International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.08 pp 01-11, 2016

The Influence of Lithovit Fertilizer on the Chemical Constituents and Yield Characteristics of Cotton Plant under Drought Stress

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Abstract : Nano-fertilizers are used recently as an alternative to conventional fertilizers for slow release and efficient use by plants. This study was carried out to evaluate the effects of Lithovit (nano-$CaCO_3$) fertilizer on chemical constituents and yield characteristics of cotton plant under drought stress. The cotton plants pre-treated with four concentrations of nano-$CaCO_3$ (3000, 6000, 9000 and 11000 ppm) then exposed to drought stress. The obtained results showed that pretreatment of cotton plants under drought stress with nano-$CaCO_3$ caused increase 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-$CaCO_3$ to alleviate the drought stress in cotton plant was 11000 ppm. Finally, it can be concluded that foliar application of nano-$CaCO_3$ can reduce the adverse effects of drought on cotton plants.
Key words: Drought stress – Cotton – Lithovit – Nanoparticles - Chemical constituents – Yield.

Introduction

Cotton is a white fibrous agricultural product that has a wide variety of uses, from textile production, to creating paper, to producing oil and food products[1]. Cotton, *Gossypium barbadense* L., is the major fiber and oil crop in Egypt and it has been playing for along time, an important role in Egyptian national economy. It is the most important cash crop and considered as the main source of foreign exchange for the country.

Water deficit is the most important factor limiting crop yield worldwide. Plant growth, including biochemical and physiological processes, is affected by water deficit stress[2]. The results of water deficit stress depend on the severity and duration of drought as well as the growth stage and genotype of the plant. The effect of drought on growth, yield and yield component and quality characters are very different and serious. In cotton plants, the sensitivity to drought stress during flowering and boll development has been well established. Lint yield is reduced by decrease in boll production due to reduction in flowering sites and increased boll abscission when the plant is exposed to extreme drought during reproductive development[3]. The turgor decrease is the first effect of drought stress that influences cell growth rate and its final volume. The phenomenon probably is the most sensitive drought-related process, resulting in decreasing the development rate, stem growth, leaf growth and also decreasing the stomatal diameter. The drought stress affect directly or indirectly photosynthesis via affecting the carbohydrate metabolism. Due to drought stress the photosynthesis decreases, flower and bud fall

increases and competition between vegetative and reproductive for obtaining carbohydrates increases. Leaf area development in cotton in the response to drought stress is more sensitive than stomatal photosynthesis and any changes in terms of decrease or increase of carbon uptake by change in photosynthesis rate, is resulted in the decrease of boll maintenance on plants[4,5,1]. Drought stress leads to accumulation of reactive oxygen species (ROS), generated mostly in chloroplast and to some extend in mitochondria, causing oxidative stress. Major ROS molecules are singlet oxygen, superoxide anion radicals, hydroxyl radicals and hydrogen peroxide (H_2O_2). 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[6,7,8].

Development and application of new types of fertilizers using innovative nanotechnology are one of the potentially effective options of significantly enhancing the global agricultural productions needed to meet the future demands of the growing population. Indeed, the review of available literature indicates that some engineered nanomaterials can enhance plant growth in certain concentration ranges and could be used as nanofertilizers in agriculture to increase agronomic yields of crops and/or minimize environmental pollution[9].

Lithovit is a natural calcium carbonate (nano-$CaCO_3$) foliar fertilizer supplemented with calcium micronutrient which delivers fine particles (<10 μm) that can easily be adsorbed directly through the stomata of plant leaves. The micronutrients supplied with Lithovit influence plant metabolism and cell wall formation (Ca), resulting in a product that has potential to increase and sustain improved plant metabolism. Inside the leaf intercellular spaces Lithovit particles break down and release gaseous CO_2 enhancing the CO_2 concentration at the photo-synthetically active area within the plant leaves. The normal concentration of CO_2 in the atmosphere is approximately 0.04 vol. %, which means that most cultivated plants fail to achieve the optimum level of photosynthetic rate, which is achieved at near to 0.1 vol. % CO_2. The Lithovit particles that enter the intracellular "compartment" dock, with its negative surface of the cell membrane, where they produce a negative potential. Lithovit's mode of action is to increase CO_2 levels within the plant leaf structure and by implication enhance photosynthetic efficiency. The additional supply of micronutrients from the Lithovit complex provides a source of key plant available elements required to aid photosynthetic activity[10]. Maswada and Abd El-Rahman[11] reported that Lithovit treatments significantly increased total chlorophyll, total carotenoids, total soluble sugar and proline concentrations under stress conditions compared to control wheat plants. Exposure to elevated CO_2 significantly affects activity of antioxidative enzymes in plants. It decreased SOD activities in spruce, pine and oak[12,13] and reduced CAT activity in spruce and tobacco[12,14]. Pritchard *et al.*[15] investigated the effects of elevated compared to current atmospheric CO_2 concentration (720 and 365 Ml/l, respectively) on antioxidative enzymatic activities of two soybean genotypes. They found that elevated CO_2 significantly decreased activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione reductase (GR) in both soybean genotypes. Lithovit has a very significant influence on the growth it had significant positive influence on stem height, crown diameter, and leaf number plants under salt stress of maize[16], *Koelreuteria paniculata*[17], wheat[11], Hayward kiwifruit[10] and tomato[18].

