

# Agronomic Biofortification of *Stevia rebaudiana* with Zinc Oxide (ZnO) Phytonanoparticles and Antioxidant Compounds

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**Abstract** Agronomic biofortification is a process used to increase the concentration of essential elements in plants through fertilization and thus improve their nutritional quality. Increasing the content of  $Zn^{+2}$  in *Stevia rebaudiana* plants would imply adding value to the crop. The use of ZnO phytonanoparticles, in addition to increasing the content of  $Zn^{+2}$ , can also generate an inductor effect that promotes the synthesis of metabolites of commercial interest. This study reports the effect of fertilization with ZnO phytonanoparticles on morphometric parameters of *S. rebaudiana* plants and the content of  $Zn^{+2}$ , stevioside, rebaudioside A, total phenols, flavonoids and the antiradical activity of leaf extracts. Biofortification was carried out in a hydroponic culture using peat moss/agrolite as a support under a completely random experimental design. The green ZnO nanoparticles were added via root at different concentrations. The physiological and morphometric

parameters of the plant were not affected with respect to the control by the use of phytonanoparticles. The biofortification of *S. rebaudiana* with ZnO phytonanoparticles at a concentration of 75 mg/L enhanced zinc content up to 406.8% with respect to the control. The total phenols (60.5%) and flavonoids (87.8%) were also increased without having a negative effect on plant growth. The biosynthetic pathway of steviol glycosides was not affected.

**Keywords** Biofortification · Zinc oxide phytonanoparticles · Steviol glycosides · Oxidative stress · Antioxidant compounds

## Introduction

*Stevia rebaudiana* (SR) is known and used industrially for its high sweetening potential, which places SR today as the best sugar substitute. Its sweetening power is attributed to steviol glycosides in the leaves. The steviol glycosides (SG) have important antihyperglycemic and antihyperlipidemic properties; therefore, they are also used as an alternative treatment in patients with type 2 diabetes mellitus. These pharmacological properties have been related to the ability of steviol glycosides to stimulate insulin secretion in the pancreas, suppress glucagon secretion and increase glucose transporters GLUT1 and GLUT4 (Jeppesen et al. 2000) and with the presence of  $Zn^{+2}$  since this mineral stabilizes the structure of insulin. In addition,  $Zn^{+2}$  is an important micronutrient because it plays important roles in crop production and human nutrition (Broadley et al. 2007). Approximately, 10% of human proteins require  $Zn^{+2}$  to maintain their catalytic activity (Andreini et al. 2006). Increasing  $Zn^{+2}$  levels in crops will lead to an

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augmentation of  $Zn^{+2}$  in humans. The biofortification of plants is a promising strategy aimed at improving the mineral content of the staple food. Agronomic biofortification defines the process of increasing the concentration of essential elements in portions of plants through fertilization and can improve the nutritional quality in the plant without it suffering genetic modifications (Sida-Arreola 2017).

One of the most innovative methods used for agronomic biofortification is the use of metal nanoparticles, which have been shown to have better assimilation and translocation throughout the plant compared with conventional fertilization (Elemike et al. 2019), and they can cause an elicitor effect that induces the biosynthesis of secondary metabolites of interest in the plant (Marslin et al. 2017). The way in which nanoparticles are absorbed and moved in the different layers of the leaves is similar to the ions/cations of mineral salts. Pérez de Luque (2017) found that positively charged metallic nanoparticles were absorbed faster by the roots than negatively charged ones, whereas the latter were more efficiently translocated to the aerial parts.

Particularly the green synthesis of nanoparticles has been of great interest because of their biocompatibility, low toxicity and ecological nature (Mafuné et al. 2001). Javed et al. (2017a) evaluated the effect of chemically synthesized ZnO nanoparticles in micropropagated shoots of *S. rebaudiana* on the content of steviol glycosides. These authors found that steviol glycosides were enhanced by using up to a 1 mg/L concentration of ZnO nanoparticles in a MS basal medium. Ramezani et al. (2019) determined the effect of chemically and green synthesis of silver nanoparticles on the growth and biochemical parameters in plants of *S. rebaudiana*. In this case, they demonstrated that increasing the AgNP concentration enhances the glycoside content in both treatments and that synthesized AgNP is more effective in accelerating the growth and improving the quality of the natural product of Stevia plants than commercial AgNP. However, there is little information about the biofortification of *S. rebaudiana* using phytonanoparticles of ZnO. The objective of this work was to evaluate the effect of the biofortification of *S. rebaudiana* plants with phytonanoparticles of ZnO on the morphometric parameters of the plant, the  $Zn^{+2}$  content, the stevioside, the rebaudioside A and the phenolic compound contents as well as their antioxidant activity in the dry leaves.

