# Biochemical Role of Some Nanoparticles in the Production of Active Constituents in *Stevia Rebaudiana* L. Callus

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**Abstract:** The use of modern techniques in the field of nanotechnology is one of the important recent trends for improving active constituents production in the field of tissue culture, for using on a commercial scale. Therefore, in this study we examined the effect of three types of nanoparticles (FeNPS, CuNPS and SiNPS) at different concentrations on the production of active constituents in *Stevia rebaudiana* L. callus. In light of the obtained results, treatment with nanoparticles had a positive effect on dry weights at all concentrations. GSH level changes in stevia callus represent a good indicator of response to treatment with nanoparticles. A slight decrease occurred in the GSH content when stevia callus treated with FeNPS and CuNPS at most concentrations. While, it was increased after treatment with all concentrations of SiNPS (except 8 ppm). With respect to antioxidant enzymes, it noticed a slight effect on enzymes activity when stevia callus treated with FeNPS and CuNPS. While, there was a clear positive effect when using SiNPS, where a new band for SOD appeared at 0.5 ppm as well as a clear increase in bands intensity at the other concentrations. Also, SiNPS had effective influence on CAT activity, especially at high concentrations. FeNPS and CuNPS had inhibitory effect on active constitute production in stevia callus with the exception of 2 and 8 ppm FeNPS and 8 ppm CuNPS, which recorded the highest values of stevioside content. While, the low concentrations of SiNPS affected positively on stevioside content, the maximum value was recorded when SiNPS applied at rate of 2 ppm. The effect of nanoparticles on minerals content in stevia callus depends on the nanoparticles type and the concentrations used, but in general, SiNPS had a negative effect on the accumulation of some minerals such as Si and Cu. The researchers in this study recommended activating the application of nanotechnology in the agricultural field for the production of active constituents in stevia callus, because of their medical and industrial importance. The study confirms the effectiveness of SiNPS (in some low concentrations), FeNPS and CuNPS (in some high concentrations) for the production of active constituents and associated with antioxidants (enzymatic and non enzymatic). Also, this study draws attention for the need to complete the work on these nanoparticles with new concentrations and new particle sizes.

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**Key words:** *Stevia rebaudiana* L. callus, Nanoparticles, Active constituents and Antioxidants.

**Abbreviations**: **FeNPS**, nano iron oxide; **CuNPS**, nano copper oxide; **SiNPS**, nano silicon dioxide; **GSH**, glutathione; **CAT**, catalase; **SOD**, superoxide dismutase.

## 1. Introduction

In recent years remarkable progress has been made in developing nanotechnology. This science has emerged into the limelight and become the focus of the most countries and it will enable us to get the materials which are characterized by high quality, purity and free of impurities. The growth of nanotechnology has led to the rapid development of commercial application which involves the use of a great variety of manufactured nanoparticles and which have the ability to revolutionize the agriculture and food industry. Although, there is a crucial urgency to perform further studies on the use of nanoparticles in the agricultural field. It is worth noting that knowledge gaps and associated uncertainties remain unaddressed on the effects of nanoparticles on plants. Nanoparticles with a size of between 1 and 100 nanometers (**Ball, 2002; Roco, 2003** and **Monica and Cremonini, 2009)** fall in the transitional zone between individual atoms (or molecules) and the bulk material. Because the physicochemical properties of material on this scale can greatly differ from the corresponding bulk material, these nanoparticles can have the potential to generate unknown biological effects in living cells. In this connection, **Monica and Cermonini (2009)** showed that nanomaterials because of their tiny size show unique characteristics (change in the physic–chemical properties) and have great surface area compared to their bulk materials, therefore increasing solubility and surface reactivity. The effect of nanoparticles on plants depending on the concentration, size, shape and other physicochemical properties as well as the kind of

genotypes. There are many researches used nanoparticles like nano silicon (**Suriyaprabha *et al.*, 2012; Kalteh *et al.*, 2014** and **Siddiqui and Al-Whaibi, 2014),** nano iron **(Afshar *et al.*, 2012; Afshar *et al.*, 2013** and **Dhoke *et al.*, 2013)** and nano copper **(Lee *et al.*, 2008; Dimkpa *et al.*, 2012** and **Sahar, 2014)** on plant species**.** However, the production of active constituents in tissue culture (like *Stevia rebaudiana* L.) using nanotechnology technique takes center late (or limited) in the uses list of this science.

Stevia is a natural non calorie sweetener plant. Stevia leaves contain sweet components (steviol glycosides) and it varies between 4 and 20% in the dry leaves. The main sweet component in stevia is stevioside and tastes about 300 times sweeter than sucrose. Stevioside is a diterpenic carboxylic alcohol with three glucose molecules **(Kohda *et al.*, 1976** and **Shibata *et al.*, 1991)** and mainly used commercially as sugar substitute. In addition to its interesting sweetening property, stevia extract shows many pharmacological properties **(Gregersen *et al.*, 2004**; **Din *et al.*, 2006** and **Ferri *et al.*, 2006)**. Other sweet compounds present in stevia leaves but in lower concentration are: steviolbioside, rebaudioside A, B, C, D, E, F and dulcoside A (**Kennelly, 2002** and **Starrat *et al.*, 2002)**. Stevia seeds are small in size and very low germination percentage (infertile), so the production of stevia through seeds is not fruitful **(Savita *et al.*, 2004)** and the seeds propagation is given great variability in sweetening levels and composition **(Nakamura and Tamura, 1985** and **Jadeja *et al.*, 2005)**. Due to the above mentioned difficulties, plant tissue culture or micropropagation can be used for rapid propagation and conservation of such valuable and endangered plant species **(Nalawade *et al.*, 2002** and **Debnath *et al.*, 2006)**, which are difficult to propagate by conventional methods. Also, tissue culture technique used in modern laboratories for the production of some active constituents in large quantities and short time for plants that have a problem to propagate them by traditional methods. The present study examined the effects of three types of nanoparticles (iron oxide, copper oxide and silicon dioxide) on the production of active constituents in *Stevia rebaudiana* L. callus.