The aim of this study was to evaluate the effects of Lithovit (nano-$CaCO_3$) 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: Lithovit was purchased from Agrolink 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 spraying cotton plants

with different concentrations of nano-$CaCO_3$ (3000, 6000, 9000 and 11000 ppm) on chemical constituents and yield characteristics under drought conditions. Seeds of cultivar Giza 94 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 irrigate plants. Plants were sprayed with Lithovit (nano-$CaCO_3$) 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 (ten plants) from each plot 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[19] and carotenoids content was determined according to method[20].

Determination of total soluble sugars: Total soluble sugars were determined in ethanol extract of cotton leaves by the phenol-sulfuric acid method according[21].

Determination of reducing sugars: Reducing sugars were determined colormetrically according to Folin and Wu method[22].

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[23].

Determination of total soluble protein: Total soluble proteins were extracted from cotton leaves according to[24] and determined by the method[25] of Lowry-Folin.

Determination of total free amino acids: Total free amino acids were determined in ethanol extract of cotton leaves by ninhydrin method[26].

Determination of proline content: Proline content of cotton leaves was determined according to method[27].

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[24].

Assay of catalase activity: Catalase (EC 1.11.1.6) activity was measured according to the method[28] 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[29] 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[30] 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; E.C. 1.6.4.2) activity was assayed according to the method[31] 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[32] as described[33] 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[34] as described by[35] 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 (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[36].

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 Lithovit on cotton plant under drought conditions

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, superoxide dismutase and glutathione

reductase), total antioxidant capacity and total reducing power. The obtained results are presented in Tables 1, 2, 3, 4 and 5.

Pigments content: Data presented in Table 1 showed that under drought conditions 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 Lithovit (3000, 6000, 9000 and 11000 ppm) under drought conditions significantly 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 as a result of application of Lithovit with the different concentration (especially 11000 ppm) may be due to those Lithovit particles can enhance the growth and productivity of crops by means of increasing the natural photosynthesis. These results are in agreement with[11,37,38]. Carmen *et al.*[39] suggested Lithovit fertilizer particles, finely sprayed on the leaf surface are absorbed and transformed in CO_2. Therefore, Lithovit fertilizer can significantly enhance photosynthesis because the external factor that limiting photosynthesis is the natural content of CO_2 in the air.

Table 1: Effect of foliar application of Lithovit (nano-$CaCO_3$) on Chlorophyll (Chl) a, b, total chlorophyll and carotenoids contents in leaves of cotton plant under drought stress

Carotenoids (mg/g DW)	Chlorophyll pigments (mg/g DW)			Concentration (ppm)	Treatment	
	Total Chl	Chl b	Chl a			
$0.62^{c}\pm0.036$	$5.10^{c}\pm0.016$	$2.46^{b}\pm0.031$	$2.64^{d}\pm0.029$	**Control (under normal conditions)**		
$0.46^{d}\pm0.047$	$3.65^{f}\pm0.035$	$1.43^{f}\pm0.034$	$2.22^{e}\pm0.021$	**Drought stress conditions**		
$0.65^{c}\pm0.026$	$3.95^{e}\pm0.024$	$1.71^{e}\pm0.018$	$2.24^{e}\pm0.022$	**3000**	**nano-$CaCO_3$**	**Drought stress conditions**
$0.70^{b}\pm0.017$	$4.56^{d}\pm0.018$	$1.79^{d}\pm0.049$	$2.76^{c}\pm0.017$	**6000**		
$0.87^{a}\pm0.012$	$5.66^{b}\pm0.022$	$1.94^{c}\pm0.029$	$3.72^{b}\pm0.033$	**9000**		
$0.90^{a}\pm0.024$	$8.24^{a}\pm0.023$	$3.64^{a}\pm0.018$	$4.61^{a}\pm0.017$	**11000**		
0.0435	0.0359	0.0393	0.0349	**L.S.D**		

-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.

