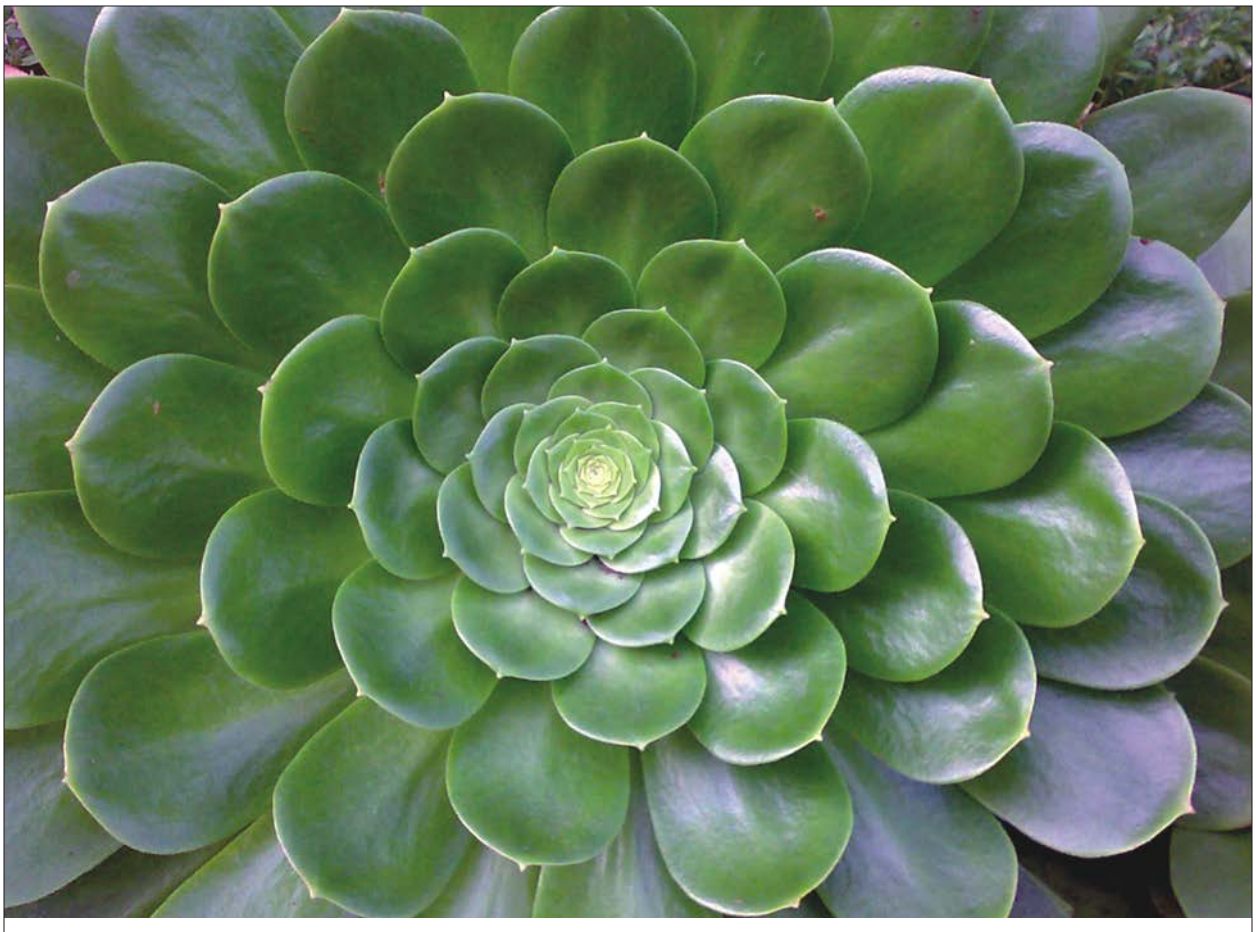

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Research Article

Nano-titanium Dioxide (Nano-TiO_2) Mitigates NaCl Stress by Enhancing Antioxidative Enzymes and Accumulation of Compatible Solutes in Tomato (*Lycopersicon esculentum* Mill.)