## 2. Materials and Methods

Stevia plants were obtained from Sugar Crop Institute, Agricultural Research Center, Giza, Egypt. The plants were maintained under greenhouse conditions of the Desert Research Center, Cairo, Egypt, for at least 30 days prior to removal of material for culture. Leaves were removed from the branches and transferred immediately to the laboratory for sterilization. The leaves were washed for 15 minutes in running tap water then rinsed in sterile distilled water and sterilized under aseptic conditions by immersion for 20 minutes in 20% (v/v) commercial bleach (Clorox) followed by 3 minutes in 0.1% (w/v) mercuric chloride solution then washed 6 times with sterile distilled water to remove the traces of mercuric chloride. Callus was induced from leaves of stevia plant according to procedures described by **Hendawey and Abo El Fadl (2014).** All sterilized leaves were cultured on basal MS medium (**Murashige and Skoog, 1962**) (Duchefea, Haarlem, Netherlands) supplemented with 2 mg/l dichlorophenoxy acetic acid (2,4-D), 0.5 mg/l naphthalene acetic acid (NAA) and 0.5 mg/l 6-benzyl aminopurine (BAP) (Sigma Cell Culture, min. 90%, St. Louis, USA), 30 g/l sucrose and solidified with 3 g/l phytagel (Duchfea, Haarlem, Netherlands). The cultures were then incubated at approximately 24ºC with 16-hours photoperiod under cool white florescent tubes (F140 t9d138, Toshiba). After six weeks callus was formed, then a piece of sub cultures callus (100mg) was placed on various concentrations of nanoparticles. The experiment included three treatments of nanoparticles with six levels compared with control as follows:

- Control (without nanoparticles application).
- Nano iron oxide ($Fe_3O_4$) at 0.1, 0.5, 1, 2, 4 and 8 ppm.
- Nano copper oxide (CuO) at 0.1, 0.5, 1, 2, 4 and 8 ppm.
- Nano silicon dioxide ($SiO_2$) at 0.1, 0.5, 1, 2, 4 and 8 ppm.

Nanoparticles were prepared at different concentrations using distilled water. Nano iron oxide (50-100 nm), nano copper oxide (< 50 nm) and nano silicon dioxide (5-15 nm) were purchased from Sigma-Aldrich. Also, fresh and dry weights of stevia callus (g) were recorded after 10 weeks of cultures.

**Chemical analysis**

**Glutathione content**

The glutathione content (GSH) was measured by the method of **Moron *et al.* (1979).**

**Antioxidant enzymes**

Superoxide dismutase (SOD) was estimated according to **Weydert and Cullen (2010).** Also, catalase activity (CAT) was determined according to the method described by **Maxwell and Bateman (1967)**.

**Sweet component (stevioside content)**

Stevia callus was extracted by mortaring in methanol according to the method of **Brandle (1998)** and **Nikolai *et al*. (2001).** The stevioside obtained by methanol extract analyzed by High Performance Liquid Chromatography (HPLC) as described by **Nishiyama *et al*. (1992)** and **Hendawey and Abo El Fadl (2014).** The HPLC system was a Dionex Ultimate 3000 equipped with an auto-sampler, quaternary pump and a

diode array detector. The analytical column was BDS Hypersil C8 column. Separation was performed with acetonitrile and water (85: 15 v/v) as the elusion solvent at flow rate of 0.7 ml/min and the detection wavelength was 205/210 nm. Under these analytical conditions, the typical retention time of stevioside was 2.32 min. It is worth mentioning that the standard addition was done using stevioside pure material to confirm the results, before the analysis of stevioside in samples of stevia callus. Standard addition is a technique that helps qualify dubious test results. The reason for using the standard addition of stevioside is due to the samples of stevia contain other components that interfere with the stevioside causing inaccuracy in the determined concentration. In addition, the separation of stevioside carried out using column C8, which differed from column C18 in their separation. The idea is to add known volume of stevioside (known concentration) to the sample and the change in peak area was noticed. The change in peak area between the sample and the sample with standard is assumed.

**Minerals content**

Constant weight of stevia callus samples were digested in nitric acid and hydrogen peroxide using Microwave Digestion Labstation closed system, Ethos Pro, Milestone, Italy. Then, raise the digested to a known volume using distilled water. Fe, Cu and Si were determined by Inductively Coupled Argon Plasma, ICAP 6500 Duo, Thermo Scientific, England. Also, 1000 mg/L multi-element certified standard solution, Merck, Germany was used as stock solution for instrument standardization.

**Statistical analysis**

The experiment was subjected to completely randomized design. Analysis of variance (**ANOVA**) and **Duncan's** multiple range test (**1955**), as modified by **Snedecor and Cochran (1982)**, were performed to analyze the obtained data.