Total soluble sugars content: Table (2) shows the effect of foliar application of Lithovit on total soluble sugar, reducing and non-reducing sugar contents in leaves of cotton plant under drought stress. The obtained results revealed that the contents of total soluble sugars, reducing sugars and non-reducing sugars of cotton leaves were increased with increasing the concentration of Lithovit. Lithovit was more effective at concentration 11000 ppm. According to Ahmed *et al.*[40] the total soluble and non-reducing sugars in cotton leaves were decreased at bolling stage under drought conditions. Lithovit application improves photosynthesis process, that due to may be related to its high content of carbonates, as source of CO_2, and calcium. However, it is found that elevated CO_2 and exogenous calcium application have positive effects on photosynthesis improvement. The effect of Lithovit, as a CO_2 reservoir, on total soluble sugar accumulation can be explained by its stimulatory effect on carbon assimilation[11,41].

Table 2: Effect of foliar application of Lithovit (nano-$CaCO_3$) on total soluble sugar, reducing 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)			$32.08^{e}\pm0.046$	$21.34^{e}\pm0.018$	$10.64^{e}\pm0.008$
Drought stress conditions			$27.57^{f}\pm0.024$	$19.73^{f}\pm0.040$	$7.83^{f}\pm0.024$
Drought stress conditions	**nano-$CaCO_3$**	**3000**	$28.80^{e}\pm0.028$	$20.16^{e}\pm0.025$	$8.64^{e}\pm0.029$
		6000	$30.54^{d}\pm0.016$	$20.36^{d}\pm0.022$	$10.18^{d}\pm0.029$
		9000	$36.24^{b}\pm0.018$	$23.07^{b}\pm0.028$	$13.17^{b}\pm0.014$
		11000	$41.43^{a}\pm0.027$	$26.38^{a}\pm0.027$	$15.05^{a}\pm0.011$
L.S.D			0.0426	0.0411	0.0317

-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.

The phenolics content: The accumulation of polyphenols, in adverse climate conditions and increasing the CO_2 concentration, are probably examples of adaptive protection strategies. Polyphenols constitute a large group of diverse molecules, implemented in ROS detoxification and in the protection of the photosynthetic apparatus. Polyphenols can also retard microbial and enzymatic decomposition by forming resistant compounds or by inhibiting microbial activity. Data presented in Table 3 indicated that the drought conditions increased the phenolics content as compared with control plants. The results revealed that the spraying of cotton plants with Lithovit (nano-$CaCO_3$) under drought conditions increased the contents total phenolics content in comparison with control plants especially at concentration 11000 ppm. The results showed improved antioxidant status indicating formation of antioxidants in all treatments. These results are in harmony with the results of Abdallah[42] mentioned 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). While, Ibrahim[5] revealed that water stress tended to reduce total phenols content significantly in wilted cotton leaves (Giza 90) as compared to turgid. This variation in results may be due to differences in plant varieties and cultivars, experimental conditions and drought stress period. The enrichment of CO_2 supply has been shown to significantly affect secondary metabolites formation including phenolics and flavonoids in various plant or cell cultures[43,44,45].

Total soluble proteins: The results in Table 3 revealed that the total soluble proteins were increased significantly in cotton leaves under drought stress as compare with plant under normal condition. The results in Table 3 indicated that the foliar application of Lithovit under drought conditions significantly increased total soluble proteins especially with concentration 11000 ppm of nano-$CaCO_3$. The increase in total soluble proteins during drought stress was due to the expression of new stress proteins[5,46]**.** The increase in Lithovit treatments may be due to any effects of elevated CO_2 on crop protein content could be ameliorated by increased use of N fertilizer. When plants grew under elevated CO_2 conditions, the rate of photosynthesis was higher, as were the nitrate reductase and glutamine synthetase activities; thus, drought had less effect on the protein content. This outcome could also result from the fact that under elevated CO_2 conditions, nitrogen uptake was higher than under ambient conditions[47,48].

Table 3: Effect of foliar application of Lithovit (nano-$CaCO_3$) on total phenol, total soluble protein, total free amino acids 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 free amino acids (mg/g FW)	Proline (µmol/g FW)
Control (under normal conditions)			11.17^{f}±0.024	8.0^{d}±0.589	12.35^{f}±0.029	3.25^{f}±0.029
Drought stress conditions			16.17^{e}±0.029	22.8^{c}±0.283	18.85^{e}±0.028	73.32^{e}±0.021
Drought stress conditions	**nano-$CaCO_3$**	**3000**	19.42^{d}±0.041	23.0^{bc}±0.432	20.55^{d}±0.023	142.79^{d}±0.018
		6000	26.18^{c}±0.032	24.0^{b}±0.632	22.75^{c}±0.018	182.64^{c}±0.022
		9000	28.95^{b}±0.024	30.0^{a}±0.432	25.06^{b}±0.025	208.55^{b}±0.040
		11000	32.12^{a}±0.045	30.8^{a}±0.365	28.79^{a}±0.038	225.55^{a}±0.014
L.S.D			0.0377	0.0552	0.0414	0.0384

-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.