## Materials and Methodology

### Synthesis and Characterization of ZnNPs from *Moringa oleifera*

To obtain the extract of *M. oleifera*, fresh leaves were used, which were washed and dehydrated at 35 °C for 24 h in a

vacuum oven (Lab-line, Squaroid, Markham, Ontario, Canada). Afterward, they were pulverized with an electric mill. Fifty grams of dried leaves was added to 500 mL of distilled water (pH 6.5) and sonicated at 360 W (Cole-Parmer, Vernon Hills, IL, USA) for 2 h at 25 °C (Luján-Hidalgo et al. 2015). The mixture was cooled to room temperature and filtered with a 0.45 µm millipore membrane. The filtered extract of *M. oleifera* was stored in refrigeration at 4 °C for further studies.

For the synthesis of ZnO phytonanoparticles, the methodology described by Chaudhuri and Malodia (2017) with some modifications was used. Fifteen milliliters of aqueous extract of *M. oleifera* was added to 1.647 g of zinc ( $ZnSO_4$ ) dissolved in 35 mL of distilled water (with a total concentration 0.2 M of solution). The reaction mixture was subjected to magnetic stirring for 6 h. Subsequently, 2 M NaOH (4 g of NaOH in 50 mL of distilled water) was added to the solution and placed in an incubator at 60 °C with constant magnetic stirring for 12 h. The colloidal solution was centrifuged (HERMLE, Germany) at 4500 rpm for 20 min. The precipitate was subjected to 2 consecutive washes with ethanol (96%) and distilled water. The precipitate was dried in an oven at 45 °C–50 °C, and finally, a fine powder was obtained with the help of a mortar.

The size and morphology of the ZnO phytonanoparticles were determined with a scanning electron microscope (SEM) (JEOL 6010) at an accelerating voltage of 10 kV according to Abdelmoteleb et al. (2016). Energy-dispersive X-ray spectroscopy analysis (EDS) was carried out to determine the chemical purity and elemental composition of the ZnO phytonanoparticles. The samples were drop-coated onto carbon film and analyzed using the Oxford instrument Thermo EDS (Ramalingam et al. 2013).

### Plant Cultivation and Biofortification with Phytonanoparticles (ZnO)

The experiment was performed in the greenhouse of the Instituto Tecnológico de Tuxtla Gutierrez, in the state of Chiapas in Mexico. *S. rebaudiana* plants were grown in plastic seedlings for 25 days with peat moss/agrolite as support (4/1 w/w). A nutrient solution (Steiner 1961) without zinc was used. After the selection of uniformity by stem length and number of shoots, the plantlets were transplanted into polyethylene bags (20 × 30) with the same support. The plants were kept in an outdoor greenhouse at a temperature not exceeding  $35 \pm 2$  °C, and the frequency of irrigation was every 48 h, with substrate humidity not exceeding 70%. Subsequently, the application of phytonanoparticles (NPs) of ZnO was carried out in a radicular way to Stevia plants at different concentrations within 5 treatments including T1 (the control without zinc),

T2 (50 mg/L ZnO NPs), T3 (75 mg/L ZnO NPs), T4 (100 mg/L ZnO NPs) and T5 (13.86 mg/L ZnSO<sub>4</sub>, equivalent to 100 mg/L of phytonanoparticles).

