International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(4): 227-231

Received: 22-05-2020
Accepted: 29-06-2020

K Manoj Kumar Reddy
Department of Biological Sciences Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Dr. PK Shukla
Department of Biological Sciences Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Corresponding Author:
K Manoj Kumar Reddy
Department of Biological Sciences Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Effect of metallic-nanoparticles on morphological and biochemical charecteristics of *Stevia rebaudiana bertoni*

K Manoj Kumar Reddy and Dr. PK Shukla

DOI: https://doi.org/10.22271/chemi.2020.v8.i4d.9694

Abstract
Nanoparticles are ≤ 100 nm in size, exhibit different properties compared to the bulk material. In most of the reported NP studies on plant both positive and negative impacts have been observed.A pot experiment was conducted at field experimentation center, Department of biological sciences, sam Higginbottom University of agriculture Technology & sciences, Uttar Pradesh. during summer season 2019 with stevia. Effect of different metallicoxide nanoparticals on stevia with thirteen treatments and three replications each, were laid out in Radomized Block design. This research was under taken to asses the impact of different concentrations of zincoxide (25,50,75 ppm), copperoxide (25,50,75 ppm), zinc (10,20,30 ppm) and silicon dioxide (10,20,30 ppm) np's on plant morphological growth, biochemical activities of Stevia. From the present investigation it was concluded that that the ZnO (50ppm) and CuO (50ppm) showed the most positive response in morphological and biochemical parameters.

Keywords: ZnO, CuO, Zn, SiO_2, morphological parameters, biochemical parameters, nanoparticles, stevia

1. Introduction

Stevia, botanically known as *Stevia rebaudiana* Bertoni (Family- Asteraceae) is a sweet herb. The leaves are mild green and intensely sweet. The compounds in the leaves which cause sweetness are stevioside and rebaudioside, they are 200 times sweeter than the sugar (Anon, 2004) [2]. These compounds play crucial role in conferring anti-diabetic,anti-cancerous and antibacterial properties of S. rebaudiana (Dey *et al.*, 2013) [8].

Nanoparticles ranging in size from 1 to 100 nm possess specific physico-chemical properties attributed to smaller size, large surface area and high reactivity compared to their bulk counterparts (Yadav, 2013) [25]. The interaction of nanoparticles with the biological system is of enormous importance, and nowadays researchers are trying to figure out the potential effects of various kinds of nanoparticles in plants, animals and humans (Boczkowski and Hoet, 2009) [5]. Nanoparticles have numerous applications in agriculture including synthesis of nano-pesticide or nano-fertilizer formulations, and their use as sensors of soil conditions and for targeted delivery of genes in transformation (Aslani *et al*., 2014) [3].

In recent years, studies encompassing field of nanotechnology for determination of the effects of environmental stress on plant physiology have been finding a fast pace (Bhattacharyya *et al*., 2015) [4]. Metallic oxide nanoparticles, specifically nano-scale zinc oxide (ZnO) and copper oxide (CuO) have gained paramount importance in this regard. Based on our literature survey ascertained ZnO nanoparticles have largely been declared phytotoxic and their phytotoxity has been manifested by the generation of reactive oxygen species (ROS), and formation of necrotic lesions as well as yellow pigmentations on the leaves of different crop plants including Lolium perenne, Glycine max, Cucumis sativus and Triticum aestivum (Lin and Xing, 2007; Lo'pez-Moreno *et al*., 2010; Kim *et al*., 2012) [15, 16, 14].

The effects of toxicity are dependent on the size of nanoparticles, dissolution of metal ions, and their uptake and translocation in plant cells (Franklin *et al*., 2007; Jiang *et al*., 2009) [9, 12]. The effect of CuO nanoparticles on the growth, photosynthesis and oxidative response has recently been studied in crop plant, *Oryza sativa, Brassica napus*

(Da Costa and Sharma 2016; Zafar *et al*. 2016) [6, 26], *Lemna minor* (Duckweed), (Song *et al*. 2016; Perreault *et al*. 2014) [22, 19], *Landolti apunctata* (Shi *et al*. 2011) [21], *Elodea nuttallii* (Regier *et al*. 2015) [20]. (Da Costa and Sharma 2016; Zafar *et al*. 2016) [6, 26], *Lemna minor* (Duckweed), (Song *et al*. 2016; Perreault *et al*. 2014) [22, 19], *Landolti apunctata* (Shi *et al*. 2011) [21], *Elodea nuttallii* (Regier *et al*. 2015) [20].

