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Effect of different quantities of CuO nanoparticles on growth, biochemical characteristics and yield of lady's finger *Abelmoschus esculentus*

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Abstract

The present study aimed at the effect of different quantities of copper oxide nanoparticles on the growth, biochemical characteristics and yield of Lady's finger *Abelmoschus esculentus*. Copper oxide nanoparticles were synthesized and characterized by using UV-Vis spectroscopy, SEM, EDAX, and FTIR. Pot culture studies on different quantities of copper nanoparticles such as 50, 100, 150,200 and 250mg for treatments T_0 (control) T_1, T_2, T_3, T_4, T_5 respectively on growth, biochemical characteristics and yield of Lady's finger *Abelmoschus esculentus* were estimated after 45 days. The UV-Visible absorption spectrum of copper oxide nanoparticles was confirmed from the peak between 300 and 600nm.SEM image of CuO nanoparticles was spherical. EDAX spectrum shows three peaks located between 0.30KeV and 9.0 KeV. The FTIR spectrum was analyzed in the range of 4000-500 cm^{-1} spectral bands. Growth, biochemical characteristics and yield of Lady's finger, *Abelmoschus esculentus* were higher in T_3 containing 150 mg of copper oxide nanoparticles.

Keywords: Different quantity; Copper oxide nanoparticles; Growth; Biochemical; Yield; Lady's finger

1. Introduction

Nanoparticles have been extensively studied over the last decade due to their characteristics of physical, chemical, electrical, mechanical, magnetic, thermal, dielectric, optical and biological properties. Nanoparticles may be considered as building blocks of the many industrial sectors, in particular, NPs are also receiving increasing attention in a large variety of applications in medical science. Metal oxide NPs are of great interest because of their efficiency as nanofluids in heat transfer applications [1]. Among the different types of metallic NPs, copper oxide nanoparticles have been mainly used as preliminary material due to their natural abundance, low-cost production, and non-toxic nature with good electrical and optical properties. Copper is a microelement required for the development of plants[2]. The Cu deficiency in plants is expressed as curled leaves, petioles bent downwards and light chlorosis as well as permanent turgor loss in the young leaves while the higher concentration of Cu leads to toxicity, growth inhibition, photosynthesis interferences, photorespiration and increases oxidative stress[3]. The effect of Cu NPs on plant cells has not been studied sufficiently and available results are uncertain. The plants grown in contaminated soil in the natural environment can transform copper into metallic nanoparticles in and near their roots, with the assistance of endomycorrhizal fungi[4]. The work related to the synthesis, characterization and effect of different quantities of CuO NPs on growth, biochemical characterization and yield of Lady's finger is totally wanting. Hence the present study was carried out.

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2. Material and methods

2.1. Materials

All the chemicals used in the experiment were analytical grade. Copper sulphate pentahydrate (0.1M), starch, ascorbic acid (0.2M) and sodium hydroxide NaOH (1M) were collected from the Dept. Laboratory. The viable seeds of Lady's finger were purchased from the Bavani store, Dindigul, Tamil Nadu, India. Cow dung was collected from the School of Agriculture and Animal Science, The Gandhigram Rural Institute-Deemed to be University, Gandhigram, Tamil Nadu, India.

2.2. Methods

2.2.1. Synthesis of copper oxide nanoparticles

The Cuo nanoparticles were synthesized by the chemical reduction process using copper sulphate pentahydrate as precursor salt with a starch capping agent. The preparation method starts with the addition of 0.1M copper sulphate pentahydrate solution into 120 ml of starch (1.2%) solution with vigorous stirring for 30 min. In the second step 50 ml of 0.2 M, an ascorbic acid solution is added under continuous rapid stirring. Subsequently, 30 ml of 1 M sodium hydroxide solution was slowly added to the prepared solution with constant stirring and heating at 80^0 C for 2h. The colour of the solution turned yellow to ocher. After the completion of the reaction, the solution was taken from the heat and allowed to settle overnight and the supernatant solution was discarded. The precipitate was separated from the solution by filtration and washed with deionized water and ethanol for three times to take out the excessive starch bound with nanoparticles ocher colour precipitates obtained and dried at room temperature. After drying nanoparticles were obtained and stored in a glass vial for further studies.