## 3. Results and Discussion

**Effect of nanoparticles on callus growth**

Data recorded in **Figs. (1 and 2) and Table (1)** showed the effect of some nanoparticles on growth of *Stevia rebaudiana* L. callus. It was clear from the data that treatment with nano iron oxide (FeNPS) had a positive effect on fresh weight when stevia callus treated only with 0.25 and 0.5 ppm compared with the control. On the other hand, FeNPS had a positive effect on dry weight at all concentrations, but the highest value was recorded at 0.5 ppm. The positive role of FeNPS on stevia callus may be due to it plays a key role in growth and development (**Miller *et al.*, 1995** and **Sheikhbaglu *et al.*, 2014)**.


**Fig. 1: Subculture of *Stevia rebaudiana* L. callus after treatment with (1) 0.5 ppm FeNPS (2) 1 ppm CuNPS (3) 8 ppm SiNPS**

The results showed that fresh weight was increased when nano copper oxide (CuNPS) applied (except 4 and 8 ppm), while there was a clear increase in dry weight at all concentrations of CuNPS. The highest value of fresh and dry weights was obtained when stevia callus treated with 1 ppm compared with the control. In this regard, copper contributes in many important physiological processes within the plants, as well as the high concentrations of copper had many adverse effects on plants (**Weckx and Clijsters, 1996; Hall, 2002** and **Monnet *et al.*, 2006 )**.

**Table 1: Effect of some nanoparticles on *Stevia rebaudiana* L. callus culture**

| Callus properties | Treatments | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | FeNPS (ppm) | | | | | | CuNPS (ppm) | | | | | | SiNPS (ppm) | | | | | |
| | Con | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 0.25 | 0.5 | 1 | 2 | 4 | 8 |
| Texture | F | F | F | F | F | F | F | F | F | F | F | F | F | C | C | C | C | C | C |
| Colour | Cr | L | L | L | B | B | B | G | G | G | B | B | B | Cr | Cr | Cr | Cr | Cr | Cr |

**Where;**
**Con=** Control**, F=** Friable**, C=** Combact**, L=** Light green**, B=** Brown**, G=** Green and **Cr=** Creamy to yellow

Although, copper is an essential micronutrient, it may become phytotoxic when present in excess in the growth medium. Exposure of plants to excess Cu generates oxidative stress, leading to cellular damage generated by reactive oxygen species, elevations in $H_2O_2$ and significant DNA impairment (**Lequeux *et al.*, 2010; Cuypers *et al.*, 2011** and **Iseri *et al.*, 2011**). Given that copper undergoes complexation with organic compounds that could modify its toxicity (**Jumg *et al.*, 2003**).

Data in the same figure describes the effect of nano silicon dioxide (SiNPS) on fresh and dry weights of stevia callus. Treatment with 4 and 8 ppm led to increase of fresh weight compared with the control. While, dry weight of stevia callus was increased with the increasing of SiNPS. The positive effect of silicon on growth of stevia callus may be due to it prevents the structural and functional deterioration of cell membranes **(Agarie *et al.*, 1998)**, also reduced osmolyte leakage and lipid peroxidation **(Shen *et al.*, 2010)**.