Metallic oxide nanoparticles, specifically nano-scale zinc (Zn) and silicon dioxide (SiO_2) have gained paramount importance in this regard. Based on literature survey ascertained Zn nanoparticles have largely been declared phytotoxic and their phytotoxity has been manifested by the generation of reactive oxygen species (ROS), and formation of necrotic lesions as well as yellow pigmentations on the leaves of different crop plants Recently, the influence of zinc (Zn) nanoparticles on physiology and stevioside production of S. rebaudiana was deciphered, and Zn np's were found to be phytotoxic at a concentration of 400 and 1000 mg L^{-1} (Desai *et al*., 2015) [7].

The production of steviol glycosides has been accomplished in the presence of abiotic stress i.e. metal np's (Jain *et al*. 2007) [11], nutrient application (Allam *et al*. 2011) [1], osmotic stress (Vives *et al.* 2017) [23]; biotic stress i.e. endophytic fungi, genetic transformation (Pandey *et al*. 2016; Kilam *et al.* 2017) [18, 13]. Copper is required for normal plant growth and development; however, it is toxic at higher levels. Due to its prominent role in development and stimulatory effect on secondary metabolites production, copper has received noticeable attention. The aim of present study is to observe the potential effects of ZnO and CuO nanopartical on the physiological, biochemical and anti-oxidant activity in leaves of S. rebaudiana.

2. Materials and methods

The study was conducted at Department of biological sciences, Sam Higginbottom University of Agriculture Technology & sciences, prayagraj. The stevia plants were grown in pots contain soil and sand. seven treatments were taken in which three treatments are different concentrations of CuO NPs *viz*., (25,50,75 ppm) of 1mg/100ml dw (Sigma, USA), another three treatments are ZnO NPs *viz*., (25,50,75 ppm) of 1mg/100ml dw(Sigma, USA) and one is kept under control, each treatment has three replications The experiment was conducted in a completely randomized design.

2.1 Morphological observation

Different morphological observations *viz.*, leaf length, plant height, were taken at flowering stage on different treatment. These observations were recorded for each repetition of treatment and expressed in terms of mean. The standard statistical analysis such as standard error of mean, critical difference and coefficient of variation were performed to decipher the significance of treatments.

2.2 biochemical parameters

2.2.1 Chlorophyll content

Chlorophyll was determined according to Wellborn (1983) [24]. 1gram leaves sample was weighed and crushed with 80% acetone made the volume to 10 ml with 80% acetone, centrifuged at 800 rpm for 5 minutes. The supernatant was read under 663, 645 nanometres. The readings were fed in the following formula and results were determined under spectrophotometer.

$$\text{Chl 'a'} = 12.7\times(A663)-2.69\times(A645)\times\frac{V}{1000\times w\times a}$$

$$\text{Chl 'b'} = 22.9\times(A645)-4.68\times(A663)\times\frac{V}{1000\times w\times a}$$

$$\text{Total chl} = 20.2\times(A645)+8.02\times(A663)\ \frac{V}{1000\times w\times a}$$

Where,
A645 = absorbance of the extract at A645 nm
A663 = absorbance of the extract at A663 nm
a = path length of cuvette (1 cm)
v = final volume of the chlorophyll extract (10ml)
w = fresh weight of the sample (0.10g)

2.2.2 Carotenoid content

Carotenoid was determined according to (Wellborn, 1983) [24]. 0.5 gm leaf homogenized in 10 ml of acetone (80% acetone).Next to the centrifuged at 1500 rpm at 10 min. The absorbance was recorded at 470 nm.

Total carotenoids = [1000×A470- (3.27 Chla+104 Chlb)]/22.

2.2.3 Total carbohydrate content

Plant extract was taken in 25 ml test tubes and 6 ml anthrone reagent (150 mg of anthrone in 72% H_2SO_4) was added, and then heated in boiling water bath for 10 min. The test tubes were ice cooled for 10 min and incubated for 20 min at 25ºC. Optical density (OD) was read at 625 nm on a spectrophotometer. The carbohydrate content was calculated from the standard curve using glucose with the same method proposed by Hedge JE, Hofreiter BT (1962) [10].