2.2.2. Characterization of copper oxide nanoparticles:

The synthesized copper oxide nanoparticles were characterized by UV-Vis spectroscopy using automated spectrometer Spectro UV-Vis double beam DUV 3500, scanning electron microscopy (SEM) using an LEO 1455 VP. An energy-dispersive X-ray detection instrument (EDAX) (HORIBA 8121-H) was used to examine the elemental composition of the sample. The possible functional groups of CuO Nps were analyzed by using Fourier transform infrared spectroscopy (FTIR) analysis with an instrument JASCO (FTIR-6200) spectrum.

2.2.3. Pot Culture studies:

For pot culture studies, 18 plastic pots (25 cm diameter, 25 cm height) were filled with a mixture of red soil, and cow dung in a ratio of 1:1. Physico-chemical parameters such as pH, electrical conductivity, organic carbon, total nitrogen, potassium, and phosphorus of red soil and cow dung were estimated [5]. (Table 1). The experimental soil was mixed with different quantities of copper oxide nanoparticles such as 0,50,100,150,200 and 250 for To (control) T1, T2, T3, T4, and T5 respectively. 10 numbers of seeds were sown in each pot for six treatments in three replicates and grown in a greenhouse for a period of 45 days. Tap water is used as an irrigant.

Table 1 Physico-chemical parameters of red soil and cow dung

Parameters	Red soil	Cow dung
pH	7.84	7.98
Electrical conductivity (ds/m)	0.37	0.412
Organic carbon (%)	52	46.1
Total Nitrogen (%)	10.7	21.2
Potassium (%)	0.26	1.12
Phosphorous (%)	0.914	0.998

2.2.4. Growth Characteristics

Growth parameters such as Germination percentage, Shoot and root length, fresh and dry weight, fresh weight, Leaf area and vigour index, biochemical characteristics such as chlorophyll a,b, total chlorophyll, carotenoid, anthocyanin,

total protein and sugar, L-proline, free amino acid and leaf nitrate(Table 2) and yield parameters such as length, weight and number of lady's finger per plants were estimated after 45 days.

Table 2 Procedures followed for Growth and Biochemical characteristics of Lady's finger (*Abelmoschus esculentus*)

S.No	Parameters	References
1.	Germination %	Carly and Watson,(1968)[6]
2.	Shoot length cm	Arts and Marks,(1971)[7]
3.	Root length „	Burris et al.,(1969)[8]
4.	Total fresh weight g	Burris et al.,(1969)[8]
5.	Total dry weight „	Burris et al.,(1969)[8]
6.	Leaf area cm^2	Ford Denison and Raymond Russotti(1997)[9]
7.	Vigor index %	Abdul Baki and Anderson,(1973)[10]
8.	Chlorophyll a mg/gfw	Arnon,(1949)[11]
9.	Chlorophyll b „	Arnon ,(1949)[11]
10.	Total chlorophyll „	Arnon,(1949)[11]
11.	Carotenoids µ mole gwf	Arnon, (1949)[11]
12.	Anthocyanin „	Mancinelli et al.,(1975)[12]
13.	Total soluble sugar „	Jeyaraman,(1981)[13]
14.	Total protein „	Lowry et al.,(1951)[14]
15.	L-Proline „	Bates et al.,(1973)[15]
16.	Free amino acids mg/g fw	Moore and Stein,(1948)[16]
17.	Leaf nitrate „	Cataldo et al.,(1978)[17]

