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Employing silicon nanoparticles and GA_3 in enhancing the productivity and biochemical attributes of sweet-leaf plant (*Stevia rebaudiana* Bert.)

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Abstract
Stevia rebaudiana Bert. is a sweetener herb with great medicinal potential the present study is aimed to elucidate the effects of combined application of silicon-nanoparticles and gibberellic acid (GA_3) on *S. rebaudiana*. The plants were applied various treatments *viz*. deionized water (Control), 50 ppm GA_3, 200 ppm Nano-200 (Aerosil), 200 ppm Nano-300 (Aerosil), GA_3 + Nano-200 (Aerosil) and GA_3 + Nano-300 (Aerosil). The application of both GA_3 and SINPs applied individually through foliage was found effective, though, the concomitant effect of the silicon-nanoparticles and GA_3 was much pronounced compared to their alone application. The data analyses revealed that combined treatment [GA_3 + Nano-300 (Aerosil)] has proved the optimum concentration for the studied plant. The applied silicon-nanoparticles together with GA_3-induced metabolic changes altogether significantly improved overall plant growth and biochemical attributes.

Keywords: Aerosil nanoparticles, gibberellic acid, productivity, *Stevia rebaudiana*

Introduction
Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are the secondary plant metabolites produced by these plants. These plant metabolites, according to their composition, are grouped as alkaloids, glycosides, corticosteroids, essential oils, etc. Stevia (*Stevia rebaudiana* Bertoni) (Family- Asteraceae), locally known as Madhupatra, is known as sweet herb. It is a perennial plant. The leaves are mild green and intensely sweet. The plant bears greenish cream flowers in autumn (Soejarto 2002 [24]; Mishra *et al*. 2010 [8]; Yadav *et al*. 2011) [28]. Stevioside and Rebaudioside are the sweetening compounds in the leaves (Kinghorn and Soejarto 1985 [12]; Yadav *et al*. 2011) [28]. These compounds might be more than 200 times sweeter than sugar (Singh and Rao 2005 [22]; Goyal *et al*. 2010 [6]; Marcinek and Krejpcio 2016) [17].
Currently consumers have more inclination towards products that are claimed to be 'All Natural 'and 'Low CHO'. Hence, the food industry could grab a major share in the market if Stevia, the natural sweetener, is used as sweetening agent in products like biscuits, jams, chocolates, ice-creams, baked foods, soft drinks, soda, candies and also common beverages like dip tea, coffee and herbal tea that are used for diabetic patients and also for health conscious consumers. The size, shape and controlled disparity of nanoparticles play a vital role in determining the physical, chemical, optical and electronic properties of substances, supporting its applications in environmental, bio technological and biomedical fields (Vuong 2019) [26]. Nano particles of silicon dioxides have marked effect on protein structure (Xu *et al*. 2010) [27]. Nanoparticles have significant effects on plants *via* interaction With cytochrome c (cyt c), deoxyribonuclease (DNA II) and haemoglobin (Hb) (Xu *et al*. 2010) [27]. Nanoparticles of silicon-dioxide exhibits growth and physiological responses in plants (Siddiqui *et al*. 2015 [20]; Vuong 2019) [26]. Gibberellic acid (GA_3) is a very potent plant growth regulator whose endogenous levels control growth and development in plants (Mander 2003 [16]; Taiz *et al*. 2014) [25]. It also exhibits a broad spectrum of physiological effects in plants (Taiz *et al*. 2014 [25]; He *et al*. 2020). GA_3 is tetracyclic diterpene acid, promoting growth and elongation of cell. It may be used to increase fruit set and is reported to inhibit formation of root in cuttings. Its higher concentration results in adversely tall and weak flower stems. Keeping this in view, it was decided to conduct a pot experiment to test whether the foliar spray of GA_3 and silicon nano-particles alone or in combination could ameliorate the growth characteristic and biochemical parameters of Madhupatra (*Stevia rebaudiana* Bert.).

Materials and Methods
The objective of this experiment was to study the effect of foliar applications of GA_3 and silicon nano-particles (aerosil 200 and aerosil 300) alone and in combination on growth parameters (shoot length per plant, root length per plant, and fresh and dry weights per plant) and physiological and biochemical parameters (total chlorophyll and carotenoids content, nitrate reductase activity, carbonic anhydrase activity and leaf-NPK contents) of the plant. Before starting the experiment, the soil samples used for filling the earthen pots (25 cm diameter × 25 cm height), were collected and analysed in the soil-testing laboratory, Government Agriculture Farm, Quarsi, Aligarh, for its physico-chemical properties. The soil analysis data are given in Table 1.