2.2.4 Total protein content (lowery *et al*, 1951) [17].

1ml of leaf extract was taken in centrifuge tube to which 1 ml of 10% trichloroacetic acid (TCA) was added to precipitate the protein. The mixture was allowed to stand on ice bath for 15 min. and then centrifuged.The supernatant was discarded. This procedure was repeated twice. The pellet was washed with ethanol–ether mixture and dissolved in 10ml of 1 N NaOH.This sample was used for protein estimation.

Procedure: 1ml of mixture was taken in a test tube to which 5 ml of freshly prepared alkaline $CuSO_4$ solution was added. After 5 minutes, 0.5ml of Folin Ciocalteu's Phenol reagent was added and the solution was immediately shaken. After 15 minutes optical density (OD) was calculated by preparing standard graph.

3 Result and discussion

3.1 plant height

The results of current study showed a pertinent role played by increasing concentration of ZnO nanoparticles in the growth of S. rebaudiana up to a certain threshold level, but once this level is reached, further increase of nanoparticles cause toxicity in S. rebaudiana. results clearly indicates that the highest plant height was observed in T_5(ZnO 50PPM)
and minimum plant height was observed in treatment T_6 (ZuO 75PPM)

3.2 No of leaves

The maximum no of leaves was observed in T_5(ZnO 50PPM), and minimum no of leaves was observed in treatment T_{12} (SiO_2 30PPM)

3.3 Chlorophyll-a (mg/g FW)
Results clearly indicates that the highest Chlorophyll -a was observed in T_2 (CuO 50PPM) and minimum Chlorophyll –a was observed in treatment T_6 (ZnO 75PPM).

3.4 Chlorophyll–b (mg/g FW)
Results clearly indicates that the highest Chlorophyll -b was observed in T_5 (ZnO 50PPM) and minimum Chlorophyll -b was observed in treatment T_9 (Zn 75PPM).

3.5Total cholorophyll (mg/g FW) Results clearly indicates that the highest total Chlorophyll was observed in T_5 (ZnO 50PPM) and minimum Total Chlorophyll was observed in treatment T_{10} (SiO_2 10 PPM).

3.6 Carotenoids (mg/g FW)
Results clearly indicates that the highest carotenoids content was observed in T_5 (ZnO 50PPM) and minimum carotenoid content was observed in treatment T_1 (CuO 25 PPM).

3.7 Carbohydrates (mg/g Fw)
Results clearly indicates that the highest carbohydrate content was observed in T_2 (CuO 50PPM) and minimum carbohydrate content was observed in treatment T_{10} (SiO_2 10 PPM).

3.8 Protein (mg/g Fw)
Results clearly indicates that the maximum protein content was observed in T_5 (ZnO50PPM) and minimum protein content was observed in treatment T7 (Zn 10PPM).

Table 1: Morphological parameters

Treatments	Plant height(cm)	No. of leaves
T0 (control)	22.66	38.33
T_1 (CuO-25 ppm)	22.00	46.00
T_2 (CuO-50 ppm)	20.83	48.33
T_3 (CuO-75 ppm)	20.16	38.83
T_4 (ZnO-25 ppm)	27.33	52.33
T5 (ZnO -50 ppm)	34.33	58.00
T6 (ZnO -75 ppm)	21.00	33.66
T7 (Zn-10 ppm)	27.00	53.23
T8 (Zn -20 ppm)	32.33	41.50
T9 (Zn -30 ppm)	30.83	54.00
T10 (SiO_2-10 ppm)	22.16	49.00
T11 (SiO_2-20 ppm)	30.16	54.00
T12 (SiO_2-30 ppm)	21.33	32.33
MEAN	25.55	46.05
C.V	7.26	5.45
S.E	1.52	2.01
C.D.5%	3.12	4.23