### Physical and Morphometric Parameters of *S. rebaudiana*

The height and diameter measurements of the main stem of the plant were performed with a digital caliper. Measurements were taken at the beginning and at the end (7 weeks) of the experimentation. In relation to the leaves and root mass, these were previously washed and dried in a vacuum oven (Lab-line, Squaroid, Markham, Ontario, Canada) at 35 °C for 24 h. Finally, an analytical balance (Discovery, Ohaus, USA) was used to determine the dry mass.

### Preparation of *S. rebaudiana* Extract

To obtain the extract of *S. rebaudiana*, dried leaves were taken and pulverized with the help of a mill until they reached the size of a 100-mesh particle. The samples (150 mg) were dispersed in 1.5 mL of 60% isopropyl alcohol. The mixture was sonicated at 360 W (Cole-Parmer, 08,855–00, Vernon Hills, IL, USA) for 18 min at 30 °C (Gasmalla et al. 2014). Finally, the mixture was centrifuged at 10,000 rpm at room temperature (HERMLE, Germany) for 10 min, and the supernatant was adjusted to 1.5 mL with isopropyl alcohol.

### Zinc Content in the Leaves

The total zinc content of the biofortified dried leaves of *S. rebaudiana* was performed under the methodology of González-Terreros et al. (2018). The method used is an adaptation of method 3052 described by the US EPA (Environmental Protection Agency), using an inductively coupled plasma optical emission spectrometry spectrometer (ICP-OES) (4300DV-PerkinElmer, USA). Three hundred milligrams of dried and powdered leaves was taken and subjected to acid digestion by refluxing with nitric acid (9 mL), 30% hydrogen peroxide (2 mL) and hydrochloric acid at 180 °C (1 mL) for 1.5 h. After digestion, the samples were filtered with Whatman No. 40 paper and adjusted to 25 mL.

### Steviol Glycosides Quantification

The total stevioside and rebaudioside A content of the biofortified leaves of *S. rebaudiana* was performed under the methodology reported by Aranda-González et al. (2014) with some modifications using HPLC-UV-Vis (High-performance liquid chromatography with a UV-Vis detector, Flexar, PerkinElmer, USA). The mobile phase

used was acetonitrile (solvent A) and Na<sub>2</sub>HPO<sub>4</sub> (buffer; 10 mM, pH 2.7 with phosphoric acid) (solvent B) with a column flow of 1 mL/min and an elution gradient from a 68% rate (A) for 0.5 min, reducing solvent (A) to 50% in 10 min. Subsequently, the solvent (A) decreased to 32% in 5 and held for 3 min. Washings were performed at the end of the injection at 80% (A)/20% (B) for 5 min and 32% (A)/68% (B) for 5 min. Chromatographic separation was performed with a Kromasil C18 5 $\mu$ -100 column with dimensions of 4.6  $\times$  150 mm (Kromasil, Bohus, Sweden). Detection was performed at 210 nm at 25 °C. External standards (Sigma Aldrich, 50956, 38462) were used for the quantification of stevioside and rebaudioside A contents expressed as mg g<sup>-1</sup> of dry leaf.

### Total Phenols Quantification

The total phenolic content was determined with the Folin–Ciocalteu reagent (Singleton et al. 1999). A 0.1 mL aliquot of the aqueous extract from *S. rebaudiana* leaves was diluted with 4.2 mL of distilled water and 0.5 mL of the Folin–Ciocalteu reagent. The mixture was vortexed (IKA 3, Wilmington, North Carolina, USA) for 1 min, and then, 1 mL of 20% Na<sub>2</sub>CO<sub>3</sub> was added and allowed to stand at room temperature in the dark for 2 h. Quantification was performed on a UV-Vis spectrophotometer (Beckman Coulter, DU 730, USA) at 765 nm. The total phenolic content was expressed as gallic acid equivalents (mg GA g<sup>-1</sup> dry leaf).

### Flavonoid Quantification

The total flavonoid content in leaves was determined by the colorimetric method of aluminum trichloride as described by Chang et al. (2002). A 0.5 mL aliquot of the ethanol extract of *S. rebaudiana* leaves was mixed with 2.8 mL of water, 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum trichloride and 0.1 mL of potassium acetate (1 M). The mixture was vortexed (IKA 3, Wilmington, North Carolina) and allowed to stand for 30 min. The absorbance was measured with a UV-Vis spectrophotometer (Beckman Coulter, DU 730, USA) at 424 nm. Quercetin was used as standard solutions. The total flavonoid content was expressed as quercetin equivalents (mg QE g<sup>-1</sup> dry leaf).