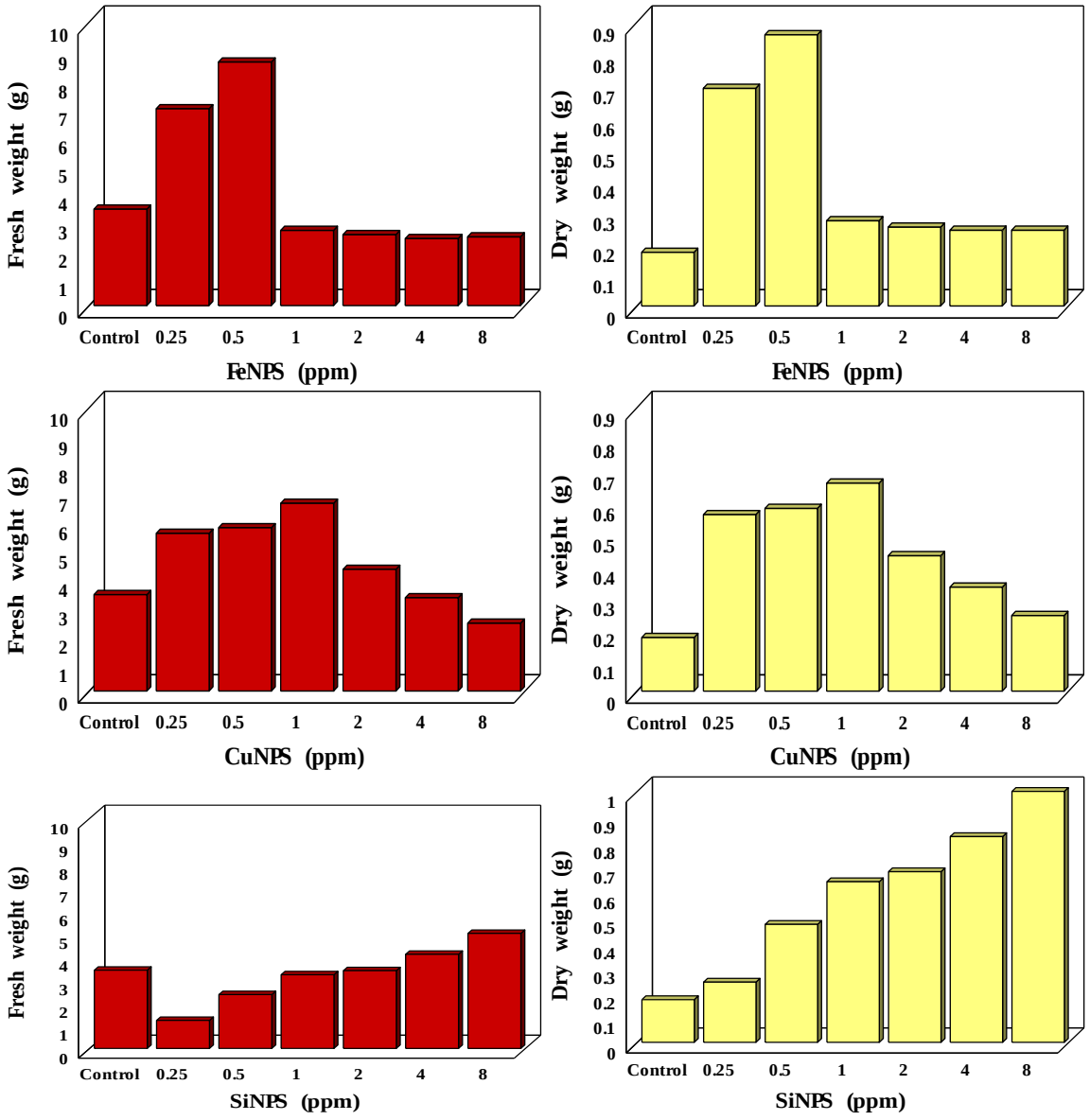


**Fig.2: Effect of some nanoparticles on fresh and dry weights in *Stevia rebaudiana* L. callus**

**Effect of nanoparticles on biochemical constituents**
**Glutathione content**

Effect of three types of nanoparticles (FeNPS, CuNPS and SiNPS) at different concentrations on glutathione content (GSH) in *Stevia rebaudiana* L. callus is presented in **Fig. (3)**. A slight decrease occurred in GSH content when stevia callus treated with FeNPS at all concentrations, except 2 ppm which recorded the highest value of GSH content compared with the control (without nanoparticles).

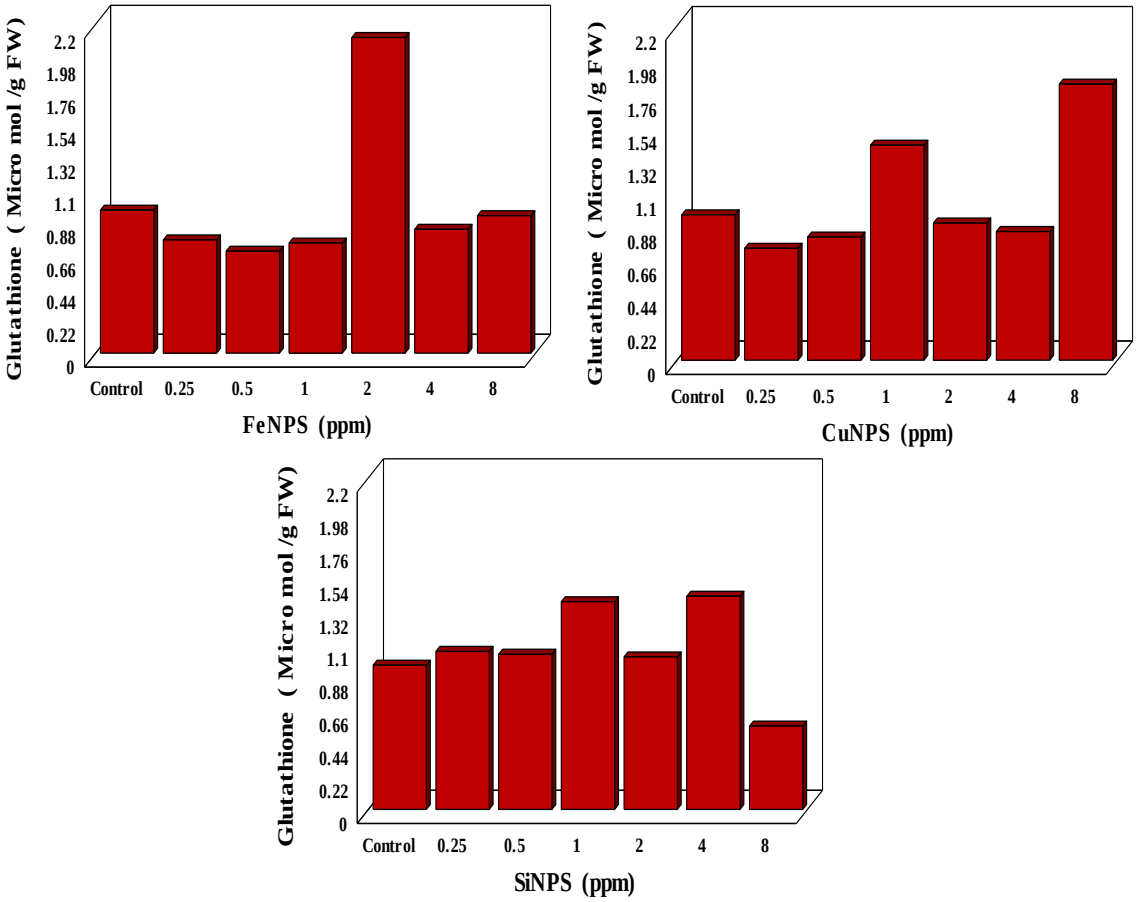


**Fig.3: Effect of some nanoparticles on glutathione content (µ mol /g FW) in *Stevia rebaudiana* L. callus**

In this regard, GSH is an important antioxidant in plants, preventing damage to important cellular components caused by reactive oxygen species (**Pompella *et al.*, 2003)**. It is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side-chain and the amine group of cysteine (which is attached by normal peptide linkage to a glycine). In addition, **Zaharieva and Abadia (2003)** and **Salama *et al.* (2009)** found marked increases in GSH content in cucumber, sugar beet and flax under iron deficiency.

