International Journal of Nanotechnology and Nanoscience, Volume 6, 2020, 16-25

Synthesis, Characterization and Influence of Differential Weight of Copper Nanoparticles on Growth, Biochemical Characteristics and Yield of Black Gram *Vigna mungo*

M.R.Rajan* **and Vasantha M**

*Department of Biology, The Gandhigram Rural Institute-Deemed to be University, Gandhigram, Tamil Nadu, Pin code: 624302 India

ABSTRACT

The present study deals with the synthesis, characterization and influence of differential weight of copper nanoparticles on growth, biochemical characteristics and yield of black gram *Vigna mungo.* Copper sulphate was utilized for the synthesis of copper oxide nanoparticles using precipitation method and characterized by using UV-Vis Spectroscopy, SEM, EDAX, FTIR, and XRD. Differential weight of copper oxide nanoparticles (0, 100, 200, 300, 400, 500 mg) were utilized for growing black gram. After 60 days, growth, biochemical and yield parameters were measured. UV-visible absorption spectrum of the synthesized copper oxide nanoparticles was confirmed from the peak at 500 nm. SEM image of copper oxide nanoparticles was observed as spherical in shape. EDAX spectrum recorded on the copper oxide nanoparticles is shown as three peaks located between 2 KeV and 10 KeV. The functional groups of synthesized copper oxide nanoparticles were confirmed by FT-IR. The crystalline structure of synthesized copper oxide nanoparticle was obtained using XRD. Germination efficiency, Shoot length, root length fresh weight, dry weight, Vigor index chlorophyll a, b, total chlorophyll, carotenoid and L-proline of Black gram *Vigna mungo* was higher in T_3 (300 mg). The protein content is higher in T_1. The weight and number of Black gram was higher in T_3.

Key words: Copper Oxide Nanoparticles, Growth, Biochemical, Yield, Black Gram

INTRODUCTION

Nanotechnology plays a very important role in modern research and it is the most capable technology that can be applied almost all fields such as pharmaceutical, electronics, health care, food and feed, biochemical science, drug, gene delivery, chemical industry, energy science, cosmetics, environmental health, mechanics and space industries. Development of nanotechnology has encouraged the production of engineered nanoparticles worldwide [1]. Nanoparticles like gold, silver, copper, zinc, aluminum, cesium oxide, zinc oxide, are used in crop production, fertilizer and irrigation. Among different nanoparticles, copper oxide nanoparticles have attracted significant attention because of its wide range of application such as super conductors, sensors, catalytic, electrical, giant magnet management resistance materials, solar energy transformation and preparation of organic and inorganic nanostructure composites. Its size typically below 100 nm have been applied in an increasing number of commercial applications, such as electronics, optics, textiles, medicine, catalysts, water treatment, and environmental remediation [2]. Black gram is considered as a significant crop that play notable role in human health

***Corresponding Author:**
mrrrajanbio[at]gmaildotcom

Receiving Date: June 12, 2020
Acceptance Date: July 13, 2020
Publication Date: July 20, 2020

ISSN 2397-5547

due to variety of vitamins, carotenoids, beneficial acids, sugar and minerals. The work related to the synthesis, characterization and influence of differential weight of CuO NPs on growth, biochemical characteristics and yield of black gram is totally wanting. Hence, the present study was carried out.

EXPERIMENTAL METHODS

1. Synthesis of Copper Oxide Nanoparticles

Synthesis of copper oxide nanoparticles was carried out by simple precipitation method. Precipitation was done by mixing 1M NaOH which is to be added in a drop wise to the 1mM of copper sulphate solution under vigorous stirring. At a particular time the solution turns dark green and indicates the formation of copper nanoparticles. Then the solution was centrifuged at 3500 rpm for 10 min followed by re-dispersion of the pellet in deionized water for purifying CuO. Then the precipitate was dried at room temperature. Finally copper oxide nanoparticles were stored in dry tube containers.

2. Characterization

The synthesized copper oxide nanoparticles were characterized by the following techniques.