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Abstract

In recent years nano materials have been emerged as potential source of plant growth and development. Of these, nano-TiO_2 plays important role in several plant developmental processes including defence against environmental stresses. Present investigation was carried five to test, whether nano-TiO_2 could improve growth, yield and quality of tomato fruits under salt stress. Tomato plants were grown with 200 mM NaCl and/or five levels of nano-TiO_2 viz., 0, 5, 10, 20 and 40 mg L^{-1}. The results show that 200 mM NaCl inhibited all the growth attributes and physiological and biochemical parameters except plant height. However, salt stress enhanced the activities of antioxidant enzymes (SOD and POX), H_2O_2 content and TBARS and compatible solutes [proline (Pro) and Glycine Betaine (GB)] content. Salt stress also suppressed all the yield attributes except fruit number. Regarding quality attributes, salt stress reduced lycopene content, whereas, total phenolics and antioxidant capacity was increased under salinity. However, foliar spray of nano-TiO_2 overcame deleterious effects of salt stress and improved most of these parameters. Out of five levels, of 20 mg L^{-1} nano-TiO_2 proved best which improved the activities of carbonic anhydrase, nitrate reductase, SOD and POX and accumulation of Pro and GB. Cumulative effect of these parameters contributed to improved growth and yield of tomato plants. Therefore, on the basis of assessment of results it can be postulated that nano-TiO_2 at the rate of 20 mg L^{-1} proved best in enhancing growth, yield and quality of tomato.

Key words: Nano-titanium dioxide, salt stress, tomato, glycine betaine, antioxidant enzyme

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tabuk is recognized as one of the hot spots for the cultivation of horticultural crops in Saudi Arabia. Tomato (*Lycopersicon esculentum* Mill.) occupies a pivotal position in the list of the horticultural crops. It is an excellent source of antioxidants, dietary fiber, minerals and vitamins and is one of the low-calorie vegetables; hold just 18 calories per 100 g. Total-Oxygen Radical Absorbance Capacity (ORAC) or antioxidant power of tomato is 367 µmol TE/100 g (http:// www.nutrition-and-you.com/tomato.html). Inspite of being such an important commodity, production and quality of tomato does not meet the requirements, because low humidity and high temperature of Tabuk region mutually accelerate the rate of evaporation, which leaves behind the accumulated salts. High level of salinity poses ionic stress which adversely affects growth, leaf area and photosynthetic capacity (Erdal *et al*., 2013), enzyme activities, productivity and quality of vegetable crops (Tari *et al*., 2015). Excessive accumulation of Reactive Oxygen Species (ROS) is one of the primary effects of salt stress. Overproduction of ROS causes peroxidation of membranes lipids and leakage of electrolytes (Khan *et al*., 2012), damages proteins, enzymes and nucleic acids (Abogadallah, 2010; Sharma *et al*., 2012; Ismail *et al*., 2014a, b). All these detrimental effects ultimately contribute to the crops with reduced yield and low nutritive value (Jannesari *et al*., 2016). Thus, the horticulture sector of Tabuk region is forced to survive under the detrimental regime of salinity. Therefore, the local farmers of the region are not getting full return of their investments and at the same time the consumers get the tomato with low nutritive value.

However, to cope with salt stress-induced oxidative stress, plants are equipped with a defence system of various antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POX). The SOD constitutes the first line of defence against ROS (Alscher *et al*., 2002) and dismutates superoxide radicals to H_2O_2, whereas POX converts H_2O_2 into water and oxygen (Weydert and Cullen, 2010). Besides oxidative stress, salt stress also creates osmotic stress, which reduces the ability of plants to take up water and minerals (Munns *et al*., 2006). Plants have been shown to resist osmotic stress by accumulating osmolytes, such as proline (Pro) and Glycine Betaine (GB) (Wutipraditkul *et al*., 2015). The Pro and GB have been reported to stabilize biological membranes against the adverse effects of abiotic stresses (Sakamoto and Murata, 2000; Khan *et al*., 2012). However, timely and precise activation of these defence systems prior to the onset of damage is vital for the endurance of plants under stressful conditions.

In present years, nano-materials are emerging as potential source for plant growth and development. Materials with particle size less than 100 nm in at least one dimension are generally classified as nano-materials. Several studies reveal that nano-materials can improve growth and yield of crop plants by enhancing seed germination, water and fertilizer absorption, antioxidant system, enzyme activities, photosynthesis and nitrogen metabolism (Hong *et al*., 2005; Yang *et al*., 2006; Siddiqui *et al*., 2015). Among the nano-materials, nano-titanium dioxide (nano-TiO_2) has been shown to play important roles in plant growth and development (Frazier *et al*., 2014). It enhances seed germination, seedling growth, photosynthetic pigments, photosynthesis and dry matter accumulation (Zheng *et al*., 2005; Morteza *et al*., 2013; Mahmoodzadeh and Aghili, 2014). Nano-TiO_2 facilitates seed germination by enhancing seed stress resistance, intake of water and oxygen (Zheng *et al*., 2005; Khot *et al*., 2012). It also acts against various abiotic stresses (Mohammadi *et al*., 2014; Akbari *et al*., 2014; Jaberzadeh *et al*., 2013; Kiapour *et al*., 2015) and protects plants through enhancing antioxidant enzymes (Song *et al*., 2012; Laware and Raskar, 2014; Mohammadi *et al*., 2014).