Total free amino acid and proline contents: The data presented in Table 3 showed that total free amino acid and proline contents of cotton plants under drought stress were increased significantly as comparison with control plants. Lithovit treatments on cotton plant under drought stress increased significantly total free amino acid and proline contents as comparison with plants untreated under normal and drought conditions. By decreasing water potentials, proline accumulation involved in osmoregulation appeared to allow additional water to be taken up from the environment, thus counteracting the influence of drought stress on the plant tissues. Accumulation of proline caused proline synthesis or decrease proline degradation or High proline concentration measured in sludge-treated nodules could also contribute to a protective role as scavenger of ROS[49]. The large amount of accumulated free proline might also be a source of energy and N for the plants used when stress is relative[50]. Proline would be a good storage form of nitrogen because of its metabolic proximity and ready conversion to glutamic acid which is considered a key compound in nitrogen metabolism. These results are in agreement with Ibrahim[5] in cotton, Hammad and Ali[8] in wheat, Lum *et al.*[51] in barley plants and Shinde and Thakur[52] in pea.

Total antioxidant capacity and reducing power: The results of total antioxidant capacity and total reducing power in cotton leaves under drought stress are presented in Table 4. The obtained results showed that the spraying of cotton plants by Lithovit (nano-$CaCO_3$) at concentration 11000 ppm increased total antioxidant capacity (1.096±0.005) and at concentration 9000 ppm increased total reducing power (1.228±0.007) in cotton plants under drought conditions. This may be related to the CO_2 concentration has a substantial influence on the stress sensitivity of plants via changes in antioxidant enzyme activity. When oxidative stresses are present, elevated CO_2 helps to increase the synthesis and activities of antioxidants that tend to alleviate the various problems caused by the stresses. In addition, the possibility exists that the greater concentrations of antioxidant compounds in plant tissues caused by the historical rise in the air's CO_2 content[53].

Table 4: Effect of foliar application of Lithovit (nano-$CaCO_3$) on the activities of 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 reducing power ($O.D_{700\ nm}$)
Control (under normal conditions)			0.683^{f}±0.003	0.481^{e}±0.006
Drought stress conditions			0.793^{e}±0.002	0.882^{d}±0.009
Drought stress conditions	nano-$CaCO_3$	3000	0.846^{d}±0.004	0.940^{b}±0.003
		6000	0.872^{c}±0.003	0.944^{b}±0.005
		9000	1.013^{b}±0.002	1.228^{a}±0.007
		11000	1.096^{a}±0.005	0.913^{c}±0.006
L.S.D			0.0059	0.0100

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

Antioxidant enzymes: Upon stress, plants have developed several antioxidation strategies to demolish the harmful effects of ROS. Several enzymatic and nonenzymatic antioxidative systems such as SOD, CAT, POD, GR and phenolic compounds, etc., are responsible for combating ROS mediated damage. The results obtained in Table 5 showed that the catalase activity was significantly increased by drought treatment in as compared to turgid leaves. The foliar application of Lithovit to cotton plants under drought conditions increased the activities of catalase, peroxidase, superoxide dismutase and glutathione reductase in comparison with control and untreated stressed plants. Ali *et al.*[53] reported that CAT and POD are also the major H_2O_2 detoxifying enzymes in plants. According to our result both CAT and POD activity continued to increase throughout the culture period. The combined action of CAT and POD is critical in mitigating the effects of oxidative stress, since both act on H_2O_2 converting it in to water and oxygen. The increased GR activity was accompanied by an increase in total glutathione content, suggesting that a predominant GSH synthesis took place. A significant increase in glutathione content was found under different stress conditions and playing an important role for ROS scavenging[54]. Frenandez-Trujillo *et al.*[55] and Ali *et al.*[53] inducted that increases in the atmosphere's CO_2 concentration may increase various plant antioxidant enzymes and thereby reduce the negative effects of various abiotic stresses.

