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Biochemical and Physiological Effects of TiO_2 and SiO_2 Nanoparticles on Cotton Plant under Drought Stress.

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ABSTRACT

Application of nanofertilizers is one of the promising methods for increasing resources use efficiency and reducing environmental pollutions. This study was carried out to investigate the effects of nano titanium dioxide (nano-TiO_2), and nano silicon dioxide (nano-SiO_2) on chemical constituents and yield characteristics of cotton plant under drought stress. The cotton plants pre-treated with four concentrations of nano-TiO_2 (25, 50, 100 and 200 ppm) or nano-SiO_2 (400, 800, 1600 and 3200 ppm) then exposed to drought stress. In general, the drought stress reduced the pigments content, total soluble sugars content, glutathione reductase activity and yield characteristics, while increased total phenolics, total soluble proteins, total free amino acids, proline content, total reducing power, total antioxidant capacity, catalase activity, peroxidase activity and superoxide dismutase activity in comparison with control. The obtained results showed that pretreatment of cotton plants under drought stress with nano-TiO_2 or nano-SiO_2 caused increasing of pigments content, total soluble sugars, total phenolics, total soluble proteins, total free amino acids, proline content, total reducing power, total antioxidant capacity and antioxidant enzyme activities and enhancement of yield characteristics. The optimum concentration of nano-TiO_2 and nano-SiO_2 to alleviate the drought stress in cotton plant was 50 ppm and 3200 ppm, respectively. Finally, it can be concluded that foliar application of nano-TiO_2 or nano-SiO_2 could improve the drought tolerance of cotton plants.

Keywords: Drought stress , Cotton, Titanium dioxide, Silicon dioxide, Nanoparticles.

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INTRODUCTION

Cotton (*Gossypium barbadense* L.) is one of the most important fiber crops playing a key role in economic and social affairs of the world. It is a soft fiber that grows around the seeds of the cotton plant *Gossypium* spp., a shrub native to tropical and subtropical regions around the world. The fiber is most often spun into thread and used to make a soft, breathable textile, which is the most widely used natural-fibre cloth in clothing today [1] and [2].

The response of plants to drought stress is complex and involves changes in their morphology, physiology and metabolism. Reduction of plant growth is the most typical symptom of drought stress [3]. Chlorophyll content decreased under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation [4]. Proline, carbohydrates, total phenols and total free amino acids accumulation is a regular response of plants exposed to environmental stresses and drought in particular [5] and [6]. Drought stress leads to accumulation of reactive oxygen species (ROS), generated mostly in chloroplast and to some extend in mitochondria, causing oxidative stress. The ROS scavenging mechanism is among the common defense responses against abiotic stresses [7]. To detoxify ROS, plants can intrinsically develop different types of antioxidants reducing oxidative damage and conferring drought tolerance. The ROS scavengers are antioxidant enzymes containing superoxide dismutase, peroxidase and catalase [8] and [9].

Nanotechnology has provided the feasibility of exploiting nanoscale or nanoparticle materials as fertilizer carriers or controlled release vectors for building of so-called “smart fertilizer” as new facilities to enhance nutrient use efficiency and reduce costs of environmental protection. Nanofertilizers will combine nanodevices in order to synchronize the release of fertilizer with their uptake by crops, so preventing undesirable nutrient losses to soil, water and air via direct internalization by crops, and avoiding the interaction of nutrients with soil, microorganisms, water, and air [10].

TiO_2 nanoparticles (TiO_2-NPs) are one of the most produced NPs in the world. Titanium has significant biological effects on plants, being beneficial at low levels but toxic at higher concentrations. Photocatalytic degradation of pesticides with TiO_2 and other catalyst has shown promise as potential water remediation method [11]. Nano titanium dioxide can improve photosynthetic apparatus and enhance a plant’s ability to capture sunlight, that affects the manufacture of pigments and the transformation of light energy to active electron and chemical activity and thus increase photosynthetic efficiency as in maize [12], especially under drough stress [13]. Nano-TiO_2 was observed to promote the growth of spinach through an increase in photosynthetic rate and nitrogen metabolism in spinach plant [14] and [15] and soybean (*Glycine max* L.) [16]. TiO_2 nanomaterial can enhance plant water and nitrogen use and stimulate some antioxidant enzyme activities, such as SOD, POD, and CAT [17]. Nano scale TiO_2 proved to be effective in improving both shoot and root length, and increase growth, yield and yield components in radish, corn, lettuce and cucumber [18], Canola plant [19] and wheat plant [20].