Table 2: Biochemical parameters

Treatments	Chl-a (mg/g FW)	Chl-b (mg/g)FW	Total chl (mg/g) FW
T0 (control)	2.76	2.16	5.35
T_1 (CuO-25 ppm)	2.78	1.77	3.73
T_2 (CuO-50 ppm)	2.95	1.94	4.74
T_3(CuO-75 ppm)	2.85	1.62	4.36
T_4(ZnO-25 ppm)	2.77	2.20	5.20
T5(ZnO -50 ppm)	2.93	2.81	5.84
T6(ZnO -75 ppm)	2.35	2.01	5.49
T7(Zn-10 ppm)	2.84	1.41	4.51
T8(Zn -20 ppm)	2.66	1.84	3.42
T9(Zn -30 ppm)	2.75	0.79	3.43
T10(SiO_2-10 ppm)	2.45	0.99	3.28
T11(SiO_2-20 ppm)	2.79	1.52	5.02
T12(SiO_2-30 ppm)	2.76	1.65	4.49
MEAN	2.74	1.75	4.53
C.V	4.66	11.9	5.39
S.E	0.10	0.18	0.20
C.D.5%	0.21	0.35	0.41

Table 3: Biochemical parameters

Treatments	Carotenoids (mg/g FW)	Protein (mg/g)	Carbohydrates (mg/g Fw)
T0(control)	10.81	0.40	0.33
T_1 (CuO-25 ppm)	7.84	0.39	0.30
T_2 (CuO-50 ppm)	11.01	0.38	0.41
T_3(CuO-75 ppm)	11.45	0.34	0.35
T_4(ZnO-25 ppm)	11.31	0.35	0.31
T5(ZnO -50 ppm)	11.85	0.51	0.29
T6(ZnO -75 ppm)	8.01	0.35	0.40
T7(Zn-10 ppm)	11.49	0.33	0.32
T8(Zn -20 ppm)	10.57	0.36	0.31
T9(Zn -30 ppm)	9.33	0.42	0.35
T10(SiO_2-10 ppm)	8.85	0.36	0.27
T11(SiO_2-20 ppm)	10.79	0.45	0.36
T12(SiO_2-30 ppm)	10.60	0.34	0.27
MEAN	10.30	0.38	0.33
C.V	5.86	6.70	6.32
S.E	0.48	0.02	0.01
C.D.5%	1.01	0.043	0.035

4. Conclusion

NPs has altered morpho-chemical properties compared to the control, small size of nanoparticles help to accelerate penetration. In the present study, the concentration dependant positive effect of ZnO NPs was observed on morphological and physiological charecteristics in stevia. The concentration of ZnO and CuO np upto 50ppm showed no phytotoxicity, hence it was effective in improving morphology and physiological aspects of stevia, nano particles like Zn and SiO_2 showed toxic effect after exceeding 10ppm when compared to control.