2.1. UV- Visible Spectroscopy

The formation of CuO NPs was initially confirmed visually and by using UV-visible spectroscopy technique, which has been frequently used to characterize the synthesized metal and metal oxide nanoparticles.

2.2. SEM and EDAX Analysis

The surface morphology and size of the nanoparticles were obtained by scanning Electron Microscopy (SEM) analysis. The morphology and composition of the copper nanoparticles were examined by scanning Electron Microscope (SEM) using HITACHI SU600 equipped with energy dispersive.

2.3. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analysis was used to identify the capping, reducing and stabilizing capacity. The FTIR spectra of synthesized copper oxide nanoparticles were analyzed for knowing the possible functional groups. The measurement was carried out by JASCO (FTIR- 6200) spectrum.

2.4. X-ray Diffraction Analysis

X-ray diffraction (XRD) analysis was carried out by a powder diffractometer (Rigaku III/A, Japan) equipped with a copper oxide target and a nickel filter to analyze the structure of the product.

3. Source of Materials used in Pot Culture

Red soil and cow dung were collected from the Gandhigram Rural Institute campus and physico-chemical parameters such as pH, electrical conductivity, nitrogen, organic carbon, potassium and phosphorus of red soil and cow dung were estimated [3]. Black gram *Vigna munga* was selected for the present study based on the relative importance of daily diet of people. The viable seeds were purchased

ISSN 2397-5547

from Rajesh Agriculture Store, Dindigul, Tamil Nadu, India.

4. Growth Studies

Red soil and cow dung was mixed in the ratio of 1:1 and were taken in 18 plastic pots (25 cm diameter, 25 cm height) for growth studies. Then differential weight of copper oxide nanoparticles such as 0, 100, 200, 300, 400, and 500 mg was mixed with 1 kg each of red soil and cow dung for T_0(control), T_1, T_2, T_3, T_4, and T_5 respectively. 10 number of black gram seeds were sown in each pot for six treatments in three replicates and grown in a Net house for 60 days. Tap water is used as an irrigant. Growth parameters such as germination percentage, shoot and root length, fresh and dry weight and vigor index, and biochemical characteristics such as chlorophyll a, b, total chlorophyll, carotenoids, anthocyanin, total soluble sugar, total protein and L-proline were estimated after 60 days and the procedures followed are presented in Table 1. The yield parameters such as length of pod, weight and number of black gram per plant were also estimated.

Table 1: Procedures Followed for Growth and Biochemical Characteristics of Black Gram

S. No	Parameters	Units	References
1	Germination	%	Carley and Watson, (1968) [4]
2	Shoot length	cm	Arts and Mark, (1971) [5]
3	Root length	,,	Burris *et al.,* (1969) [6]
4	Total fresh weight	g	Burris *et al.,* (1969) [6]
5	Total dry weight	,,	Burris *et al.,* (1969) [6]
6	Vigor index	%	Abdul Baki and Anderson, (1973) [7]
7	Chlorophyll a	mg/gfw	Arnon, (1949) [8]
8	Chlorophyll b	,,	Arnon, (1949) [8]
9	Total chlorophyll	,,	Arnon, (1949) [8]
10	Carotenoids	mole gfw	Arnon, (1949)[8]
11	Anthocyanin	,,	Mancinelli *et al.,* (1975) [9]
12	Total soluble sugar	,,	Jeyaraman, (1981) [10]
13	Total protein	,,	Lowry *et al.,* (1951) [11]
14	L- proline	,,	Bates *et al.,* (1973) [12]

RESULTS AND DISCUSSION

The UV-Vis analysis of chemically synthesized CuO Nps is presented in Figure 1. The peak at 500 nm indicates the formation of CuO NPs. Mekala *et al*., (2016) [13] also reported the green synthesis of copper oxide nanoparticles subjected to UV-spectroscopy analysis and obtained a single peak but broad at 589 nm and area in conformity with the eco-friendly synthesis of copper nanoparticles. The surface morphology and size of the nanoparticles were obtained by Scanning Electron Microscopy (SEM) analysis. The SEM image (Figure 2) showing the high density of synthesized CuO nanoparticles. It is evident that the nanoparticles are spherical is shape and size in the range of 9.10 nm (15.2 µm) and 5.19nm (26.6µm). DA Coasta and Sharma (2016) [2] reported that the surface morphology and size of the nanoparticles were obtained by Scanning Electron Microscopic (SEM) analysis. The nanoparticle characterization studies revealed that the shape of CuO NPs was spherical in size of SEM. Sathish and

