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Research Article

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IMPACT OF SELENIUM NANOPARTICLES ON GROWTH BIOCHEMICAL CHARACTERISTICS AND YIELD OF BLACK GARAM VIGNA MUNGO

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ABSTRACT

The present study aimed at the impact of selenium nanoparticles on the growth, biochemical characteristics and yield of Black gram Vigna mungo. Selenium nanoparticles were synthesized using the precipitation method and characterized by UV-VIS, SEM, EDAX and FT-IR. Physico-chemical parameters of soil and cow dung were estimated. Pot culture studies of black gram in different quantity of selenium nanoparticles such as 10,20,30,40 and 50mg for treatments such as T0(Control) T1,T2,T3,T4, and T5 respectively were conducted for 60 days. Growth characteristics such as seed germination efficiency (%), shoot length, root length, total fresh weight, total dry weight, leaf area index, and vigor index (%), biochemical characteristics such as chlorophyll a, b and total chlorophyll, anthocyanin, carotenoids, total protein, total soluble sugars, carotenoids, L- proline were measured after 60 days. Selenium nanoparticles were analyzed between 200-800nm wavelengths in UV-Vis Spectrophotometer. SEM image of selenium nanoparticles was observed as spherical in shape. EDAX spectrum recorded on purity of selenium nanoparticles. The FT-IR spectrum of selenium nanoparticles was analyzed in the range of 4000 - 400cm-1 spectral bands. Among the treatments the germination percentage, shoot length, root length, fresh and dry weight, leaf area and vigor index were higher in T3 containing 30mg of selenium nanoparticles. The chlorophyll a, b and total chlorophyll, total protein, total soluble sugar and L-proline were higher in T3. Number and weight of black gram were higher in T3.

Keywords: Impact, selenium nanoparticles, growth, biochemical, yield, black gram.

INTRODUCTION

Copper, gold, zinc, silver and iron are the most commonly used nanoparticles that have broad applications. Among various nanoparticles, selenium plays a vital role in the field of nanotechnology. Although much research has been done on metals, studies on metalloids, such as selenium, etc., are scanty [1]. Due to great potential in biomedical sciences, electronics, agriculture, and food safety, specific novel nanomaterials have gained much attention in recent years. Selenium nanoparticles emerge as promising nanomaterial that can potentially be used for a wide range of applications. It is an essential trace element for many organisms, humans and animals involving multiple biological functions, such as enhancing immunization, antioxidation, and detoxification of heavy metals [2]. Notably, a trace element of selenium is essential for proper cellular and metabolic functioning in animals and plants. Selenium in the plants triggers a variety of beneficial effects. Plants absorb Se mainly in the form of selenite using high-affinity root sulphate transporters. Therefore Se, at high doses may interfere with N- assimilation, causing a decrease in the level of N-compounds with structural and regulatory functions [3]. Selenium is biologically important because it is essential in plant metabolism. The work related to the impact of selenium nanoparticles on growth, biochemical characteristics and yield of Black gram Vigna mungo is totally wanting. Hence the present study was carried out.

MATERIALS AND METHODS

Synthesis of Selenium nanoparticles

The precipitation method is adopted for the synthesis of selenium nanoparticles. For the synthesis, 0.86g of sodium selenite were dissolved in 50ml of distilled water under stirring vigoursely using a magnetic stirrer for 20 minutes. After stirring, the precipitation achieved by adding 50ml of ascorbic acid solution dropwise under constant stirring. The initial level of pH in sodium selenite is 7 and ascorbic acid is 1 and it was increased to pH 14. Then the precipitation process was continued and the endpoint the color changed into orange color precipitation. Then this precipitate was centrifuged at 1500 rpm for 20 minutes. The centrifugation process was continued collected the pellet and dried at room temperature. Finally, selenium nanoparticles were obtained.

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Characterization of Selenium Nanoparticles:

The synthesized selenium nanoparticles were characterized by the following techniques.

UV- Visible spectroscopy

The formation of Se nanoparticles was initially confirmed visually by using UV-Visible spectroscopy technique, which has been frequently used to characterize the synthesized nanoparticles.