From the results in the previous figure, treatment with CuNPS at rates 0.25, 0.50, 2 and 4 ppm led to a slight decrease in GSH content in stevia callus compared with the control. While, the maximum values were recorded when CuNPS applied at rates of 1 and 8 ppm. Antioxidants like glutathione plays an important role in detoxification of toxic metal ions **(Singh and Sinha, 2005).** Also, **Garrido *et al.* (2010)** showed that the change in levels of reduced glutathione may represent a good indicator of the early plant response to stress due to excessive Cu supply. In this regard, **De Vos *et al.* (1992)** showed that copper caused a marked decrease glutathione in *Silene cucubalus*. While, **Aly and Mohamed (2012)** showed that level of glutathione in maize was increased with increasing of Cu stress.

In light of the results obtained, GSH content was increased in stevia callus after treatment with all concentrations of SiNPS (except 8 ppm) compared with the control. These results are in agreement with **Ali *et al.* (2013),** reporting that Si has a key role in the enhancement of plant antioxidant potentials such as GSH content in sunflower. Also, **Saqib *et al.* (2008)** showed that GSH content enhances with applications of Si in wheat.

**Antioxidant enzymes**

**Superoxide dismutase**

Superoxide dismutase (SOD) constitutes the first and one of the main links of the defense process against free radicals. Superoxide ion is the starting point in the chain production of free radicals. At this early stage, SOD inactivates the superoxide ion by transforming it into hydrogen peroxide. The latter is then quickly catabolised by catalase and peroxidases into oxygen and water (**Menvielle-Bourg, 2005** and **Angaji *et al.*, 2012).**

**Table 2: Effect of some nanoparticles on superoxide dismutase (SOD.) in *Stevia rebaudiana* L. callus**

| Band Number | Treatments / Band intensity / FeNPS (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | 0.25 | 0.50 | 1 | 2 | 4 | 8 |
| 1 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| 2 | 2.99 | 3.61 | 4.04 | 2.90 | 1.66 | 2.72 | 1.80 |
| |  | | | | | | |
| Band Number | CuNPS (ppm) | | | | | | |
| | Control | 0.25 | 0.50 | 1 | 2 | 4 | 8 |
| 1 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| 2 | 2.99 | 2.22 | 3.07 | 2.44 | 3.30 | 3.03 | 2.73 |
| |  | | | | | | |
| Band Number | SiNPS (ppm) | | | | | | |
| | Control | 0.25 | 0.50 | 1 | 2 | 4 | 8 |
| 1 | 00 | 00 | 1.00 | 00 | 00 | 00 | 00 |
| 2 | 2.99 | 4.00 | 2.98 | 4.48 | 3.51 | 3.69 | 4.24 |
| |  | | | | | | |

**Where; 00= refers to no band, 1.00= refers to lowest band intensity and 4.48 = refers to highest band intensity**

Data in the **Table (2)** showed the effect of some nanoparticles on SOD banding patterns in stevia callus and revealed the presence of about 2 bands. Band (No.2) is presented in all stevia samples after treatment with all nanoparticles and the control. However, band (No.1) was detected only in stevia callus when treated with SiNPS at rate of 0.5 ppm. Concerning band intensity, band number 2 was increased when FeNPS applied at rates 0.25 and 0.50 ppm compared with the control. Also, there was increasing in band intensity after treatment with CuNPS at rates 0.50, 2 and 4 ppm. In the same direction, treatment of SiNPS at all concentrations (except 0.50 ppm) showed increased of band intensity compared with the control. In addition, band No.2 was recorded the maximum value of band intensity when SiNPS applied at rate 1 ppm, while band No.1 was recorded the lowest value after treatment with the same nanoparticles at rate 0.5 ppm compared with the control. There are many researches that studied SOD in stevia such as **Ahmad *et al.* (2011), Sabah and Rasha (2013)** and **Arnold (2015).** In addition, **Salama *et al.* (2009)** showed that antioxidant enzymes activity can be used as reliable biochemical biomarkers for assessing the iron efficiency in flax cultivars. In the same direction, **Tewari *et al.* (2005)** and **Esfndiari and Sabaghnia (2012)** showed that SOD activity significantly decreased under iron deficiency conditions**.** The trace element copper is required as a cofactor for several processes; **Asada (1999)** found that Cu/ZnSOD requires Cu, along with Zn, as cofactors to catalyze the dismutation of superoxide radicals into hydrogen

peroxide in the chloroplast stroma. Also, **Cohu and Pilon (2007)** found that regulation of superoxide dismutase expression by copper availability in some plants. In another study**, Azooz *et al.* (2012)** showed that SOD could serve as important components of antioxidative defense mechanism against copper toxicity. Concerning the effect of silicon on SOD activity, **Ahmad and Haddad (2011)** found that Si enhanced SOD activity, also prevent the oxidative membrane damage. On the other hand, **Al-aghabary *et al.* (2004)** and **Luxova *et al*. (2009)** showed that SOD activity was decreased after treatment with silicon in maize and tomato plants under salt stress.