ISSN 2397-5547

Sharma *et al.,* (2016) [14] reported that synthesis of Cu NPs from medicinal plants and SEM analysis shows uniformly distributed and spherical.

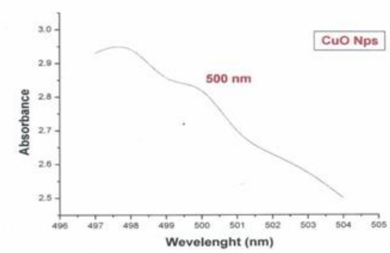

Figure 1: UV-Visible Spectroscopy of Copper oxide Nanoparticles

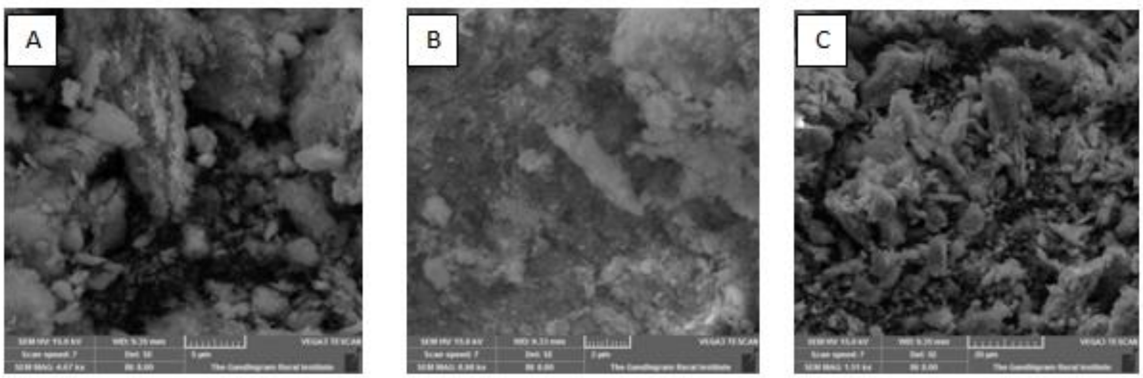

Figure 2: Scanning Electron Microscope Images of Copper Oxide Nanoparticles
A) 2µm of Copper Nanoparticles B) 5µm of Copper Nanoparticles C) 20µm of Copper Nanoparticles

EDAX spectrum recorded on the copper oxide nanoparticles is shown as three peaks located between 2 KeV and 10 KeV (Figure 3). The EDAX pattern clearly shows that copper oxide nanoparticles are crystalline in nature and it confirms the copper oxide nanoparticles weight percentage (68%). Amrut Lanje *et al.,* (2010) [15] also recorded highest percentage of elemental copper oxide peaks and were confirmed using EDAX analysis. The FT-IR results revealed that the peaks at 3429.8, 2918.2, 1619.2, 1376.9 and 1021.1cm^{-1} correspond to the functional groups of copper oxide nanoparticles and viewed the functional groups of alcohol, alkane, aromatic amine and Sulfoxide (Figure 4). Maria Isabel *et al.,* (2014) [16] reported the green synthesized copper oxide nanoparticles and showed bands at 1384 and 1528 refer to N-H bending, 2048 cm^{-1} represents alkynes, 3612 cm^{-1}, corresponds to phosphorous compound, 1228 cm^{-1} corresponding to C-O-C stretch.