Silicon is an important trace element whose presence is necessary to induce resistance to distinct stresses, diseases, and pathogens of plants. The addition of SiO_2 to plant medium reduces the penetrability of the plasma wall of the leaf cells resulting in the loss of lipid peroxidation and also, SiO_2 protects cellular wall against heat and drought stress [21] and [22]. SiO_2 nanoparticles at 400, 2000 and 4000 mg/l concentrations caused an increased content in all the photosynthetic pigments (chlorophyll a, b and carotenoids) in *Z. mays* in relation to the control [23]. Si and nano-Si applications caused significant increases in the content of soluble sugars in faba bean plants. Si-treated plants showed increased amounts of total soluble proteins [24]. Proline content significantly increased when silica nanoparticles were applied under stress, in comparison with common silica fertilizer [25]. Application of nano-Si caused a significantly increase in the activities of catalase (CAT) and peroxidase (POD) in plant leaves, but caused a decrease in the activities of superoxide dismutase (SOD) and glutathione reductase (GR) as compared to unstressed plants of faba bean [25], tomato plant [26] and alfalfa plant [27]. SiO_2 nanoparticles application significantly increased dry weight of shoot, root and seedling of tall wheatgrass. Silicon application significantly increased wheat biomass at both control as well as under saline conditions and on rice seedlings [28] and rice seedlings [29].

The aim of this study was to evaluate the effects of TiO_2 and SiO_2 nanoparticles on chemical constituents and yield characteristics of cotton plant under drought stress.

MATERIALS AND METHODS

Materials

Plant material

Cotton (*Gossypium barbadense* L. cv. Giza 94) seeds were obtained from the Plant Physiology Department, Cotton Research Institute, Agricultural Research Center, Giza, Egypt.

Chemicals

Nano titanium dioxide and nano silicon dioxide were purchased from Cornal Lab Co., Egypt. Folin reagent, Pyrogallol and trichloroacetic acid were purchased from Acmatic Co., Egypt. All other chemicals were of analytical reagent grade.

Methods

Experimental design and treatments

The experiment was conducted in two summer seasons 2014 and 2015 at Sakha Research Station of Plant Physiology Department, Cotton Research Institute, Agricultural Research Center, Kafr El-Sheikh, Egypt. This experiment was carried out to study the effects of foliar application of cotton plants with different concentrations of nano titanium dioxide (25, 50, 100 and 200 ppm) and nano silicon dioxide (400, 800, 1600 and 3200 ppm) on chemical constituents and yield characteristics under drought conditions. Seeds of cultivar Giza 86 × 10229 were sown in clay loam soils on 24th of April 2014 in the first season and on the 28th April 2015 in the second one. The experimental plot consisted of rows, 3.5 m long and 0.6 m width (plot area = 14.70 m^2) of the Agricultural Experimental Sakha Station Farm of the Agriculture Research Center, Kafr El-Sheikh, Egypt. All plots were fertilized at a rate of 60 kg N/fed in the form of urea (46.5% N) in two equal doses, the first dose was added after thinning (before the first irrigation), while the second dose was applied before the second irrigation. All plots received an adequate amount of fertilizer in order to produce healthy plants. Fertilization was carried out according to recommendation of Cotton Research Institute, phosphorus fertilizer was applied during soil preparation in form of calcium superphosphate (15.5% P_2O_5) at a rate of 15.5 kg P_2O_5/fed. Potassium fertilizer was applied after thinning at a rate of 24 kg K_2O/fed in the form of the potassium sulphate (48% kg K_2O). Irrigation was carried out regularly at the plant needs using tap water until the start of flowering stage, then the plots preventing water supply for 24 days till the appearance of sing of permanent wilting (drought stress) to take samples and back to irrigation plants. Plants were sprayed with nano titanium dioxide (nano-TiO_2) and nano silicon dioxide (nano-SiO_2) at start of flowering stage and the untreated plots (control) were irrigated with tap water continuously.

Plant samples

Plant samples (whole plant and leaves) were taken at flowering stage (74 days from sowing) during the experimental period. In this stage, 6 plants were taken from each treatment (3 plots). The soil particles were washed off the roots by a stream of tap water. At harvest stage (180 days after sowing), samples from ten plants from each plots were taken.

Chemical analysis

Cotton leaves were taken randomly after flowering stage to carry out the chemical analysis as follows:

Determination of pigments content

The chlorophyll a, b and total chlorophyll contents were determined according to the method of [30] and carotenoids content was determined according to method of [31].

Determination of total soluble sugars

Total soluble sugars were determined in ethanol extract of cotton leaves by phenol-sulfuric acid method according to [32].

Determination of reducing sugars

Reducing sugars were determined colormetrically according to Folin and Wu method as reported in [33].

Determination of non-reducing sugars

Non-reducing sugars were calculated by the difference between total soluble sugars and total reducing sugars.

Total phenolics content

Total phenolics were determined in ethanol extract of cotton leaves using Folin-Ciocalteau method according to [34].

Determination of total soluble proteins

Total soluble proteins were extracted from cotton leaves according to [35] and determined by the method of Lowry-Folin as described by [36].

Determination of total free amino acids

Total free amino acids were determined in ethanol extract of cotton leaves by ninhydrin method according to [37].

Determination of proline content

Proline content of cotton leaves were determined according to method of [38].

Assay of antioxidant enzymes activities

Extraction of antioxidant enzymes

Crude enzyme extract was prepared for assay of catalase (CAT), peroxidase (POX), superoxide dismutase (SOD) and glutathione reductase (GR) activities according to [35].