3. Results and discussion

The synthesized CuO NPs were characterized by UV-Visible analysis. The UV-Visible absorption spectrum of copper oxide nanoparticles was confirmed from the peak between 300 and 600 nm (Fig.1). The peak at 322nm indicates the presence of CuO nanoparticles. Jayalakshmi and Yogamoorthi, (2014)[18] reported that the synthesized copper oxide particles are subjected to UV- spectroscopic analysis and obtained a single peak but broad at 263nm indicating the presence of oxides of copper metal. SEM result shows that the copper oxide nanoparticles are crystal shape with agglomerated and copper oxide elements present in the synthesized copper oxide nanoparticles range from 85nm (90µm) (Fig. 2). The size of most of the nanoparticles ranges from 25.19nm to 28.73nm. However, the percentage of nanoparticles present in the synthesized sample is 8nm. From the image, it is confirmed that the sample contains various sizes of nanoparticles which indeed agreed with the result obtained from the chemical reduction method[19]. A similar result on SEM analysis of copper nanoparticles had been also reported[20]. Sorbiun et al., (2018)[21] reported the green synthesis of highly crystalline CuO nanoparticles (NPs) by oak fruit hull (Jaft) as a reducing and stabilizing agent and observed that most of the CuO nanoparticles are in nanometre scale and are mostly of quasi-spherical shape. The element composition of the synthesized nanoparticles was identified by an energy-dispersive X-ray spectroscopy system (EDAX) (Fig. 3) and the peaks are located between 0.4KeV and 2KeV. Rajan and Vasantha (2020)[22] reported that the EDAX spectrum recorded on the copper oxide nanoparticles is shown as three peaks located between 2 KeV and 10 KeV. The EDAX pattern also clearly shows that copper oxide nanoparticles are crystalline in nature and it confirms the copper oxide weight percentage (68%). Renu Sankar et al., (2014)[23] also recorded the highest percentage of elemental copper oxide peaks which were confirmed using EDXA analysis. Pulicherla Yugandhar *et al.,* (2018)[24] synthesized copper oxide nanoparticles (CuO NPs) using fruit extract of *Syzygium alternifolium* and the EDX analysis of nanoparticles showed 34.32 and 31.54% of copper oxide. Fourier Transform Infrared Spectroscopy measurements were carried out to identify the possible functional groups responsible for the reduction of the copper ions in chemically synthesized copper oxide nanoparticles. The FTIR spectrum of copper oxide nanoparticles was analyzed in the range of 500-4000 cm^{-1} (Fig. 4). The bands observed at 3421.1, 2918.73,2303.55, 1371.14, 1628.59,

1220.71, 830.2 and 583.36 which are associated with C=C stretching of alcohol, C-N stretching aromatic amine, C-F stretching and sulfonyl chloride, C-H stretching and alkane, N=C=O stretching and Isocyanate. Mekala et al., (2016)[25] reported that in chemically synthesized copper nanoparticles, peak values at 3414.35cm- 1, 2926.45cm-1, 59.92cm-1, 1633.41 Cm-1, 1390.42cm-1, and 618.074cm-1 corresponds to O-H stretching of phenolic compound, C-H bending of aldehydes compounds, C-H stretching of alkenes and N-H bending of amines.[25].In chemically synthesized copper oxide nanoparticles, FT- IR peaks correspond to O-H stretch phenolic compounds, N-H stretch of amines, O-H stretch of Carboxylic group, N-H bending of amines, C-H stretch of aliphatic amines, C-Cl stretch of alkaloids, C-H bending of alkanes[26].