Table 5: Effect of foliar application of Lithovit (nano-$CaCO_3$) on the activities of catalase and 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^{f}±0.004	0.316^{f}±0.002	0.401^{e}±0.002	0.09^{d}±0.018
Drought stress conditions			0.132^{e}±0.007	0.420^{e}±0.007	0.619^{d}±0.004	0.05^{e}±0.029
Drought stress conditions	nano-$CaCO_3$	3000	0.163^{d}±0.006	0.486^{d}±0.009	0.637^{c}±0.007	0.15^{c}±0.024
		6000	0.177^{c}±0.009	0.496^{c}±0.004	$0.660f^{b}$±0.007	0.12^{bc}±0.018
		9000	0.196^{b}±0.004	0.520^{b}±0.003	0.667^{b}±0.006	0.20^{b}±0.041
		11000	0.217^{a}±0.003	0.721^{a}±0.004	0.690^{a}±0.003	0.24^{a}±0.022
L.S.D			0.0093	0.0084	0.0080	0.0371

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

Yield characteristics: The obtained results in Table (6) showed that all yield characteristics i.e. plant height, number of fruiting branches, number of boll, boll weight (g), seed index (g), lint%, yield K/F and leaf relative water content of cotton plants were decreased under drought stress conditions in comparison with control plants of Giza 94 cultivar. CO_2 fixation is reduced and photosynthetic rate decreases, decrease in photosynthetic pigments, carbohydrates accumulation and nitrogenous compounds as showed in Tables (1 and 2). The decrease in yield and yield components in cotton crop under drought conditions has also been reported by[5,56-60].

The present study showed that pre-treatment of cotton plants under drought stress with Lithovit (nano-$CaCO_3$) decreased adverse effects of drought stress and the nano-$CaCO_3$ at 11000 ppm concentration was actively involved in the regulation of plant growth. Lithovit acts as a long term reservoir supplying plants with CO_2; thus it can enhance growth and productivity of plants, particularly C3-plants as wheat and cotton by increasing P_n (photosynthesis net) because higher CO_2 can suppress ribulose-1,5-bisphosphate (RuBP) oxygenase activity; decrease photorespiration; and increase carbon assimilates for plant growth and development. When elevated CO_2 concentrations generally increase plant growth through increased carbon assimilation, biomass and leaf area of plants[11,61]. All exogenous treatments, particularly Lithovit application, significantly enhanced grain and straw yields of plants. This effect could be related to the influence of Lithovit, as a source of calcium and CO_2 reservoir[62,63,64]. Nassef and Nabeel[65] revealed that the foliar application of Lithovit enhanced significantly plant height, main head length, main head diameter, main head weight and total head yield (ton/feddan) in favor of 0.05% concentration in the two growing seasons. The highest broccoli yield was obtained from Calabrese cultivar planted with direct seeds and subjected to 0.05% Lithovit as foliar application. Also, transplanting the same cultivar without using Lithovit fertilizer gave better growth and higher yield. Sala *et al.*[66] found that Lithovit foliar application on wheat seedlings influenced the synthesis and accumulation of fresh matter and dry matter in wheat shoots and roots. Rebalancing of the shoot: root ratio was recorded for fresh weight and dry weight.

Table 6. Effect of foliar application of nano-$CaCO_3$ on yield characteristics of cotton plant (cv Giza 94) under drought stress conditions season 2015

Relative water content (%)	Yield K/F	Lint %	Seed index (g)	Boll weight (g)	No. of open boll/plant	No. of fruiting branch/plant	Plant height (cm)	Concentration (ppm)	Treatment	
$60.74^{a}\pm1.82$	$6.67^{a}\pm0.12$	$40.70^{d}\pm0.21$	$12.12^{ab}\pm0.21$	$3.21^{a}\pm0.14$	$20.52^{a}\pm1.29$	$18.25^{b}\pm0.95$	$148.51^{c}\pm1.50$	**Control (under normal conditions)**		
$49.42^{c}\pm1.29$	$3.80^{e}\pm0.24$	$40.45^{e}\pm0.11$	$12.03^{b}\pm0.26$	$3.18^{a}\pm0.18$	$19.00^{a}\pm0.82$	$18.06^{b}\pm0.13$	$137.53^{e}\pm0.58$	**Drought stress conditions**		
$52.15^{bc}\pm1.09$	$4.51^{d}\pm0.11$	$40.88^{cd}\pm0.15$	$12.16^{ab}\pm0.22$	$3.23^{a}\pm0.14$	$19.25^{a}\pm0.96$	$18.25^{b}\pm0.50$	$144.02^{d}\pm0.50$	**3000**	**nano-$CaCO_3$**	**Drought stress conditions**
$52.93^{bc}\pm1.63$	$5.76^{c}\pm0.09$	$41.32^{b}\pm0.09$	$12.23^{ab}\pm0.21$	$3.25^{a}\pm0.11$	$19.51^{a}\pm1.29$	$18.54^{b}\pm1.29$	$145.01^{c}\pm0.82$	**6000**		
$54.78^{b}\pm1.49$	$5.96^{b}\pm0.14$	$41.70^{a}\pm0.16$	$12.31^{ab}\pm0.18$	$3.31^{a}\pm0.11$	$19.75^{a}\pm0.51$	$18.51^{b}\pm0.50$	$146.54^{b}\pm0.58$	**9000**		
$55.23^{b}\pm1.81$	$6.02^{b}\pm0.14$	$41.96^{c}\pm0.15$	$12.44^{a}\pm0.16$	$3.34^{a}\pm0.14$	$20.75^{a}\pm1.50$	$19.75^{a}\pm0.51$	$148.75^{a}\pm0.50$	**11000**		
4.313	0.168	0.219	0.315	0.208	1.652	1.110	1.237	**L.S.D**		