5. References

1. Allam A, El-Ghareeb AA, Abdul-Hamid M, Baikry A, Sabri MI. Prenatal and perinatal acrylamide disrupts the development of cerebellum in rat: biochemical and morphological studies. Toxicol Ind Health. 2011; 27(4):291-306
2. Anon. BioMed Central launches repository service. Advanced Technology Libraries. 2004; 33:9.
3. Aslani F, Bagheri S, Muhd Julkapli N, Juraimi AS, Hashemi FSG, Baghdadi A. Effects of engineered nanomaterials on plants growth: an overview. Sci World J, 2014.
4. Bhattacharyya Atanu, Firoz Mohammad H, Raja Naika, S Timothy, T Epid, Janardana Reddy *et al*. Research Journal of Nanoscience and Nanotechnology. 2015; 5(2):27-43.
5. Boczkowski J, Hoet P. What's new in nanotoxicology? Implications for public health from a brief review of the 2008 literature. Nanotoxicol. 2010; 4(1):1-14.
6. Da Costa MVJ, Sharma PK. Effect of copper oxide nanoparticles on growth, morphology, photosynthesis, and antioxidant response in *Oryza sativa*. Photosynthetica. 2016; 54(1):110-119.
7. Desai Charmi V, Desai Heta B, Suthar KP, Singh D, Patel RM, Taslim A. Phytotoxicity of zincnanoparticles and its influence on stevioside production in stevia rebaudiana bertoni, Applied Biological Research. 2015; 17(1):1-7.
8. Dey A, Kundu S, Bandyopadhyay A, Bhattacharjee A. Efficient micropropagation and chlorocholine chloride induced stevioside production of Stevia rebaudiana Bertoni. CR Biol. 2013; 336:17-28
9. Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, Casey PS *et al*. Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl2 to a freshwatermicroalga (Pseudokirchneriella subcapitata): the importance of particle solubility. Environ Sci Technol. 2007; 41:8484-8490.
10. Hedge JE, Hofreiter BT. Carbohydrate Chemistry, (Eds. Whistler R.L. and Be Miller, J.N.), *Academic Press*, New York, 1962, 17.
11. Jain JL, Jain S, Jain N. Fundamentals of biochemistry New Delhi: S. Chand & Co. Pub. Ltd. 2007; 104-107.
12. Jiang W, Mashayekhi H, Xing B. Bacterial toxicity comparison between nano- and micro-scaled oxide particles. Environ Pollut. 2009; 157:1619-1625.
13. Kilam D, Saifi M, Abdin MZ, Agnihotri A, Varma A. Endophytic root fungus Piriformospora indica affects transcription of steviol biosynthesis genes and enhances production of steviol glycosides in Stevia rebaudiana. Physiol Mol Plant Pathol. 2017; 97:40-48.
14. Kim S, Lee S, Lee I. Alteration of phytotoxicity and oxidant stress potential by metal oxide in Cucumis sativus. Water Air Soil Pollution. 2012; 223:2799-2806.
15. Lin D, Xing B. Phytotoxicity of nanoparticles. Inhibition of seed germination and root growth. Environmental Pollution. 2007; 150:243-50.
16. Lopez-Moreno ML, de la Rosa G, Hernández-Viezcas JA, Castillo-Michel H, Botez CE, dey JL Peralta-Videa JR *et al*. Evidence of the differential biotransformation and genotoxicity of ZnO and CeO2 nanoparticles on soybean (*Glycine max*) plants. Environ Sci Technol. 2010; 44(19):7315-7320.
17. Lowery OH, Roserbrough NJ, Fan AL, Randal RJ. Protein measurement with the Folin- phenol reagent. J. Biol. Chem. 1951; 193:265-275.
18. Pandey H, Pandey P, Pandey SS, Singh S, Banerjee S. Meeting the challenge of stevioside production in the hairy roots of Stevia rebaudiana by probing the underlying process. *Plant Cell* Tiss Org Cult. 2016; 126:511-521.
19. Perreault F, Samadani M, Dewez D. Effect of soluble copper released from copper oxide nanoparticles solubilisation on growth and photosynthetic processes of *Lemna gibba* L. Nanotoxicol. 2014; 8(4):374-382.
20. Regier N, Cosio C, von-Moos N, Slaveykova VI. Effects of copper-oxide nanoparticles, dissolved copper and ultraviolet radiation on copper bioaccumulation, photosynthesis and oxidative stress in the aquatic

macrophyte *Elodeanuttalli.*Int Chemospher. 2015; 128:56-61.

21. Shi J, Abid AD, Kennedy IM, Hristova KR, Silk WK. To duckweeds (*Landoltiapunctata*), nanoparticulate copper oxide is more inhibitory than the soluble copper in the bulk solution. Environ Pollut. 2011; 159(5):1277-128.
22. Song G, Hou W, Gao Y, Wang Y, Lin L, Zhang Z *et al*. Effects of CuO nanoparticles on *Lemna minor.* Bot Stud Int J. 2016; 57:3.
23. Vives K, Andújar I, Lorenzo JC, Concepción O, Hernández M, Escalona M *et al*. Comparison of different *in vitro* micropropagation methods of Stevia rebaudiana B. including temporary immersion bioreactor (BIT®). Plant Cell Tiss Organ Cult. 2017; 131(1):195-199.
24. Wellborn. Cholorophyll and Caratonoid extraction protocol, 1983.
25. Yadav V. Nanotechnology, big things from a tiny world: a review. AEEE. 2013; 3:771-778.
26. Zafar H, Ali A, Ali JS, Haq IU, Zia M. Effect of ZnO nanoparticles on *Brassica nigra* seedlings and stem explants: Growth dynamics and antioxidative response. Front Plant Sci. 2016; 20(7):535.