Scanning Electron Microscope (SEM) analysis:

The morphology and composition of the selenium nanoparticles were examined by Scanning Electron Microscope (SEM) HITACHI SU6600 equipped with energy dispersive.

Energy Dispersive X-Ray Spectroscopy (EDAX):

A minute drop of nanoparticles solution was caste on aluminum foil and subsequently dried in air before transferring it to the microscope. An energy X-ray detection instrument (EDAX) (Brucker, USA) was used to examine the electrical composition of the sample.

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectroscopy was analyzed in the range of 4000-400 cm-1. The FT-IR spectra of synthesized selenium nanoparticles were analyzed for knowing the possible functional groups. The measurement was carried out by JASCO (FT-IR-6200) spectrum.

Collection of red soil for pot culture studies:

Garden soil (Red soil) was collected from the Nursery, Department of Biology, The Gandhigram Rural Institute- Deemed to be a 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, powdered using a wooden mallet and sieved through a 2mm sieve before used for analysis. Cow dung was collected from the Dairy Farm of the Institute. Details of the analytical techniques employed for red soil and cow dung used in pot culture studies are indicated in table 1.

Table 1:Analytical techniques used in red soil and cow dung:

Parameters	Analytic method	Reference
pH	pH Meter	APHA,2012
Electrical conductivity Potassium	Conductivity Bridge	,,
Nitrogen	Flame Photo Meter	,,
Phosphorous	Micro-Kjeldhal Flame Photo Meter	,, ,,

Sources of materials used in pot culture (seeds)

Seeds of black gram were collected from Bavani store, Dindigul, Tamil Nadu, India. Black gram *Vigna mungo* was selected for pot culture studies based on their easy availability, relative importance in the daily diet of the common man, surviving capacity, growth capabilities and economic growth.

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 0,10,20,30,40, and 50 mg for treatment 0 (control), 1, 2, 3, 4, 5 respectively. Triplicates were maintained and grown in the net house for a period of 60 days. Pots were irrigated with well water. Growth parameters (germination percentage, shoot and root length, fresh and dry weight, leaf area and vigour index), biochemical characteristics (Chlorophyll a, b, total chlorophyll, carotenoid, anthocyanin, total soluble sugar and protein, and L-proline and yield parameters (number and weight) were estimated after 60 days. The procedure followed for growth and biochemical characteristics are presented in Table 2.

Table 2: Procedures followed for growth and biochemical characteristics of black gram

S.No	Parameters		References
1.	Germination	%	Carly and Watson(1968)[4]
2.	Shootlength	cm	Arts and Marks, (1971) [5]
3.	Rootlength	,,	Burris et al., (1969)[6]
4.	Totalfreshweight	g	,,
5.	Totaldryweight	,,	,,
6.	Leafarea	cm²	Ford Denison and Raymond Russotti(1997)[7]
7.	Vigorindex	%	Abdul Baki and Anderson,(1973)[8]
8.	Chlorophylla	mg/gfw	Arnon,(1949)[9]
9.	Chlorophyllb	,,	,,
10.	Totalchlorophyll	,,	,,
11.	Carotenoids	μ mole gwf	,,
12.	Anthocyanin	,,	Mancinelli et al., (1975) [10]
14.	Total solublesugar	,,	Jeyaraman,(1981) [11]
15.	Total protein	,,	Lowry et al.,(1951)[12]
16.	L-Proline	,,	Bates et al.,(1973)[13]
17.	Freeaminoacids	mg/g fw	Moore and Stein,(1948)[14]
18.	Leafnitrate	,,	Cataldoet al.,(1978)[15]

RESULTS AND DISCUSSION

The chemical synthesis of selenium nanoparticles is subjected to UV-Visible absorption spectroscopic analysis and from 200 to 800nm (Fig.1). Also reported that the characteristic peak was acquired between 200 and 300 nm[16]. Maximum absorption peak was obtained at 274nm with another peak at 212 nm which indicates the surface Plasmon resonance (SPR) forSeNPs. The peak at 212 nm can be attributed to the smaller size of SeNPs and the decline in absorption peak suggests the aggregation of synthesized nanoparticles. Sheena et al., (2017) [17] suggested UV-vis spectroscopy for the determination of selenium nanoparticles. The UV-Visible spectra of the aqueous samples of different concentrations were recorded in a Perkin Elmer Lambda 25 double beam UV-Vis spectrophotometer from 200 to 800nm[18].