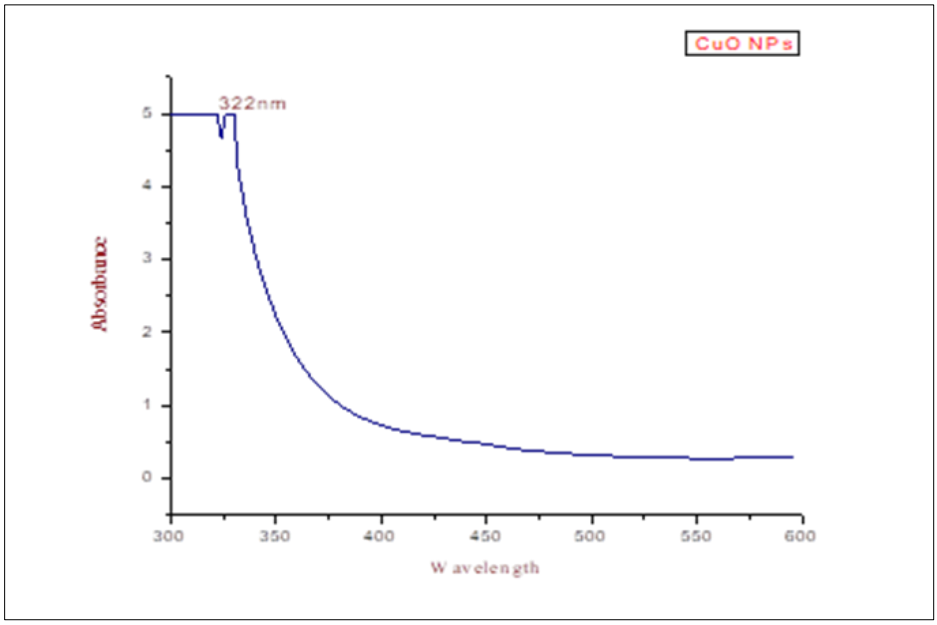

Figure 1 UV-Visible Spectroscopic image of CuO nanoparticles

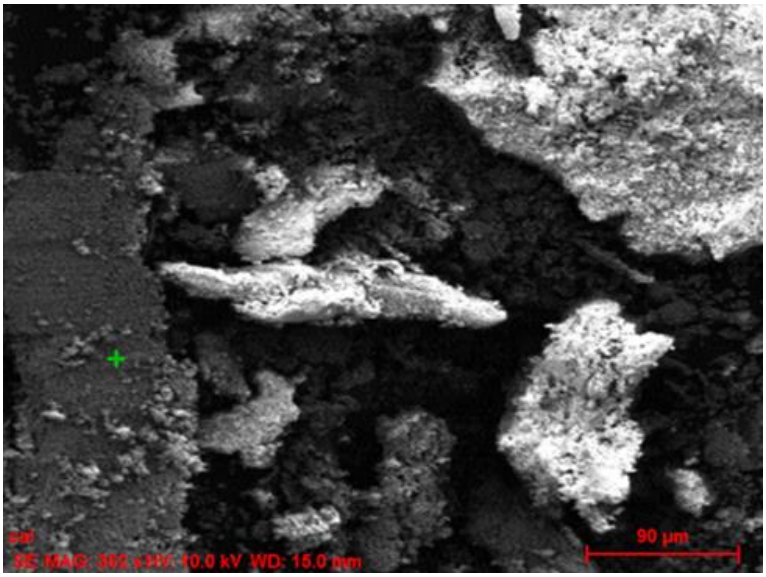

Figure 2 Scanning Electron Microscopic image of CuO nanoparticles

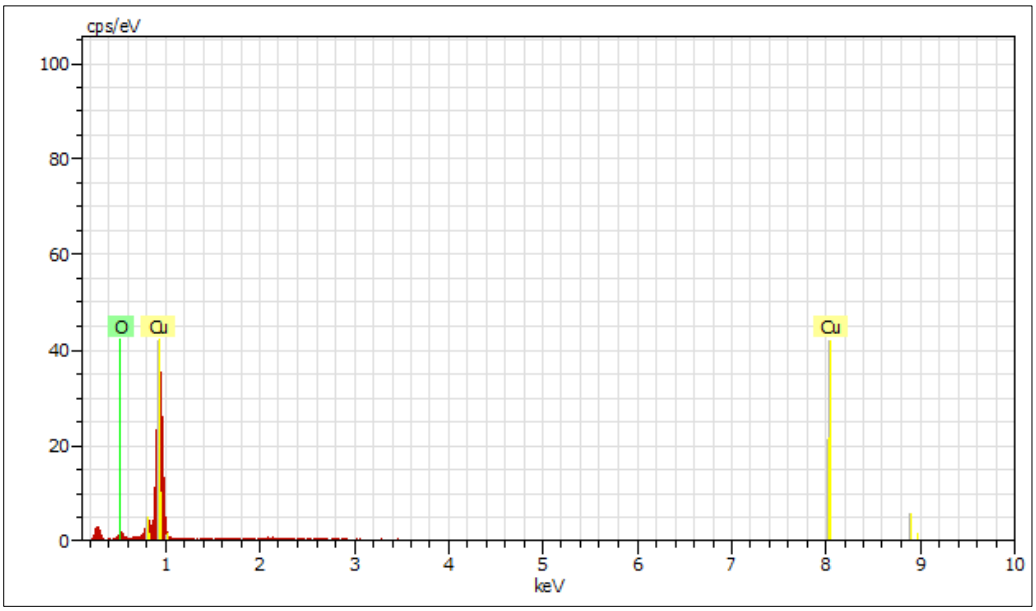

Figure 3 Energy Dispersive X-ray Spectroscopic (EDAX) image of CuO nanoparticles

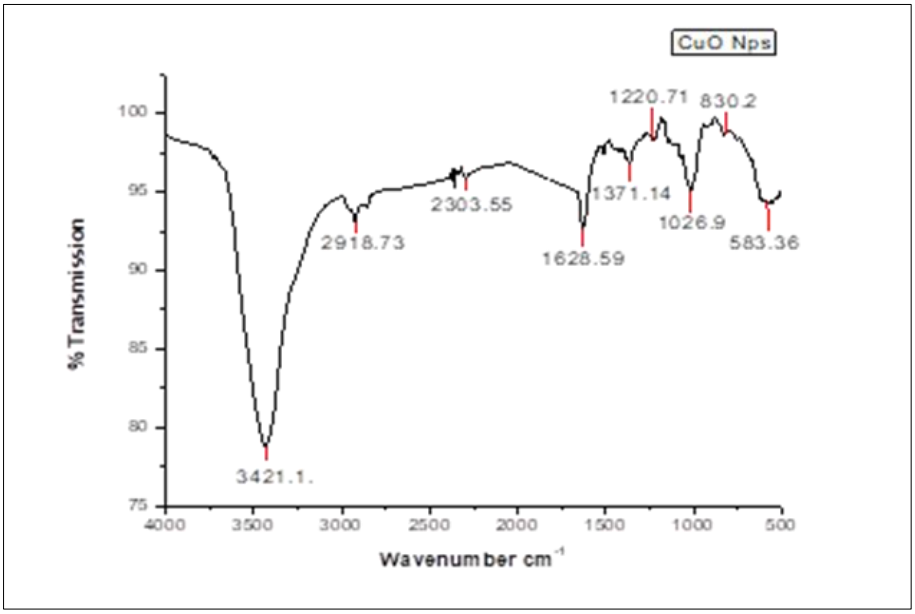

Figure 4 Fourier Transform Infrared Spectroscopic image of CuO nanoparticles

The effect of various quantities of copper oxide nanoparticles on the growth of Lady's finger *Abelmoschus esculentus* is presented in Table 3. The germination percentage of Lady's finger is higher in T3 and lower in T5. Prasand et al., (2012)[27] reported 100% germination of peanuts treated with zinc oxide nanoparticles. The shoot length of Lady's finger is higher in T3 containing 150mg of copper oxide nanoparticles and lower in T5 containing 250mg of CuO nanoparticles. Pramond Mahajan (2011)[28] reported that the shoot length of Mung and Gram increased in a lower concentration of nano ZnO nanoparticles decreasing the shoot length. Prasad et al., (2012)[27]) also reported that the shoot length of peanuts is higher in 100mg of ZnO nanoparticles. The