Assay of catalase activity

Catalase (EC 1.11.1.6) activity was measured according to the method of [39] as follows: The assay mixture contained 2.6 ml of potassium phosphate buffer solution (50 m*M*, pH 7.0), 0.4 ml of H_2O_2 solution (15 m*M*) and 0.04 ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in U/mg protein (U = 1 m*M* of H_2O_2 reduction/min/mg protein).

Assay of peroxidase activity

Peroxidase (EC 1.11.1.7) activity was assayed according to the method of [40] as follows: The assay mixture of POX contained 2 ml of phosphate buffer solution (0.1 *M*, pH 6.8), 1 ml of pyrogallol solution (0.01 *M*), 1 ml of H_2O_2 solution (0.005 *M*) and 0.5 ml of enzyme extract. The solution was incubated for 5 min at 25°C, after which the reaction was terminated by adding 1 ml of H_2SO_4 solution (1.25 *M*). The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of H_2SO_4 solution at zero time. The activity was expressed in U/mg protein. One U is defined as the change in the absorbance by 0.1 min/mg protein.

Assay of superoxide dismutase activity

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed according to the method of [41] as follows: The reaction mixture contained 2.35 ml of phosphate buffer (50 m*M*, pH 7.8), 0.30 ml of methionine solution (10 m*M*), 0.10 ml of Nitroblue tetrazolium solution (1 m*M*), 0.20 ml of EDTA solution (0.01*M*), 0.20 ml of enzyme extract and 0.05 ml of riboflavin solution (0.2 m*M*). The absorbance of reaction mixture was measured at 560 nm. The increase in absorbance in the absence of enzyme was taken as 100 and 50% initial was taken an equivalent to 1 unit of SOD activity.

Assay of glutathione reductase activity

Glutathione reductase (GR; EC 1.6.4.2) activity was assayed according to the method [42] as follow: The assay mixture was composed of 1.20 ml of phosphate buffer solution (50 m*M*, pH 7.8), 0.10 ml of extract and 0.05 ml of NADPH (83 µ*M* in 0.1% $NaHCO_3$). After incubation at 25°C for 10 min, 0.15 ml of GSSG solution (1 m*M*) was added, and the decrease of NADPH absorption was monitored for 3 min at 340 nm using a UV/Visible Spectrophotometer. The NADPH concentration change [µmol NADPH (ml extract)$^{-1}$min^{-1}] was calculated.

Determination of total antioxidant capacity

Total antioxidant capacity was determined in ethanol extract of cotton leaves using the phosphomolybdenum method of [43] as described by [44] as follows: A known volume (0.01 ml) of extract was added to test tube then completed to a constant volume (0.3 ml) with DW. 3.0 ml of reagent solution (0.6 *M* sulfuric acid, 28.0 m*M* sodium phosphate and 4.0 m*M* ammonium molybdate) were added to each tube and mixed well then incubated at 95°C for 90 min. Blank was prepared by the same procedure without extract. After cooling to room, the absorbance of the solution was measured at 695 nm using spectrophotometer against blank. Increased absorbance of the reaction mixture indicated increased total antioxidant capacity.

Determination of total reducing power

The total reducing power was determined in ethanol extract of cotton leaves according to the method of [45] as described by [46] as follows: A known volume (1 ml) of ethanol extract was mixed with 2.5 ml of phosphate buffer (0.2 *M*, pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The mixture was incubated at 50 ^{o}C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer. Increased absorbance of the reaction mixture indicates increase in reducing power.

Yield and its components

Yield and its components, including plant height (cm), number of fruiting branches/plant, number of open boll/plant, boll weight (g), lint percentage, seed index (g) and yield k/f were recorded.

Relative water content

Relative water content was determined according to the method of [47].

Statistical analysis

The results were analysed by an analysis of variance ($P<0.05$) and the means separated by Duncan's multiple range test. The results were processed by CoStat computer program (1986).

RESULTS AND DISCUSSION

Foliar application of nano-TiO_2 and nano-SiO_2 on cotton plant under drought stress

Chemical constituents of cotton leaves

Cotton leaves obtained from this experiment were employed to determine their contents of chlorophyll a, b, total chlorophyll, carotenoids, total soluble sugars, reducing sugars, non-reducing sugars, total phenols, total soluble proteins, total free amino acids and proline in addition to determine the antioxidant enzyme activities (catalase, peroxidase, suberoxide dismutase and glutathione reductase), total antioxidant capacity and total reducing power. The obtained results are presented in Tables 1, 2, 3, 4 and 5.