The phase and crystalline structure of synthesized copper oxide nanoparticles was characterized via XRD analysis. The peak position with 2θ values of 15.530, 16.240, 17.370, 18.850, 20.070, 22.360, 24.070 and 64.1800 which corresponds to crystalline structure of CuO (Figure 5). Awwad et al., (2015) [17] reported that the XRD analysis showed small distinct diffraction peaks at 32.842, 61.403 and 71.189, which indexed the copper oxide nanoparticles with a monocyclic phase. The XRD pattern revealed that the synthesized copper oxide nanoparticles are crystalline [18].

ISSN 2397-5547

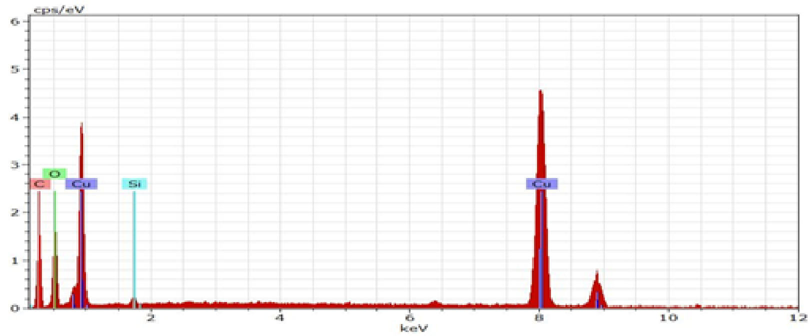

Figure 3: Energy Dispersive X- ray Spectroscopy Image

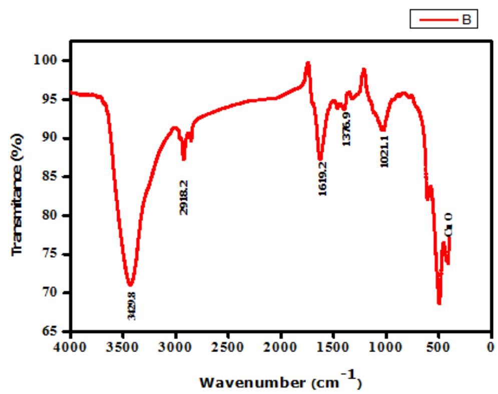

Figure 4: Fourier Transform Infrared Spectroscopy of Copper Oxide Nanoparticles

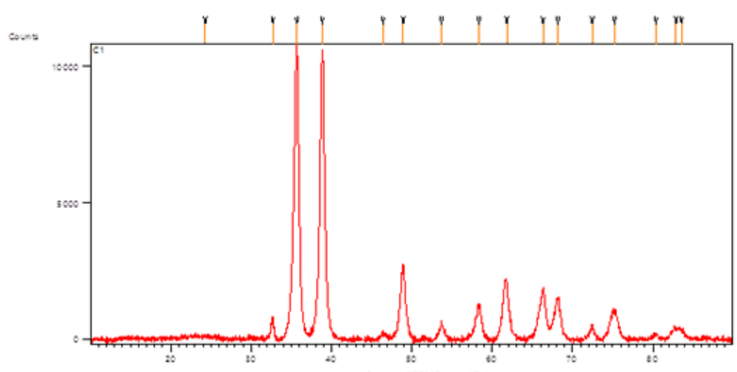

Figure 5: XRD Analysis of Copper Oxide Nanoparticles

The physico-chemical parameters of red soil and cow dung used in growth studies are presented in Table 2. The effect of differential weight of copper oxide nanoparticles on growth parameters of Black gram *Vigna mungo* is presented in Table 3. The germination percentage in T_0, T_1, T_2, T_3, T_4, T_5 is 97, 90, 93, 100, 83, 75 respectively. Prasad *et al.,* (2012) [19] reported 100% germination of pea plant nut treated with Zinc Oxide nanoparticles. Raskar et al., (2013) [20] reported that the TiO nanoparticles at the concentration ranging from 10 to 40 ppm enhance seed germination, promptness index and