### Antioxidant Activity

The total antioxidant capacity of the extract was carried out using 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) with the methodology described by Shen et al. (2010). A solution of 1 mM DPPH in methanol was prepared, and 1.0 mL of this solution was mixed with 3.0 mL of extract at different concentrations (1.0–10  $\mu$ g/mL) dissolved in

methanol. The sample was left in the dark at room temperature (25 °C) for 30 min and measured at 517 nm on a spectrophotometer (Beckman Coulter, DU 730, USA) calibrated with methanol. All tests were performed in triplicate. The blank sample was 1 mL of methanol solution with DPPH (1 mM) with 3 mL of distilled water.

The percentage of DPPH radical inhibition was calculated with the help of the following equation:

$$AAr(\%) = \frac{A_{bm} - A_m}{A_{bm}} \times 100$$

where Aar is the antiradical activity (%), A<sub>bm</sub> is the absorbance of the blank sample, and A<sub>m</sub> is the absorbance of the sample.

### Statistical Analysis

The design used was completely randomized with 15 repetitions per treatment. All experiments were repeated with three replicates. The results were analyzed using a simple ANOVA, and the means were compared using the LSD (least significant difference) test ( $p < 0.05$ ) with Statgraphics Centurion XVII statistical software.

## Results and Discussion

### SEM and EDX Analysis of ZnO Phytonanoparticles from *M. oleifera*

The synthesis of ZnO phytonanoparticles from *M. oleifera* was confirmed by the change of color from dark green to light green, suggesting that ZnSO<sub>4</sub> dissociates into Zn<sup>+2</sup> to Zn<sup>0</sup> by the reduction action of phytochemicals present in *M. oleifera* extract. The profile obtained from the SEM micrograph of ZnNPs shows that synthesized phytonanoparticles are elongated and rod-shaped, with sizes ranging from 0.1 to 60 μm and an average size of 13 μm (Fig. 1). Kuppusamy et al. (2016) reported that the shape of the phytonanoparticles depends on many factors, with among the most important being the composition of the extract (reducing agents), the type of precursor molecule, the pH of the colloidal solution, the temperature and the reduction time. There is no report, however, of any green synthesis method that generates a specific form of phytonanoparticles and to which any particular application has been attributed (Wang et al. 2016). The EDX analysis established the incidence of elemental zinc as strong signal energy peaks around 1.0 keV, successfully verifying the ZnNP biosynthesis (Fig. 1). In addition, the EDX profile of the biosynthesized sample indicates the presence of zinc by 22.62, silica 24.20, sodium 23.26, carbon 10.06 and oxygen 25.72 (weight %). The ZnNPs of *M. oleifera* used in this

experiment were previously diluted with deionized water at 100 ppm.

### Physical and Morphological Parameters

One of the most widely used culture systems for monitoring plant growth parameters is the hydroponic system (Dimkpa et al. 2013; Watson et al. 2015). In this system, it was observed how the height, stem diameter, area and root biomass had no significant statistical differences ( $p > 0.05$ ) between the treatments compared with the control (the absence of zinc) (Table 1). Therefore, it is suggested according to the results that the application of ZnO phytonanoparticles to the concentrations used in *S. rebaudiana* plants does not have a negative effect on the development and growth of the plant, even at 100 mg/L.