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

References

1. Sahito A, Baloch ZA, Mahar A, Otho SA, Kalhoro SA, Ali A, Kalhoro FA, Soomro RN, Nawaz R. Ali F. Effect of water stress on the growth and yield of cotton crop (*Gossypium hirsutum* L.). Am. J. Plant Sci., 2015, 6: 1027-1039.
2. Gardner WR, Gardner HR. Principles of water management under drought conditions. Agric. Water Manag., 1983, 7:143-155.
3. Pettigrew WT. Moisture deficit effects on cotton lint yield, yield components, and boll distribution. Agron. J., 2004, 96:377-383.
4. Alishaha O, Ahmadikhah A. The effects of drought stress on improved cotton varieties in golesatn province of Iran. Int. J. Agron. Plant Prod., 2009, 3 (1): 17-26.

5. Ibrahim AA. Biochemical studies on cotton plant under drought conditions. 2013, M.Sc. Thesis, Faculty of Agriculture, Cairo University.
6. Demiral T, Turkan I. Comparative lipid peroxidation, antioxidant defense system and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot., 2005, 53: 247–257.
7. Khan MH, Panda SK. Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. Acta Physiol. Plant, 2008, 30: 81–89.
8. Hammad SAR, Ali OAM. Physiological and biochemical studies on drought tolerance of wheat plants by application of amino acids and yeast extract. Ann. Agric. Sci., 2014, 59(1): 133–145.
9. Liu R, Lal R. Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. Sci. Total Environ., 2015, 514: 131- 139.
10. Thorn AJ, Rogan LM. The effects of Lithovit on production and returns in a Hayward kiwifruit orchard. Grower Services Manager, 2015, 1: 1-13.
11. Maswada HF, Abd El-Rahman LA. Inducing salinity tolerance in wheat plants by hydrogen peroxide and lithovit "a nano-$CaCO_3$ fertilizer". J. Agric. Res. Kafr El-Sheikh Univ., 2014, 40 (4): 696-719.
12. Polle A, Pfirrmann T, Chakrabarti, S, Rennenberg, H. The effects of enhanced ozone and enhanced carbon dioxide concentrations on biomass, pigments and antioxidative enzymes in spruce needles (*Picea abies* L.). Plant Cell Environ., 1993, 16: 311–316.
13. Schwanz P, Haberle KH, Polle A. Interactive effects of elevated CO2, ozone and drought stress on the activities of antioxidative enzymes in needles of Norway spruce trees (*Picea abies*, [L] Karsten) growth with luxurious N-supply. J. Plant Physiol., 1996, 148: 351–355.
14. Havir EA, McHale NA. Enhanced peroxidatic activity in specific catalase isozymes of tobacco, barley and maize. Plant Physiol., 1989, 91: 812-815.
15. Pritchard SG, Ju Z, Santen E, Qiu J, Weaver DB, Prior SA, Rogers HH. The influence of elevated CO_2 on the activities of antioxidative enzymes in two soybean genotypes. Aust. J. Plant Physiol., 2000, 27: 1061–1068.
16. Azevedo Neto AD, Prisco JT, Eneas-Filho J, Medeiros JVR, Gomes-Filho E. Hydrogen peroxide pretreatment induces stress acclimation in maize plants. J. Plant Physiol., 2005, 162: 1114–1122.
17. Sabina PD. Research concerning the use of some seed and material preparation method in the production of biological material in generative *Koelreuteria paniculata Laxm*. J. Hort. Forestry Biotechnol., 2013, 17: 185- 188.
18. Moisă R, Berar V. Study of the effect of several natural fertilizers on the seedlings leaf area for some tomato cultivars. J. Hort. Forestry Biotechnol., 2015, 19(2): 77-81.
19. Arnon DI. Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. J. Plant Physiol., 1949, 24:1-15.
20. Robbelen G. Ztscnr Unter Suchungen an strahlenin duzierten Blaltt arbumtaten Von *Arahidopsis thaliana* (L.) Heynt. Z. Indutet. Absbanmu Verbungaleherett, 1957, 88:189-202.
21. Cerning BJ. A note on sugar determination by the anthrone method. Cereal Chem., 1975, 52:857-860.
22. A.O.A.C. Official Methods of Analysis of the Association of Official Analytical Chemists, 12^{th}ed. 1975, Washington D.C., USA.
23. Simons TJ, Ross AF. Change in phenol metabolism associated with enclosed systemic resistance to tobacco mosaic Virus Samson N. tobacco. Phytopathol. J., 1971, 61:1261-1265.
24. Choudhury S, Panda SK. Role of salicylic acid in regulating cadmium induced oxidative stress in *Oryza sativa*L. roots. Bulg. J. Plant Physiol., 2004, 30(3-4): 95-110.
25. Dawson RMC, Elliott DC, Elliott WH, Jones KM. Data for Biochemical Research, 1986, Clarendon press, Oxford. 543p.
26. Rosen H. A modified ninhydrin colormetric analysis for amino acid nitrogen. Arch. Biochem. Biophys., 1957, 67:10-15.
27. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress. Plant Soil J., 1973, 39:205-207.
28. Sinha AK. Colorimetric assay of catalase. Anal. Biochem., 1972, 47:389-395.
29. Herzog V, Fahimi HD. A new sensitive colorimetric assay for peroxidase using 3,3'-diaminobenzidine as hydrogen donor. Anal. Biochem., 1973, 55:554-562.
30. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 1971, 44: 276-287.
31. Carlberg I, Manneryik B. Glutathione reductase. Methods Enzymol., 1985, 113:484-490.

