. The nanoparticles and lower in T_5 (12.01) containing 500mg of nanoparticles. Similar result was also reported in Cluster bean treated with 100mg of Zinc(8). The root length of the cluster bean in control is 10.4cm. Among the treatments the root length is higher in T_2(10.6cm) and lower in T_3(7.3cm).Marisamy et al.,(2015)(9)suggested that shoot length is higher in control and lower in treatment 5(10mM).Similar root length was reported when pea nut is treated
with 100mg of Zno nanoparticles(10). The fresh weight of t he cluster bean in control is 3.18g. Sanghpriya Gautam et al germination percentage of peanut is 100% when treated with ZnO nanoparticles (7). The shoot length of cluster bean in control is 21.7cm. Among the treatments the shoot length is higher (21.8) in T_1 containing 100mg of selenium .,(2015) (11)reported that fresh and dry weight of *S. oleracea* increased at treatment T4 There was maximum increase in fresh weight (38.6 %) and dry weight (78.3 %) at T4. A reduction of fresh and dry weight was reported in *Chloroxylon* swietenia treated with sugar mill effluent for a period of 90 days(12). The leaf area is higher inT_3, T_4 (16) and lower in T_1(10.6).Vijayarengan,(2013)(8) reported that total leaf area of cluster bean plants of 20th day were found to be 45.67, 58.72, 55.41, 37.40, 34.61 and 28.10 at control, 50, 100, 150, 200 and 250. But Gokila et al (2017)(13) reported that the leaf area decreased in the

increasing quantity of zinc nanoparticles treated in Lady's finger.

Table.2: Effect of different quantities of selenium nanoparticles on growth characteristics of cluster bean.

S.No.	Parameters	Treatments					
		T_o	T_1	T_2	T_3	T_4	T_5
1.	Germination (%)	100	90	80	90	100	100
2.	Shoot Length (cm)	21.7±3.17	21.8±5.80	18.5± 0.58	14.85±4.53	13.67±2.90	12.01±2.95
3.	Root Length (cm)	10.4±0.85	10.03±1.36	10.6±0.7	7.10±1.59	9.70±1.15	8.70±0.32
4.	Fresh Weight (g)	3.18±2.43	2.08±1.64	4.3±2.09	3.30±0.80	2.50±1.75	2.03±1.75
5.	Dry Weight (g)	0.60±0.37	0.40±0.2	1.17±0.6	0.70±0.3	0.23±0.1	0.28±0.23
6.	Leaf Area (cm^2)	10.6±1.08	10.6±0.9	13.3±0.05	16.0 ±0.04	16.0 ±0.26	13.3±0.05
7.	Vigor Index	1656	1933	2448	3521	4373	2313

The chlorophyll a,b and total chlorophyll of cluster bean is presented in figure 6.The chlorophyll a is higher in T_4 and lower in T1. The chlorophyll b is higher in T_4 and lower in T_1. Total Chlorophyll is higher in T_4 and lower in T_1. Similar study was also reported when Lady's finger was treated with different quantities of zinc oxide nanoparticles (13). Se treatment at the lower concentration (16µM) recorded the highest values of chlorophyll a concentration (2.68 and 1.99 mg/ g FW). Vijayarengan(2013) (8)reported that chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid content of cluster bean leaves increased at lower concentration. An increase in chlorophyll a and chlorophyll b contents of wheat seedlings may be attributed to selenium effect on protection of chloroplast enzymes and thus increasing the biosynthesis of photosynthetic pigments(6). High concentration of selenium induced reduction in photosynthetic pigments content. Marisamy et al.,(2015)(9)suggested that the chlotophyll a, and b are higher in control and lower in treatment 5(10mM) . Sanghpriya Gautam et al.,(2015)(11) reported that pigment contents (chlorophyll a, b) were increased maximum up to T3, whereas decreased at T4.