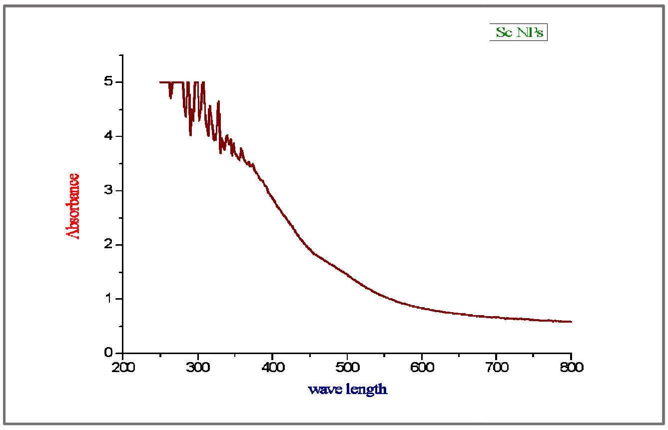

Fig. 1: UV-Visible Spectroscopy of Selenium nanoparticles

The SEM image shows the high density chemically synthesized Se NPs and further confirmed the development of selenium nanostructures. Obtained nanoparticles showed spherical in nature. The microscope image showed that the Se nanoparticles did not appear as discrete particles but form much larger dendritic flocks whose size could reach a micron-scale size range of about 14.9mm (scale bar 20µm) (Fig.2). SEM images of nano selenium where in 1hr the morphology of selenium particles is spherical agglomerated [19]. The SEM image of Se NPS is spherical in shape with an average size of 130nm[20]. The Electron microscopy studies showed that SeNPs exhibited good hexagonal shape with a clean and smooth surface and revealed a very narrow size distribution ranging from 100-200 nm [16].

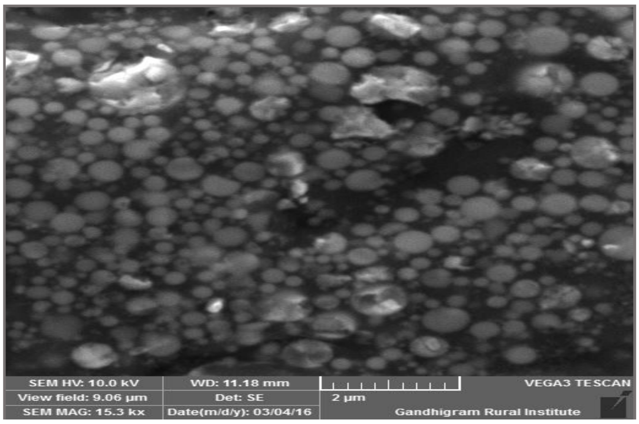

Fig.2 SEM image of Selenium Nanoparticles

EDAX spectrum recorded on the selenium nanoparticles is shown as two peaks located between 1.6Kev and 2Kev; those maxima are directly related to the selenium characterized lines. The maxima peak located on the spectrum at 1.4Kev clearly coming from selenium (Fig.3). Also reported three peaks from the energy-dispersive X-ray analysis, a strong signal from the Se atom (50.79%) together with O atom (35.55%) and C atom 13.66% [21].