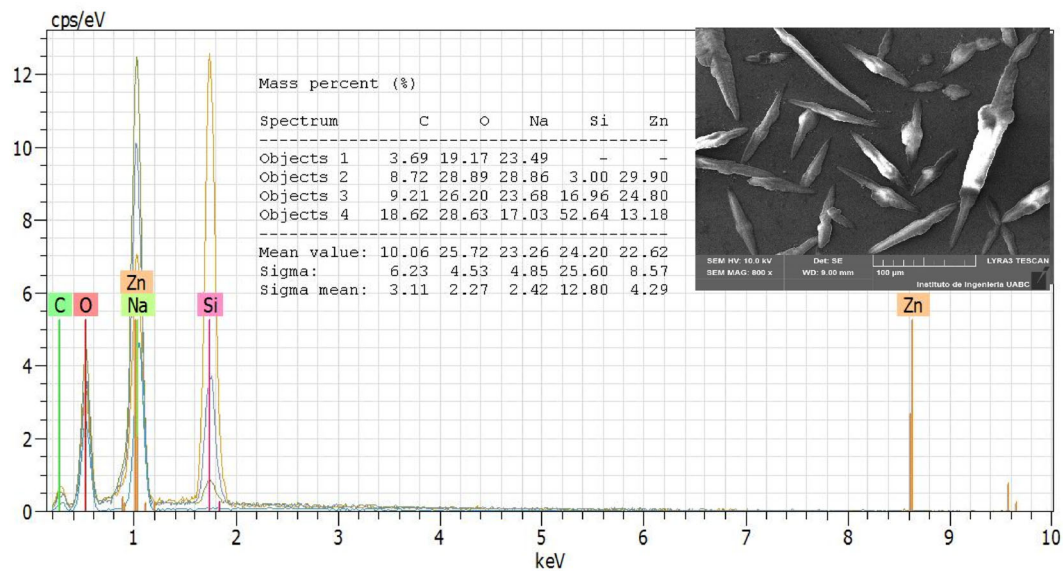
The reactivity of metallic oxide NPs depends on their size, surface, structure, concentration, dissolution and exposure routes (Chang et al. 2012). At present, there is not enough information to correlate forms of ZnO phytonanoparticles with any specific activity or biological effect in plants because during the biosynthesis of green nanoparticles (phytonanoparticles), their physical and chemical characteristics depend on the reducing compounds that are used (vegetable extract). Javed et al. (2017a) evaluated the effect of chemical nanoparticles of ZnO in the in vitro culture of *S. rebaudiana*, reporting that at high concentrations (1000 mg/L), they have phytotoxic effects, but that at 1 mg/L of the nanoparticles in the culture medium, a higher percentage of shoot formation was observed.

Raliya and Tarafdar (2013) obtained a positive effect in foliar applications of ZnO nanoparticles in *Cyamopsis tetragonoloba* plants with a threefold increase in the total chlorophyll concentration compared with the untreated treatment, suggesting an important role of Zn<sup>+2</sup> in the physiological processes of the plant such as photosynthesis and respiration and in various enzymatic reactions. Singh and Dwivedi (2019) mentioned that the leaves were larger and greener after the treatment with the foliar spraying of zinc sulfate, giving an appearance of increased leaf area in *S. rebaudiana*. Nevertheless, for all plants, ZnO nanoparticles will have different structural and functional effects; everything depends on the frequency, concentration and mode of application.

### Biofortification of *S. rebaudiana* Plant with Zn<sup>+2</sup>

The Zn<sup>+2</sup> content in the leaves of biofortified plants with phytonanoparticles increased significantly compared with the control treatment (Table 2). Results showed that Zn<sup>+2</sup> content in leaves increased significantly ( $p < 0.005$ ) up to 12 times compared with plants without biofortification





**Fig. 1** Characterization of the biosynthesized ZnNPs by *M. oleifera*. Energy-dispersive X-ray spectrometer profile (EDS) and magnified SEM micrograph (× 800)

**Table 1** Physical and morphometric parameters of *Stevia rebaudiana* Bertoni plants subjected to different treatments

Treatment	ZnO NPs content (mg L <sup>-1</sup> )	Stem length (cm)	Physical and morphometric parameters		
			Stem diameter (cm)	Foliar biomass (g plant <sup>-1</sup> DW)	Root biomass (g plant <sup>-1</sup> DW)
Control	Control (0)	18.006 ab	2.551	2.235 a	0.750 ab
ZnO NPs	50	19.021 ab	2.879 a	2.314 a	0.871 ab
ZnO NPs	75	20.561 a	2.725 a	2.376 a	0.876 ab
ZnO NPs	100	18.207 ab	2.747 a	2.520 a	1.093 a
ZnSO <sub>4</sub>	13.86	19.733 a	2.844 a	2.493 a	0.906 ab
LSD (0.05)		2.894	0.458	0.975	0.608