ISSN 2397-5547

seedling growth but result shows that lower concentration was not harmful to the seed germination and early seedling growth. The shoot length is higher in T_3 containing 300 mg of copper oxide nanoparticles and lower in T_5 containing 500mg of nanoparticles. Pramond Mahajan (2011) [21] reported that the shoot length of Mung and Gram increased in lower concentration of nanoZnO particles and higher concentration of ZnO nanoparticles decreased the shoot length. The root length of the Black gram in control is 2.33. Among the treatments the root length is higher in T_3 (5.4) and lower in T_1. Among the treatments the fresh weight is higher in T_3 (2.46). The fresh weight of the Black gram in control is 1.22 g. Sri Sindhura *et al.,* (2013) [22] reported that the fresh weight of peanut is higher in lower concentration of ZnO nanoparticles The dry weight of the Black gram is higher in T_4 and lower in T_3 and lower in T_2 and T_5. Naderi et al., (2012) [23] reported the effect of treatment of ZnO nanoparticles at different concentration and the results showed that the dry weight was influenced by different concentration of ZnO nanoparticles. The vigor index of Black gram in control is 2619. Among the treatments vigor index is higher T_3 and lower in T_5. Bhim Jyoti et al., (2016) [24] reported a vigour index 1701.3 in peanut treated with ZnO nanoparticles.

Table 2: Physico-Chemical Parameters of Red Soil and Cow Dung

S.No	Parameters	Red Soil	Cow dung
1 .	pH	7	6.99
2	Electrical conductivity (ds/m)	0.38	0.51
3	Organic Carbon (%)	30	16.7
4	Total Nitrogen (%)	11.3	23.4
5	Potassium(%)	0.27	0.8
6	Phosphorus (%)	0.31	0.83

Each value is the average of 10 individual observations.

Table 3: Influence of Differential Weight of Copper Oxide Nano Particles on Growth Parameters of Black Gram *Vigna mungo* Grown for a Period of 60 days . Each Value is the Average of 10 Individual Observations (Average ± S.D)

S. No.	Parameters	Days	Treatments					
			T_0	T_1	T_2	T_3	T_4	T_5
1	Germination Percentage	-	97	90	93	100	83	75
2	Shoot Length (cm)	60	24.6 ± 0.57	20.6 ± 1.6	24.3 ± 2.4	32.3 ± 1.6	26.3 ± 1.24	22.3 ± 1.24
3	Root Length (cm)	60	2.33 ± 0.47	3.1 ± 0.43	3.7 ± 0.50	5.4 ± 0.43	4.06 ± 0.16	4.16 ± 0.28
4	Fresh Weight (g)	60	1.22 ± 0.06	1.49 ± 0.36	1.5 ± 0.07	2.96 ± 0.44	1.97 ± 0.33	1.62 ± 0.45
5	Dry Weight (g)	60	0.29 ± 0.08	0.26 ± 0.08	0.38 ± 0.02	0.47 ± 0.08	0.31 ± 0.001	0.23 ± 0.02
6	Vigor Index	60	2619	2133	2606	3773	2519	1984

The chlorophyll a, b and total chlorophyll of Black gram *Vigna mungo* is presented in Figure 6. The chlorophyll a, b and total chlorophyll are higher in T_3 and lower in T_2. Mohammad *et al.,* (2014) [25] reported that on the effects of Magnetite Nanoparticles on Soybean and also reported that the

ISSN 2397-5547

chlorophyll content of Lettuce is increased when treated with iron oxide nanoparticles. Ragavan et al [26] reported that the chlorophyll a, b and total chlorophyll of cluster bean were higher when treated selenium nanoparticles. The carotenoids are higher in T_1 (1.07) and lower in T5 (0.93) (Figure 7). Sinha *et al.,* (2007) [27] reported an increase in carotenoids content and suggested further that is a defense strategy of the plant under metal stress. Anthocyanin is known to accumulate in stressed condition as a result of secondary metabolism [28]. The results demonstrated that the supply of copper could increase anthocyanin content of seedlings. Protein and L-proline of Black gram gradually increased in lower weight of copper oxide nanoparticles and decreased in higher weight (Figure 8). Baskaran *et al.,* (2009) [29] recorded an increase in protein content of green gram at lower concentration and then the content decreased at good concentration with sugar mill effluent was observed. Ragavan et al (2017) [26] reported similar results of L-proline in Cluster bean treated with different quantities of selenium nanoparticles.