root length is higher in T3 and lowers in T0. The fresh weight is higher in T3 and lowers in T5. Sri Sindhura et al., (2013)[29] reported that the fresh weight of peanuts is higher in lower concentrations of ZnO nanoparticles. The dry weight is higher in T3 and lowers in T2 and T5. The leaf area is higher in T3 and lowers in T0. The Vigour index is higher T3(1761). Bhim Jyoti et al., (2016)[30] reported a vigour index of 1701.3 in peanuts treated with ZnO nanoparticles.

Table 3 Effect of various quantities of copper oxide nanoparticles on Growth parameters of Lady's finger *Abelmoschus esculentus* grown for 45 days. Each value is the average of 10 individual observations (Average ±SD)

Parameters	**Treatments**					
	T_0	T_1	T_2	T_3	T_4	T_5
Germination (%)	87± 4	83± 6	93± 4	100± 0	77±6	96±4
Shoot length(cm)	33.5±11.33	34±11.45	34±11.51	53±17.54	39±13.10	32.5±10.89
Root length(cm)	9±3.10	15±5.06	17.5±5.06	18.5±6.61	10±3.36	10±3.36
Fresh weight(g)	25.0±9.35	12.5±21	18.1±5.56	32.3±10.8	21±13.42	11.15±2.84
Dry weight(g)	2.75±0.807	1.9±0.16	1.8±0.36	3.2±1.65	2.3±1.65	1.8±0.991
Leaf area (cm^2)	22.6±7.58	26.6±8.9	28.1`±9.4	42.6±14.2	32.6±11	25.5±8.56
Vigour index (%)	988	955	1079	1761	1021	1118