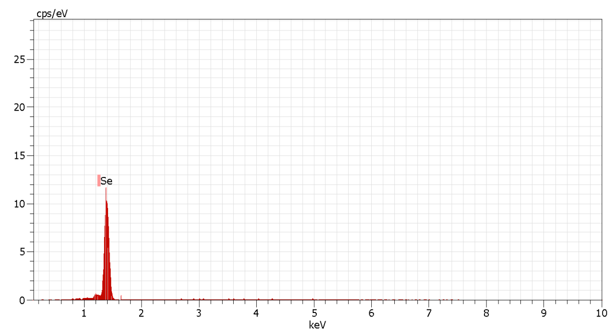

Fig. 3: Energy Dispersive X-RAY Spectroscopy (EDAX) Image

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 FT-IR spectrum of the selenium was analyzed in the range 4000-500 cm (Fig.4) and bands observed at 3992.89,3434.59,2999.73,2049.96,1632.44,1481.06,1324.85,788.74, 588.04,513.93 which are associated with O-H stretch, free hydroxyl-C-H stretch, H-C-H stretch-C-H asymmetric stretch O bond, C-O stretch, C-N stretch. The FTIR analysis was used to characterize the presence of functional groups responsible for the synthesis and stability of selenium nanoparticles [16]. The intensive absorption peak at 3623 cm-1 was assigned to a hydroxyl group (-OH) stretching of the aromatic ring and a sharp peak at 2923 cm-1 showing ether – methoxy-OCH_3 groups, while the peak at 2852 and 1740 cm^{-1} represents the stretching of C=O aldehydes group, 1602 cm-1 (amide and CH vibrations of CH_2 group), 1457 cm^{-1} (CH group), 1261 cm^{-1} (Secondary -OH bending), 1114 and 805cm^{-1} (C=O stretching vibrations, aromatic carbon vibrations and CH in-plane benching). The vibrational and stretching of functional groups at wave numbers 2,356.2, 1,618.7, 1,284.7, 2,360.1, 1,723.2 and were correspond to stretching and vibrational bending of C=C, NH_2, COOH, CH_2 and C=O, thus indicating the presence of reducing groups responsible for the reduction of nano selenium [22]. Also observed multiple bands of aromatic (C=C) bands from 1400 to 1200 cm-1 having medium stretch vibrations [23].

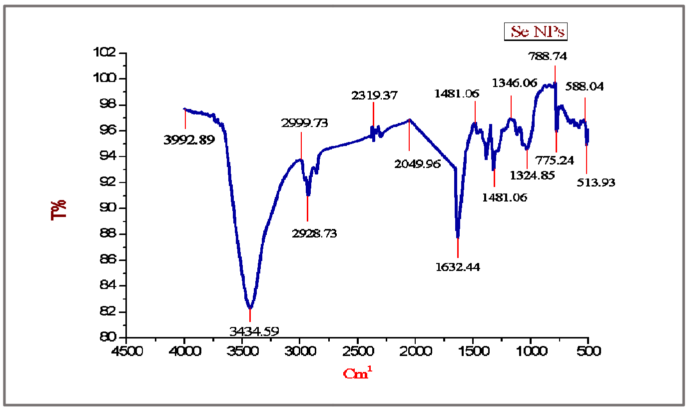

Fig.4: Fourier Transform Infrared Spectroscopy (FT-IR) Image

Physico-chemical parameters of red soil and cow dung used in pot culture studies of is presented in table 3. The impact of selenium nanoparticles on growth parameters of Black gram *Vigna mungo* is presented in table 4. The germination efficiency of Black gram T0, T1, T2, T3, T4 and T5 are 98,90,86,100,90 and 70% respectively. 100% germination was reported in pea plant nut treated with zinc oxide nanoparticles [24]. Among the treatments, the shoot length is higher (26.5cm) in T3 containing 30mg of selenium nanoparticles and lower in (19cm) T5 containing 50mg of nanoparticles. The shoot length of Mung and Gram increased in a lower concentration of nano ZnO particles and higher concentration of ZnO nanoparticles decreased the shoot length [25]. Also reported that shoot length is higher in control and lower in treatment 5(10mM) [26]. The root length of the Black gram in control is 9cm. Among the treatments, the root lengths are higher (10.5cm) in T3 and lower in T5 (5.5cm). A similar root length was also reported when peanut is treated with 100mg of zinc oxide nanoparticles [27]. The fresh weight of the Black gram in control is 0.96g. Among the treatments the fresh weight is higher in T3 (3.38g) and lower in T5 (1.39g). The dry weight of the black gram is higher in T3 (2.57g) and lower in T0 (0.52g). A reduction of fresh and dry weight was reported in the case of the *Chloroxylon swietenia* treated with sugar mill effluent for 90 days[28]. The leaf area in T3 (5.3) and lower in T0 (3.43). The leaf area of cluster bean plants of the 20th day was found to be 45.67, 58.72, 55.41, 37.40, 34.61 and

28.10 at control 50, 100, 150, 200 and 250[29]. The leaf area of Cluster bean is higher in T3 containing 200mg of selenium nanoparticles [30]. The vigour index of cluster bean in control is 126. Among the treatmentsvigour index is higher in T3 (137) and lower in T5 (91.5). A vigour index of 1701.3 was reported in peanut treated with ZnO nanoparticles [31].