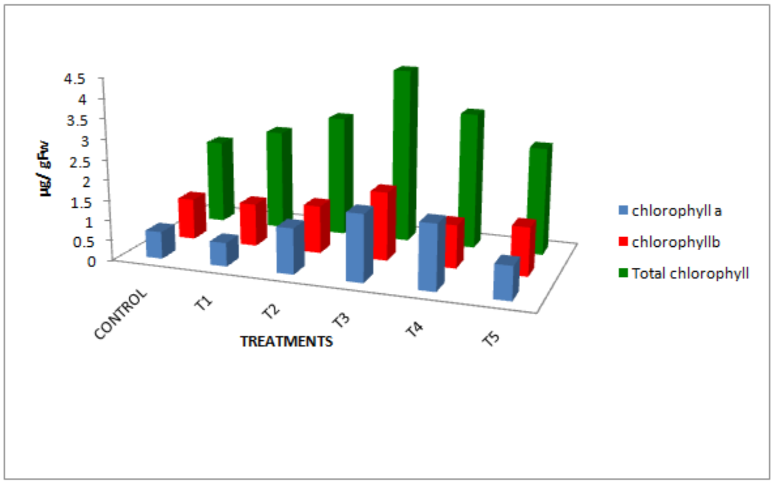

Figure 6: Chlorophyll a, b and Total Chlorophyll of Black Gram *Vigna mungo*

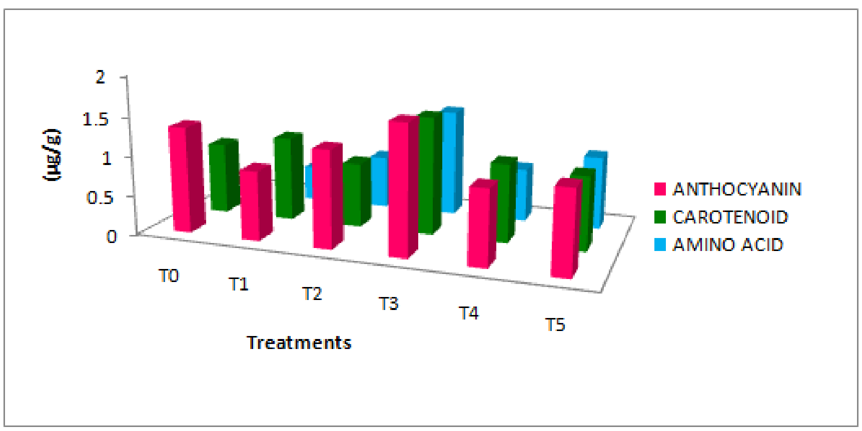

Figure 7: Carotenoid, Anthocyanin and Amino Acid of Black Gram *Vigna mungo*

ISSN 2397-5547

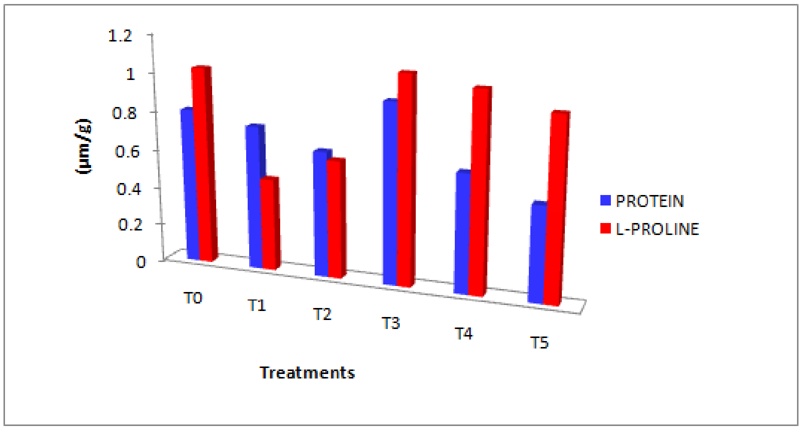

Figure 8: Protein and L. Proline of Black Gram *Vigno mungo*

The yield parameter of Black gram *Vigna mungo* is presented in Table 4. The length of black gram pod, weight, and number of black grams per plant is higher in T_3 and lower in T_1. Gokila et al (2017) [30] reported similar results when treated with differential weight of zinc oxide nanoparticles in Lady's finger.