The chlorophyll a, b, and total chlorophyll of Lady's finger were higher in T3(Fig.5). The chlorophyll a is higher in treated when compared with untreated plants[31]. CuO treated at the lower concentration (16µm) recorded the highest values of chlorophyll-a concentration (2.68 and 1.99mg/gfw). Also reported that the chlorophyll a, b, total chlorophyll and carotenoid content of cluster bean leaves increased at lower concentrations[32]. Salwa and Abbas (2012)[33] reported as an increase in chlorophyll a and chlorophyll b contents thus increasing the biosynthesis of photosynthetic pigments content. Anthocyanin and carotenoids of Lady's finger were also higher in T3(Fig.6). The results demonstrated that the supply of copper could increase the anthocyanin content of seedlings. Anthocyanin is known to accumulate in stressed conditions as a result of secondary metabolism[34]. Sinha et al., (2007)[35] reported an increase in carotenoid content and suggested further that it is a defence strategy of the plant under metal stress. Marisamy et al., (2012)[36] suggested that anthocyanin is higher in T4 and lower in T2. Protein and L-proline of Lady's finger gradually increased in lower weight of copper oxide nanoparticles and decreased in higher weight (Fig. 7). Similar studies were also reported in black gram treated with the differential weight of copper oxide nanoparticles [22]. Ragavan et al (2017)[37] reported similar results of L-proline in Cluster beans treated with different quantities of selenium nanoparticles. Total soluble sugar is higher in T3(Fig.8).

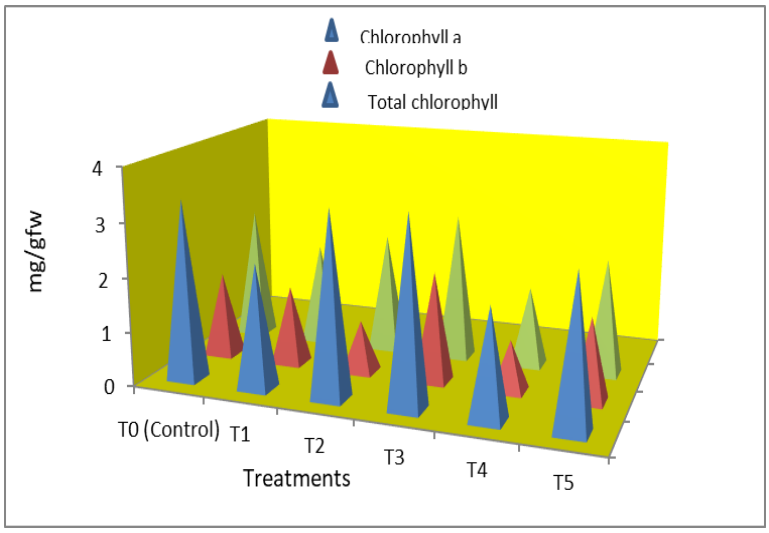

Figure 5 Chlorophyll a,b and total chlorophyll of Lady's finger *Abelmoschus esculentus*