Table 1. Effect of foliar application of nano-TiO_2 and nano-SiO_2 on Chlorophyll (Chl) a, b, total chlorophyll and carotenoid contents in leaves of cotton plant under drought stress

Treatment		Concentration (ppm)	Chlorophyll pigments (mg/g DW)			Carotenoids (mg/g DW)
			Chl a	Chl b	Total Chl	
Control (under normal conditions)			$2.64^{g}\pm0.029$	$2.46^{f}\pm0.031$	$5.10^{f}\pm0.016$	$0.62^{c}\pm0.036$
Drought stress conditions			$2.22^{i}\pm0.021$	$1.43^{i}\pm0.034$	$3.65^{h}\pm0.035$	$0.46^{e}\pm0.047$
Drought stress conditions	Nano-TiO_2	25	$2.85^{f}\pm0.024$	$2.35^{g}\pm0.023$	$5.20^{f}\pm0.026$	$0.53^{d}\pm0.018$
		50	$5.07^{a}\pm0.021$	$3.45^{b}\pm0.024$	$8.52^{a}\pm0.026$	$0.57^{d}\pm0.037$
		100	$4.14^{c}\pm0.026$	$2.87^{d}\pm0.020$	$7.01^{c}\pm0.005$	$0.63^{c}\pm0.031$
		200	$4.03^{d}\pm0.019$	$2.69^{e}\pm0.028$	$6.72^{d}\pm0.044$	$0.56^{d}\pm0.022$
	Nano-SiO_2	400	$2.37^{h}\pm0.110$	$1.15^{j}\pm0.006$	$3.52^{i}\pm0.036$	$0.54^{d}\pm0.023$
		800	$2.80^{f}\pm0.330$	$1.73^{h}\pm0.005$	$4.53^{g}\pm0.019$	$0.65^{c}\pm0.048$
		1600	$3.30^{e}\pm0.029$	$3.00^{c}\pm0.038$	$6.34^{e}\pm0.022$	$0.72^{b}\pm0.026$
		3200	$4.38^{b}\pm0.023$	$3.89^{a}\pm0.031$	$8.27^{b}\pm0.021$	$0.89^{a}\pm0.033$
L.S.D			0.0629	0.0445	0.2338	0.0487

-Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at *P*<0.05. DW: dry weight

Table 2. Effect of foliar application of nano-TiO_2 and nano-SiO_2 on total soluble sugar, reducing sugar and non-reducing sugar contents in leaves of cotton plant under drought stress

Treatment		Concentration (ppm)	Carbohydrate (mg/g FW)		
			Total soluble sugars	Reducing sugars	Non- reducing Sugars
Control (under normal conditions)			$32.08^{h}\pm0.046$	$21.34^{h}\pm0.018$	$10.64^{i}\pm0.008$
Drought stress conditions			$27.57^{i}\pm0.024$	$19.73^{j}\pm0.040$	$7.83^{j}\pm0.024$
Drought stress conditions	Nano-TiO_2	25	$32.08^{h}\pm0.048$	$19.97^{i}\pm0.031$	$12.11^{h}\pm0.042$
		50	$40.16^{c}\pm0.024$	$25.30^{c}\pm0.020$	$14.86^{e}\pm0.029$
		100	$39.87^{d}\pm0.031$	$24.14^{e}\pm0.400$	$15.73^{c}\pm0.018$
		200	$38.29^{f}\pm0.029$	$24.84^{d}\pm0.027$	$13.45^{f}\pm0.029$
	Nano-SiO_2	400	$34.95^{g}\pm0.040$	$21.74^{g}\pm0.924$	$13.21^{g}\pm0.024$
		800	$39.75^{e}\pm0.024$	$23.79^{f}\pm0.027$	$15.96^{b}\pm0.018$
		1600	$41.74^{b}\pm0.027$	$26.41^{b}\pm0.047$	$15.33^{d}\pm0.027$
		3200	$44.85^{a}\pm0.035$	$27.81^{a}\pm0.016$	$17.04^{a}\pm0.008$
L.S.D			0.0496	0.0449	0.0361

-Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at *P*<0.05. FW: fresh weight

Table 3: Effect of foliar application of nano-TiO_2 and nano-SiO_2 on total phenol, total soluble protein, total free amino acid and proline contents in leaves of cotton plant under drought stress

Treatment		Concentration (ppm)	Total Phenols (mg/g FW)	Total soluble proteins (mg/g FW)	Total amino acids (mg/g FW)	Proline (µmol/g FW)
Control (under normal conditions)			$11.17^{j}\pm0.024$	$8.0^{h}\pm0.589$	$12.35^{j}\pm0.029$	$3.25^{g}\pm0.029$
Drought stress conditions			$16.17^{i}\pm0.029$	$22.8^{g}\pm0.282$	$18.85^{i}\pm0.028$	$73.32^{f}\pm0.021$
Drought stress conditions	Nano-TiO_2	25	$20.46^{h}\pm0.026$	$26.4^{e}\pm0.163$	$21.21^{h}\pm0.041$	$130.84^{e}\pm0.029$
		50	$24.18^{c}\pm0.018$	$32.0^{b}\pm0.327$	$29.58^{b}\pm0.022$	$192.38^{cd}\pm0.021$
		100	$22.72^{e}\pm0.024$	$28.8^{d}\pm0.432$	$25.38^{e}\pm0.029$	$164.87^{e}\pm0.043$
		200	$21.01^{g}\pm 0.236$	$28.4^{d}\pm0.588$	$24.82^{f}\pm0.014$	$155.03^{e}\pm0.038$
	Nano-SiO_2	400	$21.75^{f}\pm0.029$	$24.4^{f}\pm0.673$	$24.67^{g}\pm0.040$	$163.34^{de}\pm0.025$
		800	$23.80^{d}\pm0.022$	$26.0^{e}\pm0.432$	$25.79^{d}\pm0.021$	$216.26^{bc}\pm0.018$
		1600	$26.27^{b}\pm0.018$	$30.0^{c}\pm0.516$	$27.57^{c}\pm0.034$	$238.98^{b}\pm0.023$
		3200	$30.34^{a}\pm0.035$	$36.0^{a}\pm0.800$	$32.21^{a}\pm0.022$	$276.96^{a}\pm0.028$
L.S.D			0.038	0.742	0.038	33.793

-Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at *P*<0.05. FW: fresh weight

Table 4: Effect of foliar application of nano-TiO_2 and nano-SiO_2 on the total antioxidant capacity and total reducing power in leaves of cotton plant under drought stress

Treatment		Concentration (ppm)	Total antioxidant capacity ($O.D_{695\ nm}$)	Total reduction capability ($O.D_{700\ nm}$)
Control (under normal conditions)			$0.683^{i}\pm0.003$	$0.481^{i}\pm0.006$
Drought stress conditions			$0.793^{h}\pm0.002$	$0.882^{h}\pm0.009$
Drought stress conditions	Nano-TiO_2	25	$0.982^{f}\pm0.005$	$0.958^{f}\pm0.009$
		50	$1.136^{c}\pm0.003$	$1.078^{a}\pm0.004$
		100	$0.985^{f}\pm0.004$	$0.968^{e}\pm0.003$
		200	$0.941^{g}\pm0.003$	$0.922^{g}\pm0.006$
	Nano- SiO_2	400	$1.009^{e}\pm0.010$	$0.967^{ef}\pm0.004$
		800	$1.086^{d}\pm0.004$	$0.986^{d}\pm0.007$
		1600	$1.271^{b}\pm0.009$	$1.021^{c}\pm0.005$
		3200	$1.297^{a}\pm0.003$	$1.050^{b}\pm0.003$
L.S.D			0.008	0.009

-Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at *P*<0.05.

Table 5: Effect of foliar application of nano-TiO_2 and nano-SiO_2 on the activities of catalase, peroxidase, superoxide dismutase and glutathione reductase in leaves of cotton plant under drought stress

Treatment		Concentration (ppm)	Catalase activity (U/mg protein)	Peroxidase activity (U/mg protein)	Superoxide dismutase (U/mg protein)	Glutathione reductase (U/mg protein)
Control (under normal conditions)			$0.073^{h}\pm0.004$	$0.316^{i}\pm0.002$	$0.401^{h}\pm0.002$	$0.09^{g}\pm0.018$
Drought stress conditions			$0.132^{g}\pm0.007$	$0.420^{h}\pm0.007$	$0.619^{g}\pm0.004$	$0.05^{h}\pm0.029$
Drought stress conditions	Nano-TiO_2	25	$0.153^{f}\pm0.006$	$0.459^{g}\pm0.004$	$0.653^{f}\pm0.005$	$0.78^{f}\pm0.034$
		50	$0.274^{c}\pm0.004$	$0.706^{bc}\pm0.003$	$0.728^{a}\pm0.003$	$0.99^{c}\pm0.029$
		100	$0.263^{d}\pm0.007$	$0.705^{c}\pm0.006$	$0.698^{c}\pm0.004$	$0.98^{c}\pm0.021$
		200	$0.197^{e}\pm0.005$	$0.656^{d}\pm0.002$	$0.668^{de}\pm0.003$	$0.80^{ef}\pm0.046$
	Nano-SiO_2	400	$0.257^{d}\pm0.003$	$0.557^{f}\pm0.003$	$0.667^{e}\pm0.006$	$0.83^{e}\pm0.018$
		800	$0.261^{d}\pm0.003$	$0.604^{d}\pm0.008$	$0.675^{d}\pm0.005$	$0.92^{d}\pm0.018$
		1600	$0.307^{b}\pm0.004$	$0.714^{b}\pm0.005$	$0.720^{b}\pm0.008$	$1.22^{b}\pm0.014$
		3200	$0.347^{a}\pm0.007$	$0.786^{a}\pm0.004$	$0.735^{a}\pm002$	$1.40^{a}\pm0.027$
L.S.D			0.008	0.008	0.007	0.039

-Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at *P*<0.05. FW: fresh weight