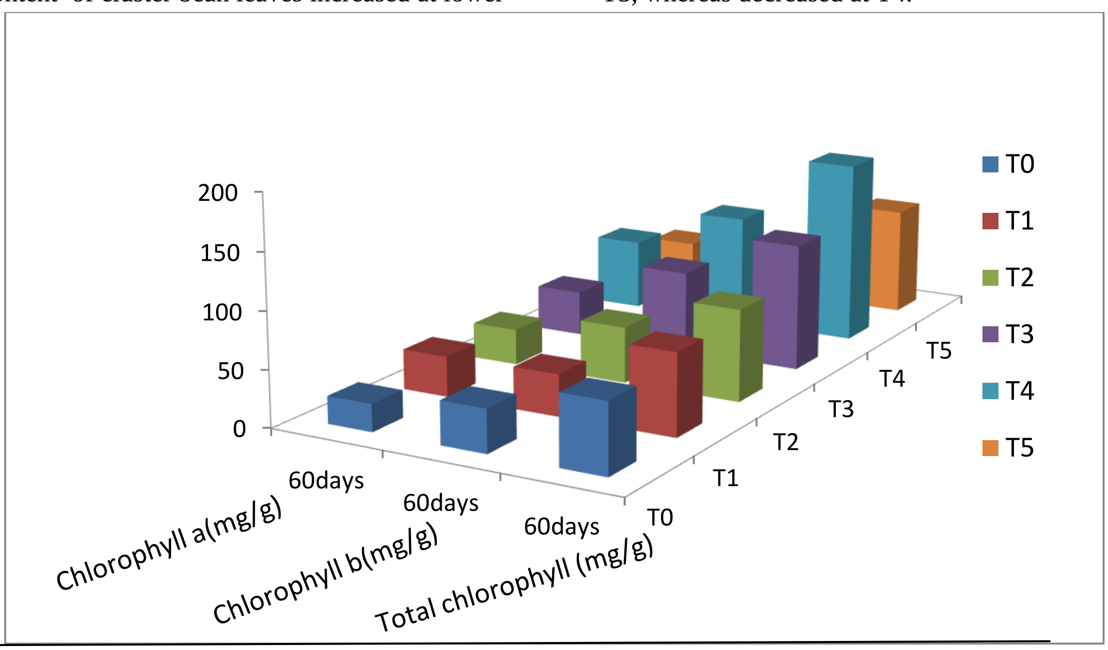


Fig.6: Chlorophyll a,b and Total Chlorophyll of Cluster bean

The carotenoids and anthocyanin of Cluster bean is presented in Figure 7. The carotenoids are higher in T_4 and lower in T_2. Salwa and Abbas.,(2012)(6) suggested that the selenium increased the contents of carotenoids and chlorophyll *a*,and in turn change in the photosynthetic pigments level is likely to have been connected with different effects of the selenium ions on the oxidation-reduction status of leaves. The carotenoids content of *Amaranthus caudatus* is higher in control and lower in treatment 5(10mM) when treated with barium(9). The

http://dx.doi.org/10.22161/ijeab/2.6.19 *ISSN: 2456-1878*

anthocyanin is higher in T_4 and lower in T_2. Anthocyanin is a pigment to protect chlorophylls from photo oxidation, compared with the other components and high concentration of sodium selenate (12 mg per litter) reduced their contents(6). The results demonstrated that Selenium supply could increase anthocyanin content of seedlings. Marisamy et al.,(2015)(9)reported that anthocyanin is higher in control and lower in treatment 5(10mM) when Amaranthus caudatus was treated with nanoparticles.