LSD: Least significant difference. Values with the same letter in each column have no statistically significant difference between treatments (LSD  $p \leq 0.05$ ). Treatments 2, 3 and 4 correspond to ZnO NPs, and treatment 5 corresponds to ZnSO<sub>4</sub>

when 100 mg/L of the phytonanoparticles was applied. Nanoparticles in general are absorbed in a similar way as micronutrients (Fraceto et al. 2016). These are assimilated by root hairs and can take the 2 proposed routes for the assimilation of macro- and micronutrients (Lambrea et al. 2015), the symplastic and/or apoplastic pathway (Tripathi et al. 2017). The main advantage of the use of nanoparticles is that they cross the Casparian strip and incorporate into the xylem, thus translocating throughout the plant (Lv et al. 2015). The xylem serves as the most important vehicle in the distribution and translocation of nanoparticles (Aslani et al. 2014).

Treatment 5 (13.86 mg/L ZnSO<sub>4</sub>) is equivalent to the concentration of treatment 4 (100 mg/L) of phytonanoparticles according to Chen et al. (2018). The zinc content (Table 2) in the leaves biofortified with

phytonanoparticles (treatment 4), however, was 155% higher than the treatment with salts of ZnSO<sub>4</sub> (treatment 5). This behavior can be attributed to the fact that phytonanoparticles can be assimilated and translocated more efficiently by their size and charge throughout the plant. This fact is reinforced given that the biofortification was carried out via the roots and the quantification of zinc was evaluated in the leaves.

### Steviol Glycosides Content

In relation to the content of stevioside and rebaudioside A, in Table 2 it is observed that there is a significant statistical difference ( $p < 0.05$ ) between treatments. It was found that 50 mg/L of phytonanoparticles (treatment 2) promotes higher concentrations of stevioside in the leaves compared

**Table 2** Stevioside and rebaudioside A content in *Stevia rebaudiana* Bertoni plants subjected to different treatments

Treatment	ZnO NPs (mg L <sup>-1</sup> )	Zinc (mg kg <sup>-1</sup> )	Stevioside (mg g <sup>-1</sup> )	Rebaudioside A (mg g <sup>-1</sup> )
Control	0	2.312 c	2.421 b	51.244 c
ZnO NPs	50	7.488 bc	4.143 a	43.853 c
ZnO NPs	75	11.717 b	2.787 ab	56.467 a
ZnO NPs	100	28.157 a	2.917 ab	39.759 c
ZnSO <sub>4</sub>	13.86	11.023 bc	2.345 b	53.227 ab
LSD (0.05)		9.238	1.565	12.685

LSD: Least significant difference. Values with the same letter in each column have no statistically significant difference between treatments (LSD  $p \leq 0.05$ ). Treatments 2, 3 and 4 correspond to ZnO NPs, and treatment 5 corresponds to ZnSO<sub>4</sub>

with the control, while to 100 mg/L of phytonanoparticles (treatment 4), the rebaudioside A content decreases. Javed et al. (2017b) reported similar results in the steviol glycosides content employing 100 mg/L of CuO NPs during the in vitro culture of *S. rebaudiana*.

Several authors have reported that the use of compounds such as methyl jasmonate, spermidine, salicylic acid, some auxins and nanoparticles (ZnO and CuO) of chemical synthesis has an effect on the biosynthesis of secondary metabolites mainly in the synthesis of steviol glycosides and phenolic compounds (Ramezani et al. 2020; Mohar-ramnejad et al. 2019; Lucho et al. 2018; Yoneda et al. 2018; Javed et al. 2017a, b; Javed et al. 2018). Therefore, ZnO phytonanoparticles could be acting similarly in the synthesis of steviol glycosides. Table 2 also shows that treatment 3 with 75 mg/L of ZnO NPs had no statistically significant differences with the control, suggesting that at this concentration, biofortification did not have a negative effect on steviol glycoside biosynthesis.