32. Prieto P, Pineda M, Anguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. Anal. Biochem., 1999, 269:337-341.
33. Kumaran A, Karunakaran RJ. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT-Food Sci. Technol., 2007, 40(2): 344-352.
34. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucoamine. Jpn. J. Nutr., 1986, 4: 307-315.
35. Mathew S, Abraham TE. In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies Food Chem. Toxicol., 2006, 44: 198-205.
36. Schonfeld MA, Johnson RC, Carver BF, Mornhinweg DW. Water relations in winter wheat as drought resistance indicators. Crop Sci., 1988, 28: 526-531.
37. Agrawal M, Deepak SS. Physiological and biochemical responses of two cultivars of wheat to elevated levels of CO_2 and SO_2, singly and in combination. Environ. Pollut., 2003, 121:189–197.
38. Wang L, Feng Z, Schjoerring JK. Effects of elevated atmospheric CO_2 on physiology and yield of wheat (*Triticum aestivum* L.): A meta-analytic test of current hypotheses. Agric. Ecosyst. Environ., 2013, 178: 57– 63.
39. Carmen B, Radu S, Ştefania G, Sorin V. Physiological indicators study involved in productivity increasing in tomato. ProEnvironment, 2014, 7: 218 – 224.
40. Ahmed FM, Ismail MS, Abdel-Al M. Effect of drought conditions at bolling stage on some chemical constituents of cotton plant. J. Agron. Crop Sci., 1989, 163:167–173.
41. Tan W, Meng QW, Brestic M, Olsovska K, Yang X. Photosynthesis is improved by exogenous calcium in heatstressed tobacco plants. J. Plant Physiol., 2011, 168: 2063-2071.
42. Abdallah MM. Effect of drought conditions at different stages on phenol and carbohydrate content of cotton leaves. Z Ack Pflanzenbau, 1985, 155:246-252.
43. Ibrahim MH, Jaafar HZE. Involvement of carbohydrate, protein and phenylalanine ammonia lyase in up-regulation of secondary metabolites in *Labisia pumila* under various CO_2 and N_2 levels. Molecules, 2011, 16: 4172-4190.
44. Ali G, Jaafar HZE. Effect of CO_2 enrichment on synthesis of some primary and secondary metabolites in ginger (*Zingiber officinale* Roscoe). Molecules, 2011, 12: 1101-1114.
45. Jang YS, Baque MA, Shiragi HK, Hyun S, Lee, EJ, Paek KY. CO2 enriched microenvironment induces biosynthesis of anthraquinones, phenolics and flavonoids in bioreactor cell suspension cultures of Morinda citrifolia (L.): the role of antioxidants and enzymes. Aust. J. Crop Sci., 2013, 7(11):1606-1616.
46. Mohammadkhani N, Heidari R. Drought-induced accumulation of soluble sugars and proline in two maize varieties. World Appl. Sci. J., 2008, 3(3):448-453.
47. Mena-Petite A, Lacuesta M, Munoz-Rueda A. Ammonium assimilation in *Pinus radiate* seedlings: effects of storage treatments, transplanting stress and water regimes after planting under simulated field conditions. Environ. Exp. Bot., 2006, 55: 1-14.
48. Robredo A, Pérez-López U, Miranda-Apodaca J, Lacuesta M, Mena-Petite A, Munoz-Rueda A. Elevated CO_2 reduces the drought effect on nitrogen metabolism in barley plants during drought and subsequent recovery. Environ. Exp. Bot., 2011, 71: 399–408.
49. Silveira JA, Viegas RA, da Rocha IM, Moreira AC, Moreira RA, Oliveira JT. Proline accumulation and glutamine synthetase activity and increased by salt-induced proeolysis in cashew leaves. J. Plant Physiol., 2003, 160:115-123.
50. Janagoudar BS, Venkatasubbaiah K, Janardhan KV, Panchal YC. Effect of short term stress on free proline accumulation, relative water content and potassium content in different plants parts of three cotton genotypes. Indian J Plant Physiol., 1983, 26(1):82-87.
51. Lum SM, Hanafi MM, Rafii YM, Akmar ASN. Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. The J. Animal Plant Sci., 2014, 24(5): 1487-1493.
52. Shinde BP, Thakur J. Influence of Arbuscular mycorrhizal fungi on chlorophyll, proteins, proline and total carbohydrates content of the pea plant under water stress condition. Int. J. Curr. Microbiol. App. Sci., 2015, 4(1): 809-821.
53. Ali MB, Dewir YH, Hahn E, Peak K. Effect of carbon dioxide on antioxidant enzymes and ginsenoside production in root suspension cultures of Panax ginseng. Environ. Exp. Bot., 2008, 63: 297-304.
54. Noctor G, Gomez L, Vanacker H, Foyer CH. Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signaling. J. Exp. Bot., 2002, 53: 1283–1304.