Table 1: Physico-chemical properties of soil

S. No.	Characteristics	Value
1.	Texture	Sandy loam
2.	pH	7.8
3.	Conductivity	0.57 d Sm^{-1}
4.	Available nitrogen (mg/kg soil)	98.5
5.	Available phosphorus (mg/kg soil)	7.15
6.	Available potassium (mg/kg soil)	141.8

Prior to transplanting, 5 kg homogeneous mixture of soil and cow dung manure in the ratio of 5:1 was filled in the earthen pots. The soil was maintained at proper moisture to ensure better growth of the plants. 30 days old seedlings of *Stevia rebaudiana* were obtained from CIMAP, Lucknow. Healthy seedlings of uniform size were selected for transplanting and one seedling per pot was maintained. The experiment was conducted according to simple randomized design using a net-house of the Department of Botany, Aligarh Muslim University, Aligarh. There were five replicates for each treatment. The plants were kept free from weeds and irrigated as and when required. Sampling was carried out at 90 days after planting (DAP). All the spray treatments were given at seven days interval. Spray treatments of the pot experiment are displayed Table 2.

Table 2: Experimental treatments

S.N.	Treatment	Detail
1.	Control	Spray of deionized water only
2.	Treatment 1	Spray of 50 ppm solution of GA_3
3.	Treatment 2	Spray of 200 ppm of Nano-200 (Aerosil)
4.	Treatment 3	Spray of 200 ppm of Nano-300 (Aerosil)
5.	Treatment 4	Spray of GA_3 + Nano-200 (Aerosil)
6.	Treatment 5	Spray of GA_3 + Nano-300 (Aerosil)

A. Growth characters

The above ground plant height (shoot length) and root length were measured by meter scale. Fresh plants were weighed using electronic balance. The plants were dried in an oven at 80 °C for 48 h and subsequently the dry weighed of plants were measured with the help of the electronic balance.

B. Physiological and biochemical parameters

a. Estimation of chlorophyll and carotenoid content

The total chlorophyll content was estimated by the method of Mac Kinney (1941) [14]. 100 mg of fresh leaf tissue from interveinal leaf area was grinded with mortar-pestle using 10 mL of 80% acetone. The suspension was filtered through Whatman filter paper No.1 and the filtrate was collected in a volumetric flask. Optical density (OD) was recorded at 645 and 663 nm wavelength using a spectrophotometer (UV-1700 Shimadzu, Japan). The absorbance value used for the calculation of total chlorophyll content was estimated. Carotenoids content was also estimated by the method of Mac Lachlan and Zalik (1963) [15]. The procedure for the preparation of extract was same as that of chlorophyll content. However, the OD was recorded at 480 and 510 nm.

b. Estimation of carbonic anhydrase activity

Carbonic anhydrase activity in fresh leaves was analyzed using the method described by Dwivedi and Randhawa (1974).

The fresh copped leaf pieces (200 mg) were transferred to Petri plate, containing 10 mL of 0.2 M cysteine hypochloride. They were left for 20 min at 4 °C. Thereafter, the leaf pieces were blot-dried and transferred to a test tube containing 4 mL of phosphate buffer (pH 6.8). To this it, 4 mL of 0.2 M sodium carbonate and 0.2 mL of 0.022% bromothymol blue were added. The test tubes were shaken gently and left for 20 min at 4 °C. CO_2 liberated by catalytic activity of carbonic anhydrase on sodium bicarbonate ($NaHCO_3$) was estimated by titrating the reaction mixture against 0.05 N hydrochloric acid (HCl), using methyl red as indicator. In each sample, the quantity of HCl used to neutralise the reaction mixture, was noted and the difference was calculated. A blank consisting of all the above components of reaction mixture except leaf sample was run simultaneously with each set of sample.

c. Estimation of nitrate reductase (NR) activity

The activity of nitrate reductase in fresh leaves was estimated by the method of Jaworski (1971) [9]. The leaves were chopped into small pieces. 200 mg of these chopped leaves were transferred to plastic vial. To it, 2.5 mL of phosphate buffer (pH 7.5), 0.5 mL of potassium nitrate solution and 2.5 mL of 5% isopropanol was added. The reaction mixture was incubated at 30°C in a BOD for 2 h. Later, to a test tube carrying 0.4 mL of the incubated reaction mixture, 0.3 mL each of sulphanilamide solution and NED-HCl was added. The test-tubes were left for 20 min for maximum colour development. The OD of the coloured reaction mixture was recorded at 540 nm using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). A blank solution was run simultaneously with each sample. Standard curve was plotted by using the graded concentration of $NaNO_2$ (sodium nitrite) solution. The absorbance (OD) of each sample was compared with that of calibration curve.