**Catalase activity**

Catalase (CAT) is involved in the destruction of hydrogen peroxide that is generated in cells. This enzyme catalyzes the decomposition of hydrogen peroxide to $O_2$ and water and thus provides protection against the toxic effects of hydrogen peroxide**.** As shown in **Fig. (4),** it is obvious that treatment with FeNPS tended to increase CAT activity in stevia callus (except 0.5 and 8 ppm) compared with the control. The maximum value was recorded when FeNPS applied at rate of 4 ppm. There are many researches that studied CAT enzyme in stevia such as **Ahmad *et al.* (2011), Sabah and Rasha (2013) and Arnold (2015).** Also**, Esfndiari and Sabaghnia (2012)** showed that CAT was decreased in wheat leaves under iron deficiency conditions. Regarding the effect of CuNPS on CAT activity, it was noticed that a positive effect on the activity of enzyme when stevia callus treated with 2 and 8 ppm. Also, application of 2 ppm gave the highest value of CAT activity compared with the control. In this regard, CAT can serve as an important component of antioxidative defense mechanism against copper toxicity (**Azooz *et al.*, 2012)**. Also, **Lombardi and Sebastiani (2005)** showed that CAT activity and expression can be modulated in response to copper excess. In addition, there is a clear correlation between CAT and copper concentrations in *Jatropha curcas* L. seedlings, also differed between plant tissues **(Gao *et al.*, 2008)**. It is quite clear from results that treatment with SiNPS enhanced CAT activity (except 0.25 ppm). In addition, the highest value of CAT activity was obtained by stevia callus after treatment with 1 ppm compared with the untreated callus. In light of the previous results, nano silica particles prevent oxidant damages via increasing of antioxidant enzymes activity and decreasing of free radicals, as well as, protect the plant's physiological processes against stresses **(Roohizadeh *et al.*, 2014)**. Also, **Ahmad and Haddad (2011)** found that Si enhanced CAT activity, also prevent the oxidative membrane damage.

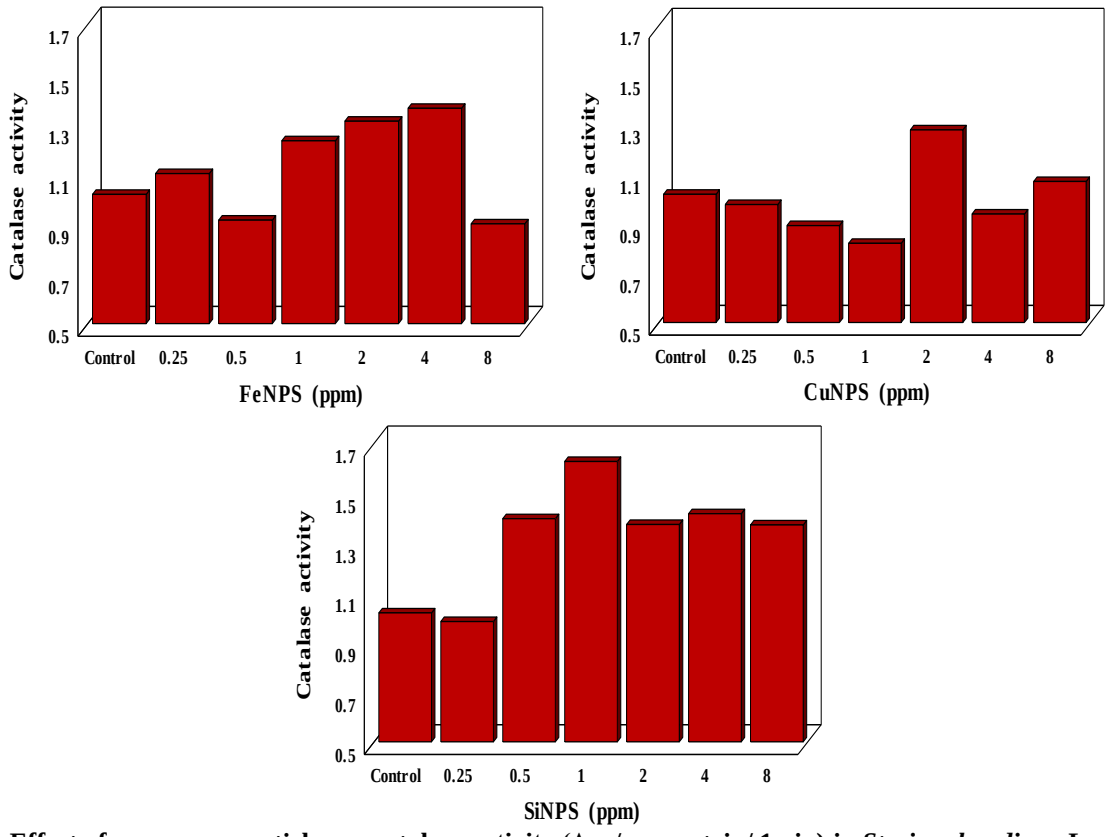


**Fig. 4: Effect of some nanoparticles on catalase activity ($\Delta_{240}$/mg protein/ 1min) in *Stevia rebaudiana* L. callus**

**Stevioside content**

The effect of some nanoparticles on stevioside content in stevia callus is presented in **Fig. (5)**. Data showed that stevioside content was increased when callus treated with the high concentrations of FeNPS. The highest value was recorded at rate of 8 ppm compared with the control. On the other hand, a clear reduction occurred in stevioside content at low concentrations (0.25, 0.50, 1 and 4 ppm). Iron is needed in very small quantities for adequate plant growth and production, their deficiency may cause great disturbance in physiological and metabolic processes involved in the plant. According to **Brittenham (1994)**, iron is a cofactor for approximately 140 enzymes that catalyze unique biochemical reactions. In this regard, iron fills many essential roles in plant growth and development (**Miller *et al.*, 1995** and **Sheikhbaglu *et al.*, 2014)**.