55. Frenandez-Trujillo JP, Nock JF, Watkins CB. Antioxidant enzyme activities in strawberry fruit exposed to high carbon dioxide atmospheres during cold storage. Food Chem., 2007, 104: 1425-1429.
56. Gorham J, Bridges J, Malik MN, Khan IA. Physiological response of cotton to water deficit in Pakistan. Proceedings of the 2nd World Cotton Conference, 6-12 September, 1998, Athens, Greece, pp. 316-328.
57. Gebaly SG. Effect of foliar application of methanol under two levels of irrigation regime on cotton productivity. Egypt J. Agric. Res., 2007, 85(2): 615–628.
58. Namich AAM. Response of cotton cultivar Giza 80 to application of glycine betaine under drought conditions. Minufiya J. Agric. Res., 2007, 32 (6), 1637–1651.
59. Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi Y. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust. J. Crop Sci., 2010, 4(8):580-585.
60. Shallan MA, Hassan HMM, Namich AM, Ibrahim AA. Effect of sodium niroprusside, putrescine and glycine betaine on alleviation of drought stress in cotton plant. Am. Eurasian J. Agric. Environ. Sci., 2012, 12 (9): 1252-1265.
61. Rebbeck J, Scherzer AJ. Growth responses of yellow-poplar (*Liriodendron tulipifera* L.) exposed to 5 years of O_3 alone or combined with elevated CO_2. Plant Cell Environ., 2002, 25:1527–1537.
62. Reddy KR, Hodges HF, Kimball BA. Crop ecosystem responses to global climate change: cotton. In: Climate Change and Global Crop Productivity (Eds Reddy, K.R. and Hodges, H.F.) CAB International, 2000, Wallingford, UK., pp. 162–187.
63. Zhao D, Reddy KR, Kakani VG, Read JJ, Sullivan JH. Growth and physiological responses of cotton (*Gossypium hirsutum* L.) to elevated carbon dioxide and ultraviolet-B radiation under controlled environmental conditions. Plant Cell Environ., 2003, 26: 771–782.
64. Kumar P. Nanotechnology in agriculture. Financing Agric., 2011, 34: 8-10.
65. Nassef DMT, Nabeel AHM. Response of two broccoli cultivars to foliar application of Lithovit fertilizer under two planting methods. Assiut J. Agric. Sci., 2012, 43:(6): 27-45.
66. Sala F, Rawashdeh H, Boldea M. Biofortification and shoot: root ratio in wheat seedlings under the influence of certain mineral elements. AgroLife Scientefic J., 2015, 4(2): 106-113.

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