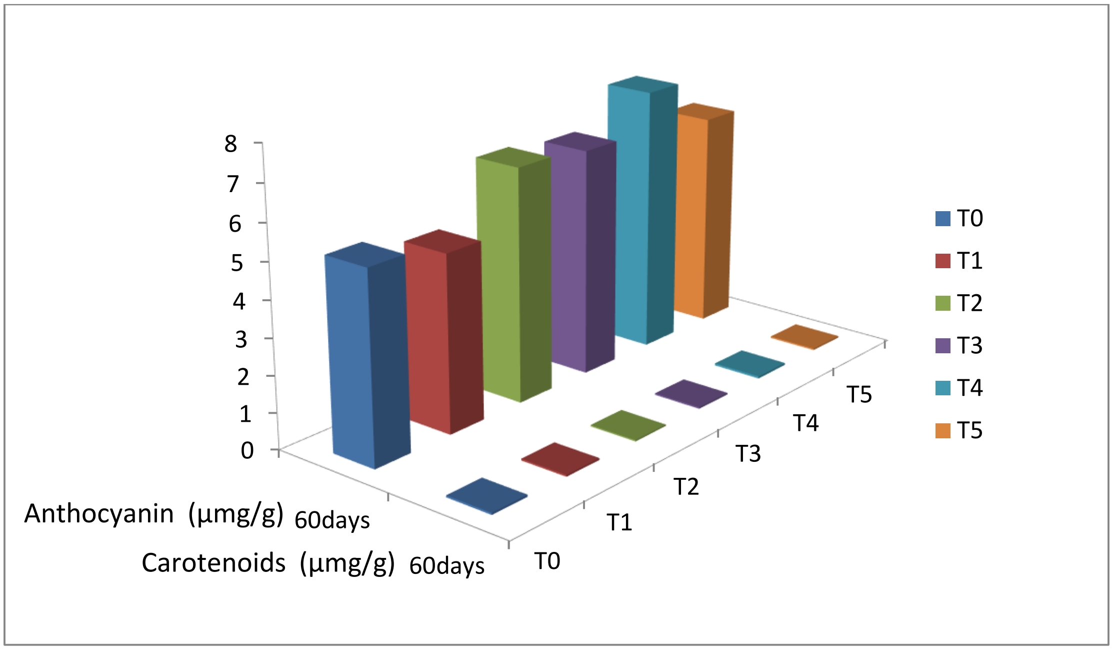


Fig.7: Anthocyanin and Carotenoids of Cluster bean

The protein and L-proline of the cluster bean is presented in Figure 8.The protein content is higher in T_4(5.5mg/g) lower in T_5(3.4mg/g). Vijayarengan (2013)(8) reported that the protein content of leaves was maximum at 50 mg of zinc treated with cluster bean. Suresh kumar and Total soluble protein content was found to be 35 % in 90 days at 50% treatment when compared to control in sugarcane effluent(12). The L-proline is higher in T_4(5.2mg/g) and lower in T_1 (4.1mg/g). Proline concentration was significantly increased in Lady's finger treated with Zinc oxide nanoparticles when compared to untreated ones(14). Also reported that L-proline accumulates in the leaves of many tree species when subjected to stress(12).

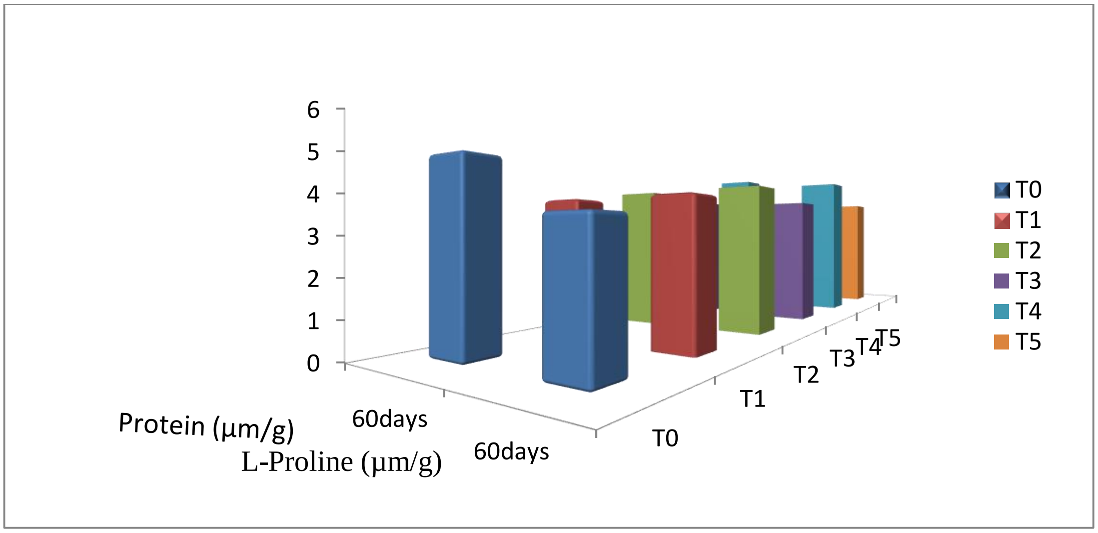


Fig.8: Protein and L-proline of Cluster bean

The free amino acid and leaf nitrate of the cluster bean is presented in Figure 9.The free amino acids are higher in T4 (6.8mg/g) lower in T_2(5.3mg/g).). The free amino acids content in *Helianthus annuus* significantly increased with increasing concentration of barium(9). Suresh Kumar and Mariappan.,(2013)(12) reported a reduction in soluble protein level eventually leads to increase in free amino acids content. The leaf nitrate is higher in T4(5.9mg/g) and lower in T_1(4.12mg/g). Marisamy et al.,(2015)(9)suggested that leaf nitrate content increased in control when compared to barium treatment. Suresh Kumar and Mariappan (2013) (12) reported that leaf nitrate accumulated in all the effluent treated tree species.