d. Estimation of leaf-N, -P and -K content

Leaf N, P and K content was estimated in dry powder of leaves. For estimation of N, P and K content, the leaf powder was digested according to standard technique described below.

e. Digestion of leaf powder

One hundred mg of the oven-dried leaf powder was transferred to 50 mL digestion tube, to which 2 mL of concentrated sulphuric acid (H_2SO_4) was added. The tube was kept in the digestion assembly at 80 °C for about two h to allow complete reduction of nitrates present in the leaf tissue. Ultimately, the content of digestion tube turned black. After cooling the content for about fifteen minutes, 0.5 mL of 30% hydrogen peroxide (H_2O_2) was added drop by drop and the solution was heated again till the colour of the solution turned from black to light yellow. Again, after cooling for about 30 min 3-4 drops of H_2O_2 were added, followed by heating for another 5 minutes. The addition of H_2O_2, followed by heating, was repeated until the content of the flask turned colourless. The peroxide digested material was transferred from the digestion tube to a 100 mL volumetric flask with three washings of distilled water (DW). The volume of the flask was then made up to the mark using DW.

f. Estimation of leaf nitrogen (N) content

The method of Lindner (1944) was adopted for the estimation of leaf-N. 10 mL aliquot of the digested material was taken in a 50 mL volumetric flask. To it, 2 mL of 2.5N NaOH solution (appendix) and 1 mL of 10% sodium silicate solution (appendix) were added to neutralize the excess of

acid and to prevent turbidity, respectively. The volume of the solution was made up to 50 mL using DW. 5 mL of this solution was taken in a test tube and 0.5 mL of Nessler's reagent (appendix) was added. The content of the test tube was allowed to stand for five min for maximum colour development. The OD of the solution was then recorded at 525 nm using the spectrophotometer. The reading of each sample was compared with the standard calibration curve. Thus, the percent leaf-N was determined on dry weight basis.

g. Estimation of leaf phosphorus (P) content

The method of Fiske and Subba Rao (1925) was adopted for the estimation of leaf-P. 5 mL of the peroxide digested sample was taken in a 20 mL graduated test tube and 1mL of molybdic acid was added carefully followed by the addition of 0.4 mL of 1-amino-2-naphthol-4-sulphonic acid. The colour of the solution turned blue. The final volume was made up to 100 mL using DW. The solution was shaken well and allowed to stand for about 5 min for maximum colour development. The O.D. of the solution was then recorded at 620 nm using the spectrophotometer. A blank consisting of DW, molybdic acid and 1-amino-2-naphthol-4-sulphonic acid was run simultaneously. The reading of each sample was compared with a standard calibration curve, determining the percent leaf-P on the dry weight basis.

h. Estimation of leaf potassium (K) content

Leaf-P content in the aliquot was determined according to (Hald 1947) with the help of a flame-photometer (Model, C150, AIMIL, India), using a specific filter for K emission spectrum. The test solution (aliquot) was discharged through an atomizer in the form of a fine mist into a chamber, where it was drawn into the flame. Combustion of the element (potassium) produced light of a particular wavelength [λmax for K = 767 nm (violet)]. The light produced was passed through the appropriate filter to impinge upon a photoelectric cell that activated a galvanometer leading to a digital display of the potassium content in the leaf as per the emission spectrum constructed.

i. Statistical analysis

One-way ANOVA (Analysis of variance) was applied to test differences among the treatments. Duncan's multiple range test (DMRT ≤ 0.05%) was also used to identify different treatment followed by ANOVA. Standard error of replicates was also presented in the Tables. Statistical significance level was considered as 0.05%, using SPSS (ver: 22) statistical program.

Experimental Results

In a pot experiment, the effect of foliar spray of GA_3 and silicon nano-particles was studied on growth characteristics, physiological and biochemical attributes.

A. Growth characters

The spray of GA_3 + Nano-200 (Aerosil) resulted in maximum shoot length. The next value was given by GA_3 in this regard. Treatment GA_3 + Nano-200 (Aerosil) gave 32.60% higher value than the water sprayed control (Table 3). The spray of GA_3 + Nano-300 (Aerosil) gave maximum value for root length. The next value was given by GA_3 in this regard. Treatment GA_3+Nano-300 (Aerosil) resulted in 23.44% longer roots compared to the water sprayed control (Table 3).