### Total Phenolic Content, Total Flavonoid Content and Antiradical Activity

The total phenolic compounds and total flavonoid contents increased significantly compared with the control treatment ( $p < 0.05$ ) in the presence of ZnO phytonanoparticles and ZnSO<sub>4</sub>. Table 3 shows an increase of 60.38% in the total

phenolic compounds and 87.79% in the flavonoids with respect to the control. The antioxidant activity in the leaves biofortified with 75 mg/L of ZnO phytonanoparticles showed the lowest IC<sub>50</sub> value, which is the minimum concentration necessary for inhibiting 50% of free radicals and may indicate that the use of ZnO phytonanoparticles and ZnSO<sub>4</sub> promotes the biosynthesis of compounds with antioxidant activity.

The content of phenolic compounds has been shown to increase after nanoparticle treatments (Comotto et al. 2014; Ghorbanpour and Hadian 2015; Vecerova et al. 2016; Javed et al. 2017b). The increase in these compounds may be due to lipid peroxidation and oxidative stress (production of H<sub>2</sub>O<sub>2</sub>) caused by the abiotic stress to which the plant is subjected; one of the strategies to reduce this damage is the activation of the enzymatic antioxidant defense system, where the production of phenolic compounds is included. González-Chavira et al. (2018) reported similar results when evaluating hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as inducers in *S. rebaudiana*, reporting an increase in the percentage of free radical inhibition.

In addition, the antioxidant capacity could be influenced by the abundance of certain metals since many of them, including Zn<sup>+2</sup>, act as cofactors for enzymes, transcription factors and signaling proteins (Zhu et al. 2013). These include several enzymes with the function of eliminating reactive oxygen species (Sida-Arreola et al. 2017; Rico

**Table 3** Content of total phenols, flavonoids and their antioxidant activity in extract leaves from *Stevia rebaudiana* Bertoni plants subjected to different treatments

Treatment	ZnO NPs (mg L <sup>-1</sup> )	Total Phenols (mg GA g <sup>-1</sup> )	Flavonoids (mg QE g <sup>-1</sup> )	DPPH IC <sub>50</sub> (µg mL <sup>-1</sup> )
Control	0	19.438 c	2.655 b	9.226 a
ZnO NPs	50	28.863 b	3.202 b	8.439 b
ZnO NPs	75	31.198 a	4.986 a	7.531 c
ZnO NPs	100	30.487 a	4.624 a	7.951 bc
ZnSO <sub>4</sub>	13.86	31.075 a	4.634 a	7.556 c
LSD (0.05)		1.549	0.850	0.765

LSD: Least significant difference. Values with the same letter in each column have no statistically significant difference between treatments (LSD  $p \leq 0.05$ ). Treatments 2, 3 and 4 correspond to ZnO NPs, and treatment 5 corresponds to ZnSO<sub>4</sub>

et al. 2015; Sharma et al. 2012). The enrichment of Zn<sup>+2</sup> could increase nutritional quality and antioxidant activity as functional foods.

## Conclusion

The agronomic biofortification of *S. rebaudiana* with ZnO phytonanoparticles did not show a negative effect on the physical and morphometric parameters compared with an ionic biofortification (ZnSO<sub>4</sub>); however, the zinc content was 240.31% higher with NPs. Thus, it is possible to suggest that zinc in the form of nanoparticles is more assimilable than zinc in ionic form (ZnSO<sub>4</sub>). In addition, the steviol glycosides content was not affected. The biofortification with zinc (NPs, ZnSO<sub>4</sub>) showed an increase in the production of compounds with antioxidant activity. Therefore, the biofortification of *S. rebaudiana* plants with ZnO NPs helps to improve the nutritional quality of the crop and possibly potentiate its pharmaceutical effects; however, it is still necessary to continue with research aimed at evaluating the cytotoxic effect of the extract obtained from biofortified plants with phytonanoparticles.

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**Author contributions** LH conceived the idea. VG, RH, VS, GM and GT did experimental work and wrote the manuscript. LH, AA, GM and GM analyzed the data, critically reviewed the manuscript and added to its technical part. All authors have contributed, seen and approved the manuscript.

## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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