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

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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 metallic oxide nanoparticles on stevia with thirteen treatments and three replications each, were laid out in Randomized Block design. This research was under taken to assess the impact of different concentrations of zinc oxide (25,50,75 ppm), copper oxide (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, SiO2, 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) [3]. 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) [20]. 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) [3]. 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 phytotoxicity has been manifested by the generation of reactive oxygen species (ROS), and formation of necrotic lesions as well as yellow pigmentation on the leaves of different crop plants including Lulium 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*
Metallic oxide nanoparticles, specifically nano-scale zinc (Zn) and silicon dioxide (SiO₂) have gained paramount importance in this regard. Based on literature survey ascertained Zn nanoparticles have largely been declared phytotoxic and their phytotoxicity has been manifested by the generation of reactive oxygen species (ROS), and formation of necrotic lesions as well as yellow pigments 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 concentration of 400 and 1000 mg L⁻¹ (Desai et al., 2015) [7]. The production of steviol glycosides has been accomplished.

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]. lgram 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.

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

2.2.2 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 CuSO₄ 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 T₅(ZnO 50PPM) and minimum plant height was observed in treatment T₃(ZnO 75PPM)

3.2 No of leaves
The maximum no of leaves was observed in T₄(ZnO 50PPM), and minimum no of leaves was observed in treatment T₁(ZnO 30PPM)
3.3 Chlorophyll-a (mg/g FW)
Results clearly indicates that the highest Chlorophyll-a was observed in T2 (CuO 50PPM) and minimum Chlorophyll-a was observed in treatment T6 (ZnO 75PPM).

3.4 Chlorophyll-b (mg/g FW)
Results clearly indicates that the highest Chlorophyll-b was observed in T5 (ZnO 50PPM) and minimum Chlorophyll-b was observed in treatment T9 (Zn 75PPM).

3.5 Total chlorophyll (mg/g FW)
Results clearly indicates that the highest total Chlorophyll was observed in T5 (ZnO 50PPM) and minimum Total Chlorophyll was observed in treatment T10 (SiO2 10 PPM).

3.6 Carotenoids (mg/g FW)
Results clearly indicates that the highest carotenoids content was observed in T5 (ZnO 50PPM) and minimum carotenoid content was observed in treatment T1 (CuO 25 PPM).

3.7 Carbohydrates (mg/g FW)
Results clearly indicates that the highest carbohydrate content was observed in T2 (CuO 50PPM) and minimum carbohydrate content was observed in treatment T10 (SiO2 10 PPM).

3.8 Protein (mg/g FW)
Results clearly indicates that the maximum protein content was observed in T5 (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        |
| T1 (CuO-25 ppm)    | 22.00             | 46.00        |
| T2 (CuO-50 ppm)    | 20.83             | 48.33        |
| T3 (CuO-75 ppm)    | 20.16             | 38.83        |
| T4 (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 (SiO2-10 ppm)  | 22.16             | 49.00        |
| T11 (SiO2-20 ppm)  | 30.16             | 54.00        |
| T12 (SiO2-30 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/gFW) | Total chl (mg/g FW) |
|--------------------|-----------------|---------------|---------------------|
| T0 (control)       | 2.76            | 2.16          | 5.35                |
| T1 (CuO-25 ppm)    | 2.78            | 1.77          | 4.33                |
| T2 (CuO-50 ppm)    | 2.95            | 1.94          | 4.89                |
| T3 (CuO-75 ppm)    | 2.85            | 1.62          | 4.47                |
| T4 (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          | 5.50                |
| T7 (Zn-10 ppm)     | 2.84            | 1.41          | 4.25                |
| T8 (Zn-20 ppm)     | 2.66            | 1.84          | 4.49                |
| T9 (Zn-30 ppm)     | 2.75            | 0.79          | 3.54                |
| T10(SiO2-10 ppm)   | 2.45            | 0.99          | 3.24                |
| T11(SiO2-20 ppm)   | 2.79            | 1.52          | 4.31                |
| T12(SiO2-30 ppm)   | 2.76            | 1.65          | 4.49                |
| MEAN               | 2.74            | 1.75          | 4.53                |
| C.V                | 4.66            | 11.90         | 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                    |
| T1 (CuO-25 ppm) | 7.84                  | 0.39          | 0.30                    |
| T2 (CuO-50 ppm) | 11.01                  | 0.38          | 0.41                    |
| T3(CuO-75 ppm) | 11.45                  | 0.34          | 0.35                    |
| T4(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(SiO2-10 ppm) | 8.85                  | 0.36          | 0.27                    |
| T11(SiO2-20 ppm) | 10.79                  | 0.45          | 0.36                    |
| T12(SiO2-30 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 characteristics in stevia. The concentration of ZnO and CuO up to 50 ppm showed no phytotoxicity, hence it was effective in improving morphology and physiological aspects of stevia, nano particles like Zn and SiO2 showed toxic effect after exceeding 10 ppm when compared to control.

5. References

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