Table 3: Physico - chemical parameters of red soil and Cow dung

Parameters	Red Soil	Cow dung
pH	6.7	6.4
Electrical conductivity(ds/m)	0.59	5.43
Organic carbon (%)	42.3	32.8
Total nitrogen (%)	3.8	11.5
Total potassium (%)	0.26	0.96
Phosphorous (%)	0.172	0.544

Table 4: Impact of Selenium nanoparticles on Growth parameters of Black gram *Vigna mungo* grown for period of 60days. Each value is the average of 10 individual observations (Average ±SD)

	Treatments					
Parameters	T_0	T_1	T_2	T_3	T_4	T_5
Germination Efficiency (%)	98±2	90±4	86±2	100±0	90±2	70±4
Shoot length (cm)	19±6.41	23.5±7.90	17.7±5.90	26.5±8.93	23.5±7.85	16±5.39
Root length (cm)	9±3.00	8.6±2.90	7.6±2.55	10.5±2.52	6.7±2.26	5.5±1.96
Fresh weight (g)	0.96±0.28	1.88±0.19	1.94±0.77	3.38±0.90	1.5±0.08	1.39±0.92
Dry weight (g)	0.52±0.22	1.33±0.11	1.68±1.02	2.57±0.54	1.77±0.20	1.27±0.60
Leaf area	3.43±0.55	3.43±0.51	2.7±0.68	5.3±2.92	3.2±0.26	3.6±0.15
Vigor index	126	122.1	102	137	120.2	91.5

The chlorophyll a, chlorophyll b and total chlorophyll is presented in fig 5. The chlorophyll a is higher in T3. Chlorophyll a, chlorophyll b and total chlorophyll and carotenoid content of cluster bean leaves increased at lower concentration of chitosan-PMAA-NPK nano fertilizer [32]. An increase in chlorophyll a, chlorophyll b and total chlorophyll contents of wheat seedling may be attributed to the selenium effect on the protection of chloroplast enzymes and thus increasing the biosynthesis of photosynthetic pigments[33]. High concentration of selenium induced reduction in photosynthetic pigments content. Also reported that the chlorophyll a, chlorophyll b and total chlorophyll are higher in control and lower in treatment 5 with barium(10mM)[26]. The carotenoids and anthocyanin are higher in T3(Fig.6). Salwaand Abbas (2012)[33] reported that the selenium increased the contents of carotenoids and chlorophyll a and in turn change in the level of the photosynthetic pigment is likely to have been connected with different effects of the selenium ion on the oxidation-reduction status of leaves. The anthocyanin can protect chlorophylls from photo-oxidation, compared with the other components and high concentration of sodium selenite (12mg per litter). The results demonstrated that selenium supply could increase the anthocyanin content of seedlings. The L-proline is higher in T3 (2.42mg/g) and lower in T0 (1.800mg/g). The total protein is higher in T3 (4.371mg/g) and lower in T1 (3.429mg/g) (Fig.7). The total soluble sugar is higher in T3 (0.775mg/g) and lower in T0 (0.435mg/g)(Fig.8). A similar result was reported when different quantities of selenium nanoparticles treated Cluster bean[30]. Yield parameters of Black gram *Vigna mungo* are presented in table 5. The weight and number of black grams were higher in T3. The application of 30mg of Cu-NPs in the soil increased the yield of the wheat crop significantly [34].