Pigments content

Data presented in Table 1 showed that the contents of chlorophyll a, b, total chlorophyll, carotenoids of stressed cotton plants were decreased in comparison with control plants under normal conditions. Foliar application of cotton plants with different concentrations of nano-TiO_2 (25, 50, 100 and 200) and nano-SiO_2 (400, 800, 1600 and 3200) under drought conditions increased chlorophyll a, b, total chlorophyll and carotenoid contents of cotton plants to be more than control plants. This increasing in pigments content of cotton plants is varied between treatments. In general, the results indicated that chlorophyll a, b, total chlorophyll and carotenoid contents were significantly increased as a result of foliar application of nano-SiO_2 (3200 ppm) in comparison with nano-TiO_2 at the highest concentration. The maximum effective concentration of nano-SiO_2 on pigments content was 3200 ppm, while the maximum effective of nano-TiO_2 was 50 ppm. Similar results were obtained by [48] and [26]. The obtained results also supported by the suggestion of Abdul Qados [24] reported that the nano-Si or Si reduced the damage effects of stress on photosynthetic pigments through decreasing the electrolyte leakage and increasing the membrane stability compared with those of the control. The nano-Si can improve structure of chlorophyll and can facilitate manufacture of pigments and protect chloroplasts from ageing in faba bean cells [12]. Lei *et al.* [49] reported that nano TiO_2 increased photosynthesis and plant growth in spinach and serves to enhance absorption and transmission of the sun's energy to electron energy and active chemical energy. Nano TiO_2 could greatly improve plant processes such as whole chain electron transportation, photoreduction activity of photosystem II, O_2^- evolving and photophosphorylation activity of spinach Chl, not only under visible light but also energy enriched electrons from nanoanatase TiO_2, which entered the Chl and was transferred by a photosynthetic electron transport chain to produce $NADP^+$ reduce into NADPH, and coupled to photophosphorylation and transferred electron energy to ATP.

Total soluble sugars content

Carbohydrates that represent one of the main organic constituents of the dry matter were found to be affected by water stress. As shown in Table 2, the foliar application of nano-TiO_2 and nano-SiO_2 on cotton plants under drought stress conditions increased the contents of total soluble sugars, reducing sugars and non-reducing sugars as compared with untreated stressed cotton plants (control). The results generally showed that the spraying of cotton plants with nano-SiO_2 (3200 ppm) was more effective in increasing the contents of total soluble sugars, reducing and non-reducing sugars in comparison with nano-TiO_2 at highest concentration (200 ppm). These results are in line with the findings of [50], [51] and [52]. The results are in agreement with the possible mechanism by which silicon plays a positive role in alleviation of the harmful effects of water stress on faba bean plants, which silicon synergistically increased the amounts of soluble sugars than in untreated stressed ones which indicated that accumulation of these compounds by silicon plays a key role in retaining the water capacity of stressed cells which thereby can tolerate severe drought and salinity stress Jaberzadeh *et al.* [20] and Abdul Qados [24] who found the positive effects of titanium treatment were found on plant development (an increase of chlorophyll content and photosynthesis intensity) and sugar content.

The phenolics content

Data presented in Table 3 indicated that the foliar application of nano-TiO_2 and nano-SiO_2 on cotton plants under drought conditions increased the contents total phenolics content in comparison with control plants. The results revealed that the spraying of cotton plants with nano-SiO_2 at different concentration (800, 400, 1600 and 23000 ppm) was more effective in increasing total phenolics content than nano-TiO_2 at different concentrations (25, 50, 100 and 200 ppm). These results are in accordance with those of Sakihama *et al.* [53] who reported that plants can accumulate phenolic compounds under various stress conditions such as light, low temperature, hydric deficit. Also, Abdallah [54], Agastian *et al.* [55] and Muthukumarasamy *et al.* [56] noted that drought conditions tended to increase total phenols of cotton leaves at all stages of growth in Giza 70 (all stages), Dandara (seedling) and Giza 69 (squaring).

Total soluble proteins

The results in Table 3 revealed that the total soluble proteins were increased significantly in cotton leaves and recorded the highest value (36±0.8 mg/g FW) after spraying with nano-SiO_2 at 3200 ppm, whilst the spraying of cotton leaves with nano-TiO_2 recorded the highest value of total soluble proteins was 32±0.327 mg/g FW at concentration 50 ppm. In this respect, Hong *et al.* [57] found that, nano-TiO_2 attendances could persuade nitrate absorption, accelerate non organic nitrogen (Like: NNO^-_3 and NNH^+_4) change to organics (such as protein and chlorophyll) and rising spinach yield up, too. Also, silicon is able to increase soluble protein content of plants' leaves, which helps plants to overcome stress by replacing the lost soluble protein content under stress [58].

Total free amino acid and proline contents

Data presented in Table 3 showed that nano-TiO_2 and nano-SiO_2 treatments caused increase in total free amino acid and proline contents of cotton plant under drought stress. The role of total free amino acid and proline accumulation is considered as a compatible solute involved in osmotic adjustment, which accumulates in majority of cotton plants under stress. The induction of proline accumulation may be due to an activation of proline synthesis through glutamate pathway. It has been shown that accumulation of proline is a common response to a wide range of biotic and abiotic stresses such as salt, drought and high temperature [59], [60], [61], [5] and [62].

Total antioxidant capacity and reducing power

Drought stress on plants increased total reducing power and total antioxidant capacity in comparison with control plants (Table 4). The results showed that the spraying of cotton plants by nano-SiO_2 at concentration 3200 ppm and nano-TiO_2 at concentration 50 ppm increased total reducing power (1.050±0.003 and 1.078±0.004, respectively) and total antioxidant capacity (1.297±0.003 and 1.136±0.003, respectively) in cotton plants under drought conditions; this may be related to the induction of antioxidant responses enzymatic and non- enzymatic that protect the plant from oxidative damage. This results agreement with Sacaáa [63] who reported that, Si-alleviated effects have been associated with an increase in antioxidant defense abilities under drought stress [64], [21] and [65].