Regarding the effect of CuNPS on stevioside content, the results showed that it had inhibitory effect on active constitute production in stevia callus with the exception of high concentration (8 ppm), where it recorded the highest value of stevioside content compared with the control. Copper is an essential element for plants because it is involved in a number of physiological processes, but in excess it is also a proven inhibitor of various physiological functions (**Monnet *et al.*, 2006**). In other words, **Weckx and Clijsters (1996)** and **Hall (2002)** found that copper toxicity led to the generation of harmful reactive oxygen species, which can damage biological molecules and membranes. In the same direction, **Hansch and Mendel (2009)** showed that excess of Cu concentrations may induce a significant toxic effect by altering the protein function and enzymes activity.

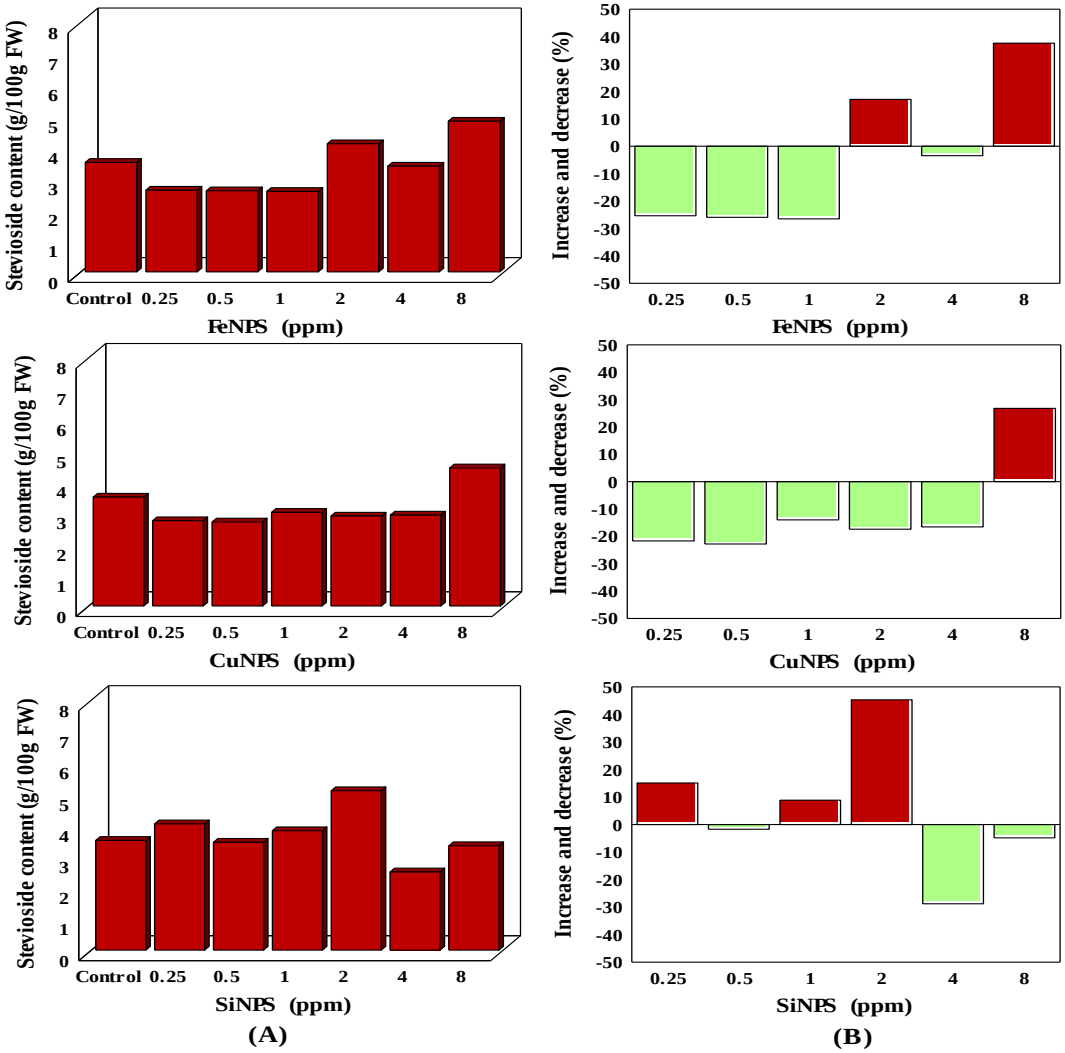


**Fig. 5:(A) Effect of some nanoparticles on stevioside content (g /100g fresh weight) in *Stevia rebaudiana* L. callus. (B) Increase and decrease (%) of stevioside content compared with the control (without nanoparticles)**