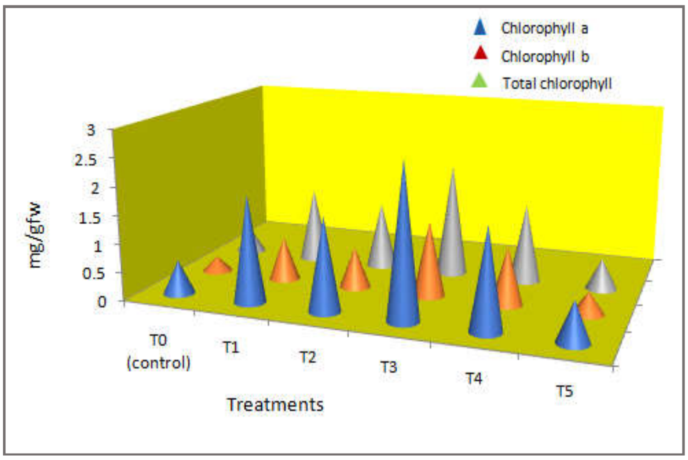

Fig. 5: Chlorophyll a, Chlorophyll b and Total Chlorophyll of Black gram *Vigna mungo*

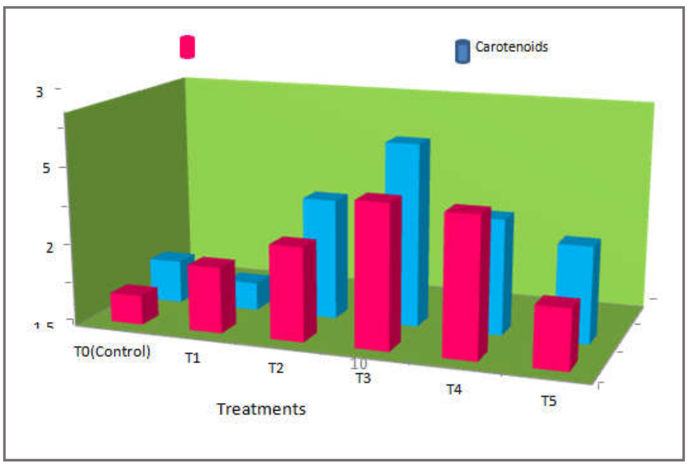

Fig. 6: Anthocyanin and Carotenoids of Black gram *Vigna mungo*

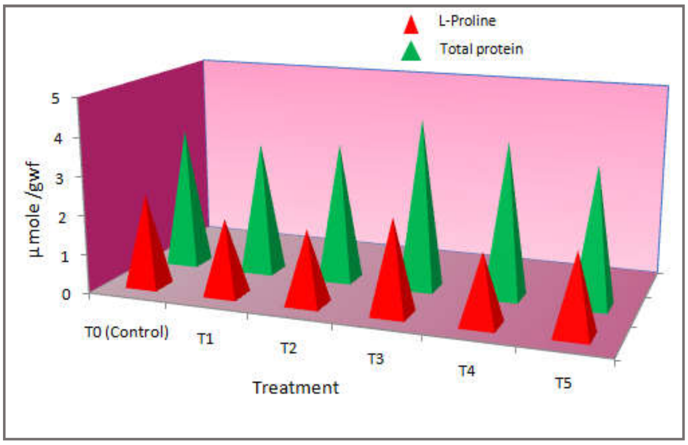

Fig.7: L-proline and Total Protein of Black gram *Vigna mungo*

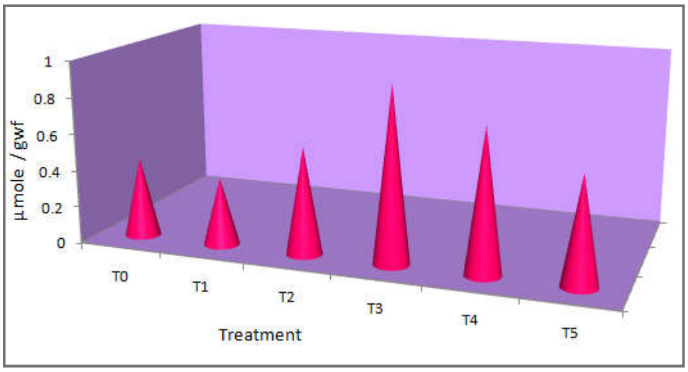

Fig.8: Total soluble sugar of Black gram *Vigna mungo*

CONCLUSION:

The present study successfully demonstrates that 30mg of selenium nanoparticles are suitable for the growth, biochemical characteristics and yield of Black gram *Vigna mungo.*

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