The spray of GA_3 + Nano-200 (Aerosil) gave the maximum value for plant fresh weight.

The next value was given by GA_3 in this regard. Spray of GA_3 + Nano-200 (Aerosil) increased the plant fresh weight by 76.60% over the water sprayed control (Table 3). The spray of GA_3 + Nano-200 (Aerosil) gave the maximum value for plant dry weight. The next value was given by GA_3 in this regard. Treatment GA_3 + Nano-200 (Aerosil) improved the plant dry weight by 72.02% as compared to the water sprayed control (Table 3).

Table 3: Effect of GA_3 and silicon nano-particles on growth parameters of *Stevia rebaudiana* (Means of five replicates+SE). Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$).

Treatments/ parameters	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
Control	59.33 ± 2.91^{b}	21.33 ± 1.76^{b}	31.33 ± 0.88^{b}	8.33 ± 0.88^{c}
GA_3	75.00 ± 2.89^{a}	26.00 ± 1.52^{a}	54.00 ± 4.16^{a}	12.33 ± 1.45^{ab}
Nano-200 (Aerosil)	60.00 ± 2.89^{b}	22.66 ± 1.20^{b}	34.66 ± 2.40^{b}	7.33 ± 1.20^{c}
Nano-300 (Aerosil)	60.00 ± 4.00^{b}	21.00 ± 1.00^{b}	31.33 ± 0.88^{b}	8.33 ± 1.20^{c}
GA_3+Nano-200 (Aerosil)	78.67 ± 2.40^{a}	26.00 ± 1.15^{a}	55.33 ± 4.37^{a}	14.33 ± 0.88^{a}
GA_3+Nano-300 (Aerosil)	74.66 ± 2.60^{a}	26.33 ± 1.20^{a}	49.66 ± 5.20^{a}	10.83 ± 0.60^{bc}

B. Physiological and biochemical parameters

The spray of GA_3 + Nano-300 (Aerosil) gave the maximum value for leaf chlorophyll content. The beneficial effect of this treatment was followed by that of GA_3 + Nano-200 (Aerosil) that increased the chlorophyll content by 58.12% as compared to the water sprayed control (Table 4). The treatment of GA_3 + Nano-300 (Aerosil) gave the maximum value for leaf carotenoids content. Its beneficial effect was followed by that of GA_3 + Nano-200 (Aerosil), which enhanced the carotenoids content by 11.11% in comparison to the water sprayed control (Table 4). The spray of GA_3 + Nano-300 (Aerosil) gave the maximum value for NR activity. The beneficial effect of this treatment was followed by that of GA_3 + Nano-200 (Aerosil). The treatment of GA_3 + Nano-300 (Aerosil) gave 10.46% higher activity of NR than the water sprayed control (Table 4).

The spray of GA_3 + Nano-300 (Aerosil) gave the maximum value for CA activity. The beneficial effect of this treatment was followed by that of GA_3 + Nano-200 (Aerosil). The treatment GA_3 + Nano-300 (Aerosil) gave 13.05% higher activity of CA as compared to the water sprayed control (Table 4). The treatment GA_3 + Nano-200 (Aerosil) gave the maximum value for the leaf-N content. The beneficial effect of this treatment was followed by that of GA_3 + Nano-200 (Aerosil). The treatment GA_3 + Nano-300 (Aerosil) gave 10.37% higher content of leaf-N than the water sprayed control (Table 5). The spray of GA_3 + Nano-300 (Aerosil) increased the leaf-P content to the highest extent. The beneficial effect of this treatment was followed by that of Nano-300 (Aerosil). Treatment GA_3 + Nano-300 (Aerosil) gave 5.40% higher content of leaf-P than that given by water sprayed control (Table 5). The spray of GA_3 + Nano-

300 (Aerosil) gave maximum value for leaf-P content. The beneficial effect of this treatment was followed by that of GA_3 + Nano-200 (Aerosil).

Spray of GA_3 + Nano-300 (Aerosil) gave 1.70% higher content of leaf-K compared to the water sprayed control (Table 5).

Table 4: Effect of GA_3 and silicon nano-particles on biochemical parameters of *Stevia rebaudiana* (Means of five replicates ± SE). Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$).