Table 4: Yield Parameters of Black Gram *Vigna mungo*

Treatment	Length	Weight	Number
T_0	13.46 ± 11.13	0.75 ± 0.46	12 ± 2
T_1	11.85 ± 8.75	0.93 ± 0.56	12 ± 3
T_2	14.01 ± 10.31	0.87 ± 0.65	9 ± 1
T_3	18.86 ± 13.46	1.71 ± 1.24	17 ± 1
T_4	15.18 ± 11.12	1.14 ± 0.83	8 ± 1
T_5	13.23 ± 9.7	0.92 ± 0.69	7 ± 1

CONCLUSION

This study successfully demonstrates the acceptable application of copper oxide nanoparticles and subsequent utilization as nutrient for the growth, biochemical characterization, and yield of Black gram *Vigna mungo.*

ACKNOWLEDGMENT

Authors thank the Department of Biology, The Gandhigram Rural Institute-Deemed to be University, Gandhigram, Tamil Nadu, India for offering laboratory facilities to carry out this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ISSN 2397-5547

REFERENCES

1. Zhao L, Peralta-Videa JR, Rico CM, Hernandez-Viezcas JA, Sun Y, Niu G, Servin A, Nunez JE, Duarte-Gardea M, Gardea-Torresdey JL. CeO_2 and ZnO nanoparticles change the nutritional qualities of cucumber (*Cucumis sativus*). Journal of agricultural and food chemistry. 2014 Apr 2;62(13):2752-9.
2. Da Costa MV, Sharma PK. Effect of copper oxide nanoparticles on growth, morphology, photosynthesis, and antioxidant response in *Oryza sativa*. Photosynthetica. 2016 Mar 1;54(1):110-9.
3. APHA AW, Greenberg WI, Clesceri L, Eaton A. Standard methods for the examination of water andwastewater. Wasghington DC American Public Health Association. 2012.
4. Carley HE, Watson RD. Effect of various aqueous plant extracts upon seed germination. Botanical gazette. 1968 Mar 1;129(1):57-62.
5. Art HW, Marks PL. summary table of biomass and net annual primary production in forest ecosystems of the world. Maine Agr Exp Sta Misc Rep. 1971.
6. Burris JS, Edje OT, Wahab AH. Evaluation of various indices of seed and seedling vigor in soybeans [Glycine max (L.) Merr.]. InProceedings of the Association of Official Seed Analysts 1969 Jan 1 (pp. 73-81). The Association of Official Seed Analysts.
7. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria 1. Crop science. 1973 Nov;13(6):630-3.
8. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant physiology. 1949 Jan;24(1):1.
9. Mancinelli AL, Yang CP, Lindquist P, Anderson OR, Rabino I. Photocontrol of anthocyanin synthesis: III. The action of streptomycin on the synthesis of chlorophyll and anthocyanin. Plant physiology. 1975 Feb 1;55(2):251-7.
10. Jayaraman J, Jayaraman J. Laboratory manual in biochemistry. Delhi: Wiley Eastern; 1981.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. Journal of biological chemistry. 1951;193:265-75.
12. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and soil. 1973 Aug 1;39(1):205-7.
13. Mekala J, Rajan MR, Ramesh R. Green synthesis and characterization of copper nanoparticles using tulsi (*ocimum sanctum*) leaf extract, PARIPEX. Ind J. Res.. 2016;5:14-6.
14. Sathish, Sharma. Comparative study of the effect of paper mill effluent on Lycopersicum esculentum. Journal of Bioscience Research. 2007;3:14-23.
15. Lanje AS, Sharma SJ, Pode RB, Ningthoujam RS. Synthesis and optical characterization of copper oxide nanoparticles. Adv Appl Sci Res. 2010 Sep;1(2):36-40.
16. Morales MI, Rico CM, Hernandez-Viezcas JA, Nunez JE, Barrios AC, Tafoya A, Flores-Marges JP, Peralta-Videa JR, Gardea-Torresdey JL. Toxicity assessment of cerium oxide nanoparticles in cilantro (*Coriandrum sativum L.*) plants grown in organic soil. Journal of agricultural and food chemistry. 2013 Jul 3;61(26):6224-30.
17. Awwad AM, Albiss BA, Salem NM. Antibacterial activity of synthesized copper oxide nanoparticles using *Malva sylvestris* leaf extract. SMU Med J. 2015;2(1):91-101.
18. Sankar R, Manikandan P, Malarvizhi V, Fathima T, Shivashangari KS, Ravikumar V. Green synthesis of colloidal copper oxide nanoparticles using *Carica papaya* and its application in photocatalytic dye degradation. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014 Mar 5;121:746-50.
19. Prasad TN, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Reddy KR, Sreeprasad TS, Sajanlal PR, Pradeep T. Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. Journal of plant nutrition. 2012 Apr 1;35(6):905-27.
20. Raskar S, Laware SL. Effect of titanium dioxide nano particles on seed germination and germination indices in onion. Plant Sciences Feed. 2013;3(7):103-7.
21. Mahajan P, Dhoke SK, Khanna AS. Effect of nano-ZnO particle suspension on growth of mung (*Vigna radiata*) and gram (*Cicer arietinum*) seedlings using plant agar method. Journal of Nanotechnology. 2011 Jan 1;2011.