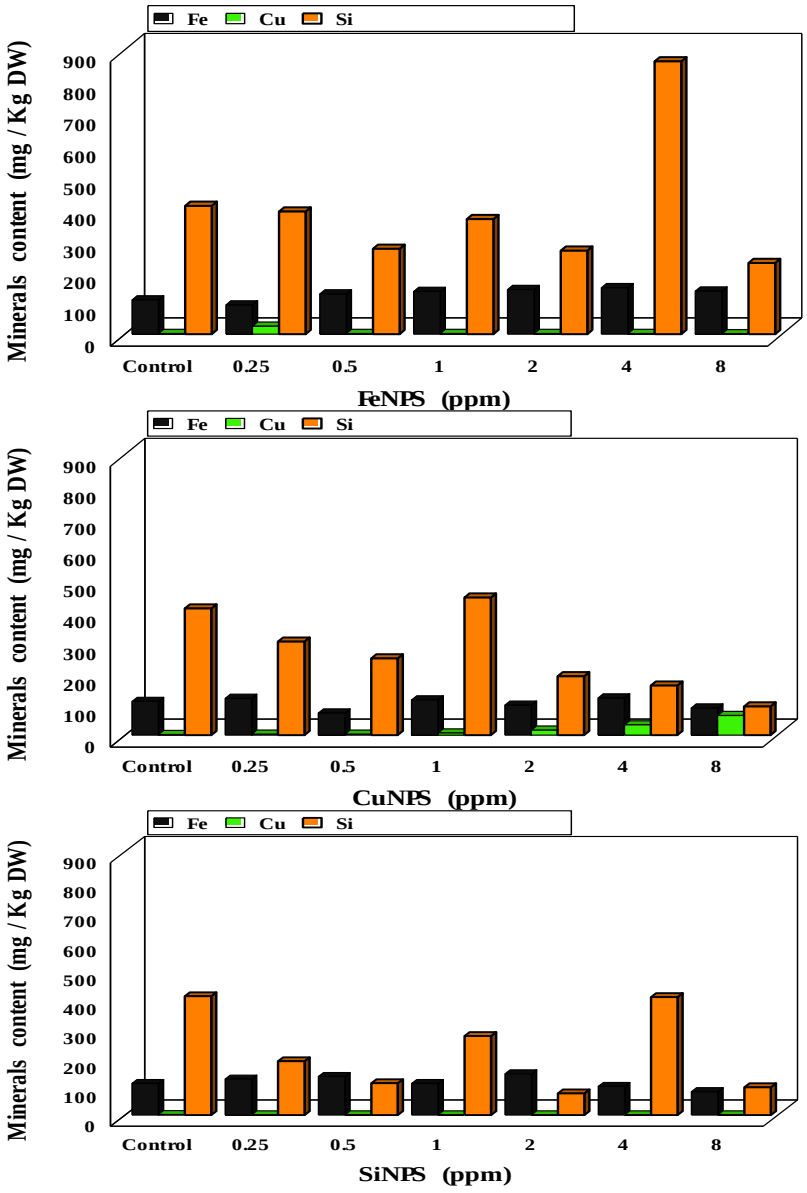


**Fig. 6: Effect of some nanoparticles on minerals content in *Stevia rebaudiana* L. callus**

Results in the same figure showed that the low concentrations of SiNPS (0.25, 1 and 2 ppm) affected positively on stevioside content, while the high concentrations (0.5, 4 and 8 ppm) affected negatively compared with the control. The maximum value was recorded when SiNPS applied at rate of 2 ppm. The important role of silicon in the plant cell is probably due to: i) The positive effect of silicon on growth where produced the greatest biomass yield **(Eneji *et al.*, 2008** and **Bakhat *et al.*, 2009)**. ii) Effect of silicon on tissue strength where it prevents the structural and functional deterioration of cell membranes **(Agarie *et al.*, 1998)**, also silicon reduced osmolyte leakage and lipid peroxidation **(Shen *et al.*, 2010)**. iii) Si may be involved in the metabolic or physiological and/or structural activity **(Liang *et al.*, 2003)**, as well as, increased antioxidant defense activities, alleviated oxidative damage and maintained many physiological processes **(Gong *et al.*, 2005)**.

There are also researches that study the production of stevioside in stevia callus **(Chen and Li, 1993; Sivaram and Mukundan, 2003** and **Das *et al.*, 2006).** In the same direction, **Hendawey and Abo El Fadl (2014)** used some chemical inducers to produce

stevioside in stevia callus. They showed that treatments with inducers had a promotive role in enhancing active constituent (stevioside content) in stevia callus.

**Minerals content**

The results found in **Fig. (6)** describes the effect of some nanoparticles on minerals pattern in stevia callus. Under FeNPS treatment conditions, it was noticed a clear decrease in silicon content (except 4 ppm) compared with the control. On the other hand, it was observed a positive effect on iron content (except treatment with 0.25 ppm). While, application of FeNPS had a clear negative effect on the content of Cu (except 0.25 ppm) compared with the control. In this regard, **Celik et *al.* (2010)** showed that the highest concentrations of iron had a negative effect on some macronutrients element contents in maize. Also, **Pooladvand *et al.* (2012)** found that iron content in soybean plants increased with the increase of iron concentration.

With respect the effect of CuNPS on minerals content in stevia callus, it was noticed a clear increase in copper content at all concentrations used. Also, there was increasing in iron content (except 0.5, 2 and 8 ppm) compared with the control. In contrast, a clear reduction occurred in silicon content (except 1 ppm) after treatment with CuNPS. There are many researches that show the effect of copper on the minerals content in plants (**Farias *et al.*, 2013** and **Azeez *et al.*, 2015**).

Data presented in the same figure showed that iron content was increased in stevia callus after treatment with SiNPS at rates 0.25, 0.5 and 2 ppm compared with the control. On the other hand, SiNPS treatment had inhibitory effect on the accumulation of Si and Cu in stevia callus. There are many researches that show the effect of silicon on some minerals content in plants such as **Matoh *et al.* (1986)** and **Rogalla and Römheld (2002).**

In this study, it is worth mentioning that there is no published researches on the effect of nanoparticles (FeNPS, CuNPS and SiNPS) on the accumulation of active constituents (stevioside content), antioxidants (GSH, CAT and SOD) and minerals (iron, copper and silicon) in *Stevia rebaudiana* L. callus.

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