Treatments/ parameters	Total chlorophyll (mg/g FW)	Total carotenoids (mg/g FW)	Nitrate reductase activity (n mol NO_2 g/FW/h)	Carbonic anhydrase activity [µmol (CO_2)/Kg(FW)/s]
Control	0.917 ± 0.055^{c}	0.270 ± 0.03^{c}	281.2 ± 3.22^{e}	202.3 ± 2.21^{c}
GA_3	1.103± 0.058^{bc}	0.274 ± 0.51^{c}	293.9± 4.63^{c}	216.3 ± 2.48^{b}
Nano-200 (Aerosil)	1.240 ± 0.081^{ab}	0.280 ± 0.32^{b}	287.7 ± 4.41^{d}	205.7 ± 2.33^{c}
Nano-300 (Aerosil)	1.230 ± 0.12 ^{ab}	0.283 ± 0.32^{b}	290.4 ± 4.52^{c}	207.7 ± 2.83^{c}
GA_3+Nano-200 (Aerosil)	1.400± 0.045^{a}	0.287± 0.36^{b}	304.4 ± 4.61^{b}	219.3 ± 2.48^{b}
GA_3+Nano-300 (Aerosil)	1.450 ± 0.416^{a}	0.300 ± 0.44^{a}	310.6 ± 4.21^{a}	228.7 ± 2.78^{a}

Table 5: Effect of GA_3 and silicon nano-particles on biochemical parameters of *Stevia rebaudiana* (Means of five replicates ± SE). Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$).

Treatments/ parameters	Leaf-nitrogen content (%)	Leaf-phosphorus content (%)	Leaf-potassium content (%)
Control	3.18 ± 0.076^{e}	0.277 ±0.014^{a}	2.31 ±0.71^{a}
GA_3	3.42 ± 0.079^{b}	0.282 ±0.017^{a}	2.30 ±0.73^{a}
Nano-200 (Aerosil)	3.25 ± 0.082^{d}	0.286 ±0.019^{a}	2.25 ±0.72^{a}
Nano-300 (Aerosil)	3.32 ± 0.078^{c}	0.287 ±0.022^{a}	2.22 ±0.81^{a}
GA_3+Nano-200 (Aerosil)	3.51 ± 0.091^{a}	0.283 ±0.017^{a}	2.31 ±0.82^{a}
GA_3+Nano-300 (Aerosil)	3.45 ± 0.082^{b}	0.292 ±0.014^{a}	2.35 ± 0.77^{a}

Discussion

The present study describes the positive effect of foliar combined application of GA_3 + silicon nanoparticles (Aerosil) on growth characteristics, physiological attributes and biochemical parameters of sweet plant (*Stevia rebaudiana*). The enhancement in values for most of the parameters studied due to application of the foliar spray of GA_3 + silicon nanoparticles (Aerosil) (Tables 3-5) might be considered a valuable observation. This improvement in the performance may be attributed to the effect of GA_3 + Nano-300 (Aerosil), followed by that of GA_3 + Nano-200 (Aerosil). A significant effect of PGRs and nanoparticles application on other plants has been reported by various workers (Stefanani and Rodriques 1999 [23]; Singh and Misra; 2001 [21]; Moaveni *et al*., 2011 [19]; Javed *et al*. 2017 [8]; Castro-González *et al*. 2019) [3]. The optimum concentration of GA_3 and nano-particles treatments (50 to 200 ppm), by virtue of their growth stimulating role might be considered helpful in promoting photosynthesis, uptake of mineral nutrients, and biosynthesis of chlorophyll and carotenoids, etc. Hence, higher values (compared to the water-spray control) of CA activity, chlorophyll content, carotenoid content and leaf-N, -P and -K contents were observed (Tables 4 and 5). The improvement in these parameters was, in turn, manifested in the enhancement of plant shoot and root length as well as plant fresh and dry weights (Table 3). These results corroborate the findings of several workers, who have explored that plant growth was significantly increased by GA_3 treatment as compared to control (Stefanani and Rodriques 1999 [23]; Singh and Misra., 2001 [21]; Aftab *et al*. 2010 [1]; Aparna *et al*. 2018 [2]; Khalid and Aftab 2020) [10]. In conformation with these results, the effect of spray of nanoparticles was positively significant on *Stevia rebaudiana* and other plants as also reported by others workers (Moaveni *et al*., 2011 [19]; Kiapour *et al*. 2015; Javed *et al*. 2017 [8]; Castro-González *et al*. 2019) [3].

It is concluded that the combined foliar application of GA_3 and silicon nanoparticles [GA_3 + Nano-300 (aerosil)] proved much effective and successful over their individual use in exploring the overall performance of this medicinal herb.

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