ISSN 2397-5547

22. Sindhura KS, Prasad TN, Selvam PP, Hussain OM. Synthesis, characterization and evaluation of effect of phytogenic zinc nanoparticles on soil exo-enzymes. Applied Nanoscience. 2014 Oct 1;4(7):819-27.
23. Naderi MR, Abedi A. Application of nanotechnology in agriculture and refinement of environmental pollutants. J Nanotechnol. 2012;11(1):18-26.
24. Jyoti B, Gaurav SS, Pant U. Use of growth regulators as priming agent for improvement of seed vigour in tomato (*Lycopersicum esculentum*). Journal of Applied and Natural Science. 2016 Mar 1;8(1):84-7.
25. Muthulakshmi S, Pandiyarajan V. Effect of Iaa on the Growth, Physiological and Biochemical Characteristics in *Catharanthus roseus* (L). G. Don. International Journal of Science and Res. 2015;4(3):442-8.
26. Ragavan P, Ananth A, Rajan MR. Impact of selenium nanoparticles on growth, biochemical characteristics and yield of cluster bean *Cyamopsis tetragonoloba*. International Journal of Environment, Agriculture and Biotechnology. 2017;2(6):238983.
27. Sinha S, Gupta AK, Bhatt K. Uptake and translocation of metals in fenugreek grown on soil amended with tannery sludge: involvement of antioxidants. Ecotoxicology and Environmental Safety. 2007 Jun 1;67(2):267-77.
28. Jayarambabu N, Kumari BS, Rao KV, Prabhu YT. Germination and growth characteristics of mungbean seeds (*Vigna radiata L.*) affected by synthesized zinc oxide nanoparticles. International Journal of Current Engineering and Technology. 2014;4(5):2347-5161.
29. Baskaran L, Sundaramoorthy P, Chidambaram AL, Ganesh KS. Growth and physiological activity of greengram (Vigna radiata L.) under effluent stress. Bot. Res. Int. 2009;2(2):107-14.
30. Gokila B, Keerthika V, Rajan MR. Impact of zinc oxide nanoparticles on growth, biochemical characteristics and yield of lady's finger abelmoschus esculentus. Ind J of Appl Res. 2017;7(8):53-56..

ISSN 2397-5547