Antioxidant enzymes

Catalase (CAT), peroxidase (POX), superoxide dismutase (SOD) and glutathione reductase (GR) are antioxidant enzymes that protect cells from oxidative stress of highly reactive free radicals. Catalase is mainly responsible for eliminating H_2O_2 from the peroxisomes. Peroxidase is the major key enzyme for the removal of H_2O_2 from the chloroplasts and superoxide dismutase for catalyzing the dismutation of O^-_2 to O_2 and H_2O_2. The results obtained in Table 5 showed that the foliar application of nano-SiO_2 and nano-TiO_2 on cotton plants under drought conditions increased the activities of catalase, peroxidase, superoxide dismutase and glutathione reductase enzymes in comparison with control plants. The obtained results revealed that the spraying of cotton plant with nano-SiO_2 at concentration 3200 ppm was more effective in increasing the activities of antioxidant enzymes than other concentrations (400, 800 and 1600 ppm). Whilst the spraying with nano-TiO_2 at concentration 50 ppm was more effective concentration in increasing the activities of antioxidant enzymes than other concentrations (25, 100 and 200 ppm). Application of nano-SiO_2 and nano-TiO_2 improve the activities of antioxidant enzymes such as CAT, POX, SOD, GR, in addition to total reducing power and total antioxidant capacity were observed in plants under stress [66], [17] and [52]. Abdul Qados [24] suggested that nano-Si and Si treatments might be due to induction of antioxidant responses that protect the plants from oxidative damage, increased membrane stability and tolerance of plants which in turn enhanced scavenging of harmful free radicals and elevated Ca uptake that protects the plant from the oxidative damage by silicon treatments.

Yield characteristics

Data in Table 6 show the effect of foliar application of nano-SiO_2 and nano-TiO_2 on yield characteristics of cotton plants under drought conditions. As shown in results, many differences in yield characteristics of cotton plants in response to different concentrations of nano-SiO_2 and nano-TiO_2 were reported. Data revealed that the best concentrations of nano-SiO_2 and nano-TiO_2 for the maximum values of most yield characteristics of cotton plants under drought conditions were with nano-SiO_2 (1600 and 3200 ppm) and nano-TiO_2 (50 and 100 ppm) respectively as compared with the plants grown under normal conditions. The yield characteristics of cotton plants (plant high, number of fruiting branches/plant, number of open boll/plant, boll weight, lint%, seed index and yield k/f) were affected by concentrations 1600 and 3200 ppm of nano-SiO_2 more than the concentrations 50 and 100 ppm of nano-TiO_2. In general, it could be concluded that spraying cotton plants with 3200 ppm of nano-SiO_2 and 50 ppm of nano-TiO_2 under drought stress conditions increased yield characteristics compared to untreated plants under the same drought stress.

Table 6. Effect of foliar application of nano-TiO_2 and nano-SiO_2 on yield characteristics of cotton plant (cv Giza 94) under drought stress conditions season 2015

Treatment		Concentration (ppm)	Plant hight (cm)	No. of fruiting branch/plant	No. of open boll/pl	Boll weight (g)	Seed index (g)	Lint %	Yield K/F	Relative water cont (%)
Control (under normal conditions)			$148.51^{b}\pm1.50$	$18.25^{bc}\pm0.95$	$20.52^{a}\pm1.29$	$3.21^{ab}\pm0.14$	$12.12^{bc}\pm0.21$	$40.70^{f}\pm0.21$	$6.67^{b}\pm0.12$	$60.74^{ab}\pm1.82$
Drought stress conditions			$137.53^{d}\pm0.58$	$18.06^{c}\pm0.13$	$19.00^{b}\pm0.82$	$3.18^{b}\pm0.18$	$12.03^{c}\pm0.26$	$40.45^{g}\pm0.11$	$3.80^{g}\pm0.24$	$49.42^{c}\pm1.29$
Drought stress conditio	Nano-TiO_2	25	$142.25^{c}\pm1.26$	$18.75^{abc}\pm0.51$	$19.75^{ab}\pm0.96$	$3.22^{ab}\pm0.12$	$12.29^{abc}\pm0.11$	$41.22^{e}\pm0.10$	$4.88^{f}\pm0.14$	$55.24^{b}\pm2.01$
		50	$143.75^{c}\pm1.48$	$19.25^{abc}\pm0.96$	$21.12^{a}\pm0.91$	$3.37^{ab}\pm0.13$	$12.47^{a}\pm0.12$	$42.07^{b}\pm0.19$	$6.03^{d}\pm0.13$	$57.82^{ab}\pm1.13$
		100	$143.51^{c}\pm1.29$	$19.04^{abc}\pm0.82$	$20.75^{a}\pm1.25$	$3.35^{ab}\pm0.28$	$12.45^{a}0.14$	$41.77^{c}\pm0.23$	$5.56^{e}\pm0.23$	$57.65^{ab}\pm2.1$
		200	$142.53^{c}\pm1.33$	$18.91^{abc}\pm0.14$	$20.03^{ab}\pm0.82$	$3.30^{ab}\pm0.12$	$12.32^{ab}\pm0.21$	$41.53^{d}\pm0.12$	$5.48^{e}\pm0.16$	$55.33^{b}\pm2.37$
	Nano-SiO_2	400	$142.50^{c}\pm1.24$	$19.03^{abc}\pm0.18$	$20.04^{ab}\pm1.15$	$3.29^{ab}\pm0.19$	$12.19^{abc}\pm0.13$	$40.89^{f}\pm0.15$	$5.52^{e}\pm0.11$	$59.83^{ab}\pm1.75$
		800	$147.25^{b}0.96$	$19.25^{abc}\pm0.95$	$20.25^{ab}\pm0.96$	$3.34^{ab}\pm0.12$	$12.28^{abc}\pm16$	$41.33^{de}\pm0.13$	$6.33^{c}\pm0.18$	$60.08^{ab}\pm1.62$
		1600	$153.52^{a}\pm1.38$	$19.52^{ab}\pm1.29$	$20.75^{a}\pm0.54$	$3.35^{ab}\pm0.14$	$12.34^{ab}\pm0.21$	$41.89^{bc}\pm0.24$	$7.32^{a}\pm0.23$	$60.67^{ab}\pm1.73$
		3200	$153.75^{a}\pm0.97$	$19.83^{a}\pm0.13$	$21.25^{a}\pm0.52$	$3.51^{a}\pm0.29$	$12.48^{a}\pm0.19$	$42.41^{a}\pm0.09$	$7.53^{a}\pm0.13$	$62.18^{a}\pm2.30$
L.S.D			1.604	1.183	1.315	0.262	0.258	0.234	0.228	5.184

-Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at *P*<0.05.

In conclusion, results obtained indicated that foliar application of nano-SiO_2 and nano-TiO_2 on cotton plants under drought conditions decreased the adverse effects and enhanced growth and yield characteristics. In the present study, yield characteristics of cotton plants were reduced due to water stress. The reduction in yield characteristics of stressed cotton plants can be attributed to the plants grown under drought condition have a lower stomatal conductance in order to conserve water. Consequently, CO_2 fixation is reduced and photosynthetic rate decreases, decrease in photosynthetic pigments, carbohydrates accumulation and nitrogenous compounds [67]. The decrease in yield and yield components in cotton crop under drought conditions has also been reported by [68], [69], [60] and [61]. Our finding showed that pre-treatment of cotton plants under drought stress with nano-SiO_2 and nano-TiO_2 decreased adverse effects of drought stress. Previous studies have demonstrated that the exogenous nano-SiO_2 and nano-TiO_2 mitigated decrease in plant yield component caused by drought is through increasing antioxidant system, alleviating oxidative damage and accelerate proline accumulation, augmented the synthesis of compatible solutes, enhance photosynthesis.

Finally, it can be concluded that the exogenous application of nano-SiO_2 and nano-TiO_2 to cotton plant resulted in enhancement of yield characteristics and increasing of pigments content, total soluble sugars, proline content, total free amino acids, total phenols, total soluble proteins, total reducing power, total antioxidant capacity and antioxidant enzyme activities during water stress as compared to untreated plants. The nanomaterials, because of their tiny size, may show unique characteristics. For example, they can change physico-chemical properties compared to bulk materials. They have greater surface area than bulk materials, and due to this larger surface area, their solubility and surface reactivity tend to be higher [24]. Similar results were recorded in variously nano-SiO_2 or nano-TiO_2 treated plants. Suriyaprabha *et al.* [70] noted that Si nano-particles were found to increase the growth of different species i.e., maize. Romero-Aranda *et al.* [71] reported that silicon was also found helpful in removing toxic substances like salts from plants by increasing water storage in plant tissues which in turn increases growth and contribute in dilution of solutes in plants. Ma and Yamaji [72] suggested that silicon priming could be indirectly useful in aspect that it facilitates the plant with increased growth and production by decreasing the chances of biotic and abiotic stresses like insect pest attack, diseases, drought and nutrient losses. Zheng *et al.* [73] reported that the significant effect of titanium nanoparticles on spinach is probably attributed to the small particle size, which allows its penetration into the seed during the treatment period. It seems that bulk titanium could not penetrate into the plants; therefore, the results were not as marked as those of the treatment with nanoparticles. Increase of growth and yield may be due to the positive effects of titanium in different cellular mechanisms. Titanium nanoparticles helped the water absorption by the spinach and improved growth. Owolade *et al.* [74] reported that the seed yield of cowpea (*Vigna unguiculata Walp*) was increased when treated (as foliar application) with nano-sized titanium dioxide. They concluded that it may be due to the photocatalyst ability of the nano-sized titanium dioxide, which leads to an increased photosynthetic rate. Nano scale TiO_2 at 100 mg/l proved to be effective in improving both shoot and root length. At higher concentration of nano scale TiO_2 (more than 100 mgl^{-1}), shoot and root length decreased and these results were in accordance with the reports on radish, rape, corn, lettuce and cucumber by [75].

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