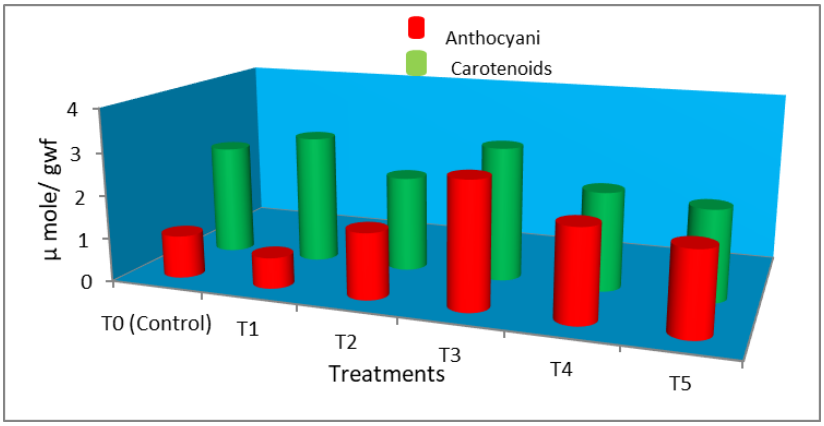

Figure 6 Anthocyanin and Carotenoids of Lady's finger *Abelmoschus esculentus*

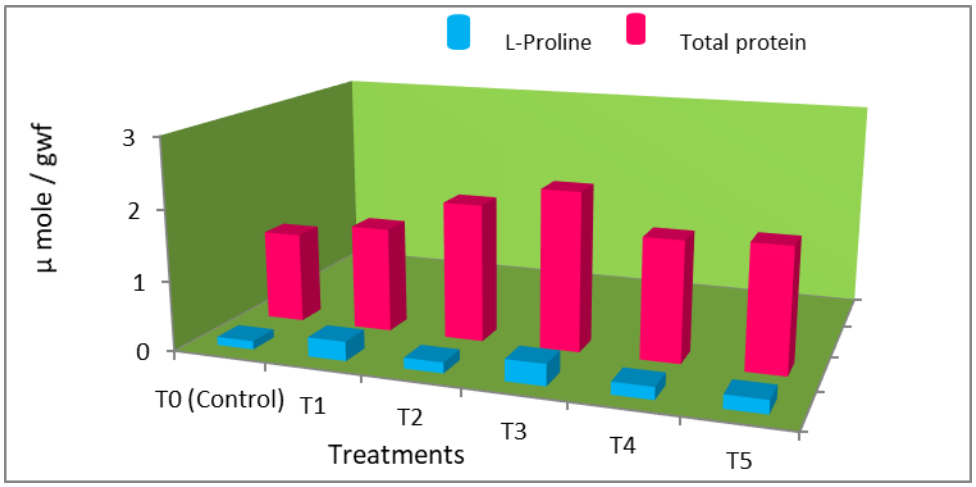

Figure 7 L-Proline and total protein of Lady's finger *Abelmoschus esculentus*

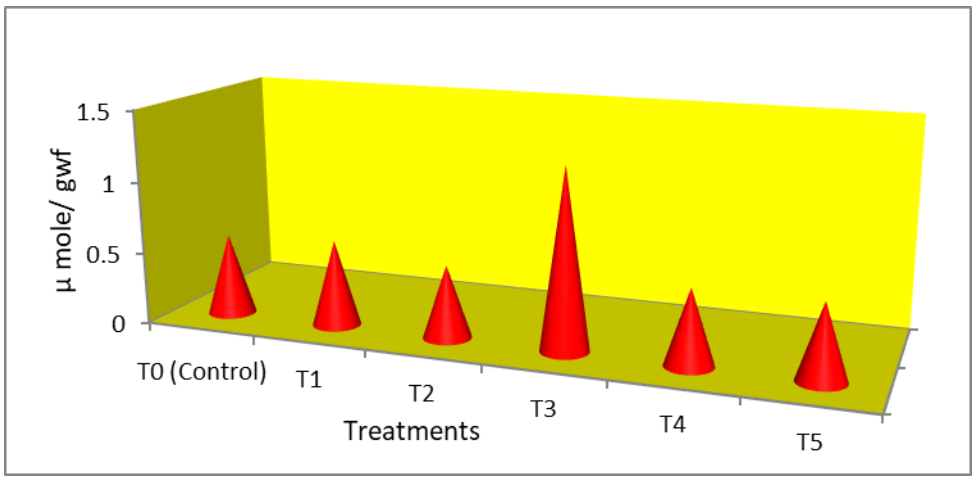

Figure 8 Total soluble sugar of Lady's finger *Abelmoschus esculentus*

The yield parameters of Lady's finger *Abelmoschus esculentus* are presented in table 4. The length, weight and number of Lady's fingers per plant are higher in T3. Abdul Hafeez et al., (2015)[38] reported the soil application of 30ppm Cu-

NPs may increase the yield of wheat crops significantly to match the food demand of the growing population. Similar results were reported when treated with the differential weight of zinc oxide nanoparticles in Lady's finger[39].

Table 4 Yield parameters of Lady's finger *Abelmoschus esculentus*

Treatment	Length(cm)	Weight(g)	Number
T0	24±0.34	3.75±3.27	6.0±1.0
T1	20±0.95	4.61±3.53	5.00±1.00
T2	21.2±3.86	5.9±4.00	9.00±1.00
T3	25.2±5.79	11.89±4.90	19.00±6.0
T4	23±1.95	3.13±0.40	16.0±2
T5	21±0.42	3.7±1.27	2.00±1.00

4. Conclusion

The study successfully demonstrates the utilization of copper oxide nanoparticles as a nutrient for the growth, biochemical characteristics and yield of Lady's finger *Abelmoschus esculentus.*

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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