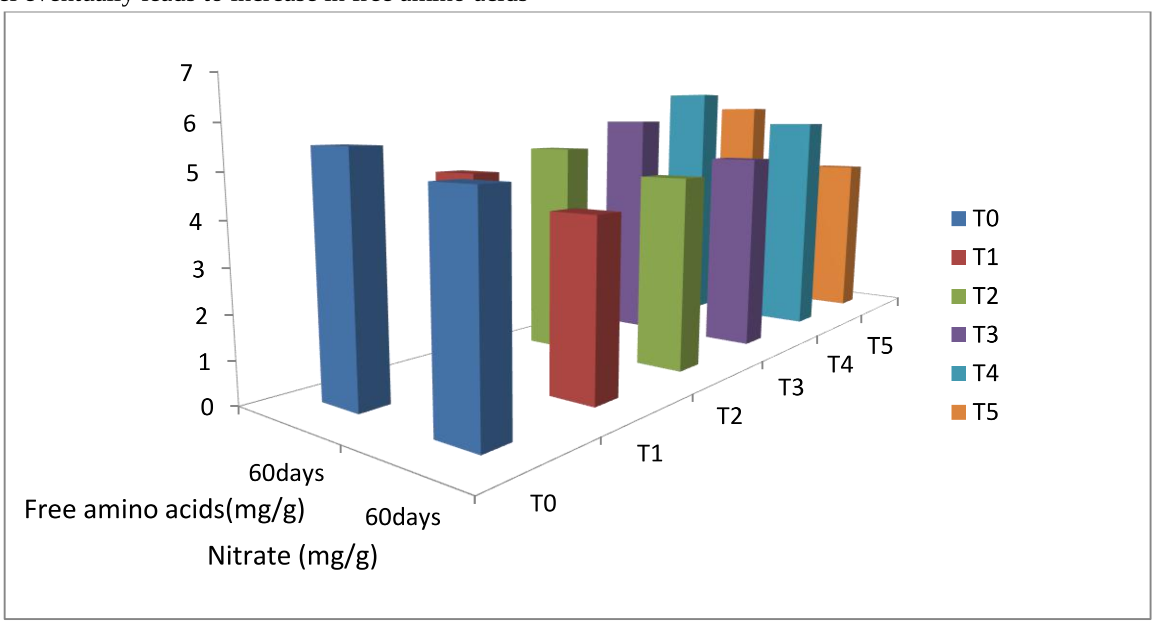


Fig.9: Free Amino acids and Nitrate of Cluster bean

Yield performance of cluster bean is presented in figure Table 3.Yield performance of cluster bean is higher in T_4(1.41g) and lower in T_2(0.69g). Sharma and Kansal(1984)(14) suggested that the yield parameters such as length, weight and number of the cluster bean showed increase over control up to 800mg and decrease gradually in the further higher concentrations. Manal et al.,(2014)(15) reported that imposition of drought stress reduced plant height, number of tillers, 1000-grain weight and grain yield of both rice cultivars. The treatment with either Se has a favorable effect on1000-grain weight which up to 9.5% and 5.2%.

Table.3: Yield performance of cluster bean

Treatment	Length(cm)	Weight(g)	Number
T0	13.11±5.39	5.4±1.03	8.0±1.00
T1	32.1±10.95	1.9±1.09	6.0±0.00
T2	29.1±5.58	7.3±0.65	10.0±1.00
T3	21.95±5.48	8.04±0.69	8±1.00
T4	28.37±6.34	2.45±1.41	15.0±1.0
T5	20.71±2.34	0.25±2.60	5.0±1.0

IV. CONCLUSION

The present study concluded that 400 mg of selenium nanoparticles influences the growth, biochemical characteristics and yield of Cluster bean.

V. ACKNOWLEDGEMENTS

Authors thank the Department of Biology, School of Sciences, **T**he Gandhigram Rural Institute
(Deemed to be University), Gandhigram-624302, Dindigul District, Tamilnadu, India for offering facilities to carry out the present study.

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