Therefore, to cope with detrimental situation of salt stress, induction of salinity tolerance capacity within the plant, through the up-regulation of antioxidant enzymes, accumulation of compatible solutes by some means would be of considerable importance for better growth and yield without compromising the quality of tomato fruits. Considering important roles of nano-TiO_2 in plants, the present study was carried out to test whether nano-TiO_2 could mitigate the adverse effects of salt stress and improve yield and quality of tomato grown under NaCl stress.

MATERIALS AND METHODS

Plant culture: Surface sterilized healthy seeds of BL-1076 genotypes of tomato were sown in 25 cm diameter plastic pots filled with acid-washed sand. In each pot 20 seeds were sown at the depth of approximately 2 cm, later on thinning was performed and 4 plants were maintained in each pot. Plants grown in pots were kept under natural illuminated conditions (General climatic conditions, such as humidity, day/night temperature were recorded during the experiment). To fulfill nutrient requirement of growing plants, all the pots were supplied with 50 mL of Raukura's nutrient solution (Smith *et al*., 1983) every day. Salinity treatment at the level of 200 mM was started 30 Days After Sowing (DAS). To avoid osmoti c shock, NaCl concentration was increased by 25 mM

every two days until the desired concentration was achieved. Nano-TiO_2 was applied as foliar spray one week after completion of NaCl treatment. The treatments were comprised of (i) Double Distilled Water (DDW) (T0: Control), (ii) 200 mM NaCl (T1), (iii) 200 mM NaCl+5 mg L^{-1} TiO_2 (T2), (iv) 200 mM NaCl+10 mg L^{-1} TiO_2 (T3), (v) 200 mM NaCl+20 mg L^{-1} TiO_2 (T4) and (vi) 200 mM NaCl+40 mg L^{-1} TiO_2 (T5).

Plants treated with DDW only were considered as control. Each pot was considered as one replicate and all the treatments were repeated five times. At 60 DAS the response of the plants to salt stress and nano-TiO_2 was assessed in terms of growth parameters viz., plant height, number of leaves, leaf area per plant (LA), fresh and dry weight of shoot and root and physiological and biochemical parameters viz., Carbonic Anhydrase (CA) activity, Nitrate Reductase (NR) activity, leaf chlorophyll content (Chl), Leaf Relative Water Content (LRWC), hydrogen peroxide (H_2O_2) content, content of thiobarbituric acid reactive substances (TBARS), activities of antioxidant enzymes [superoxide dismutase (SOD) and peroxidase (POX)] and proline (Pro) and Glycine Betaine (GB) content. Yield attributes were studied at harvest (120 DAS) in terms of fruit number, fruit fresh weight and dry weight and total yield, whereas, quality parameters were studied in terms of lycopene content and total phenolics and antioxidant capacity.

Measurement of growth attributes: Growth of plants in response to NaCl and nano-TiO_2 was assessed in terms of plant height, fresh weight, dry weight, number of leaves and leaf area per plant (LA). Dry weight was recorded by drying the plants in oven at 80°C for 24 h. The LA was measured by outlining about 10% of leaves on a graph paper and dry weight of these leaves was recorded. The LA was determined using leaf dry weight per plant and dry weight of those leaves for which the area was estimated (Watson, 1958).

Determination of physiological and biochemical parameters

Assay of Carbonic Anhydrase (CA) activity: The activity of CA (E.C. 4.2.1.1) was measured using the method as described by Dwivedi and Randhawa (1974). Fresh leaves (chopped leaf-pieces) were transferred to petri plates. The leaf pieces were dipped in 0.2 M cystein hydrochloride solution for 20 min at 4°C. To each test tube, 0.2 M sodium bicarbonate solution and 0.022% bromothymol blue were added. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. The enzyme was expressed as μM CO_2 kg^{-1} leaf FW sec^{-1}.

Assay of Nitrate rate Reductase (NR) activity: Activity of NR (E.C. 1.6.6.1) was estimated by the intact tissue method developed by Jaworski (1971). In this method fresh chopped leaves were transferred to plastic vials containing phosphate buffer (pH 7.5), potassium nitrate solution and 5% isopropanol. The vials, containing the reaction mixture were incubated for 2 h at 30°C. After incubation, 1% sulphanilamide and 0.02% N-(1-naphthyl) ethylenediaminedihydrochloride (NED-HCL) was added. The test tubes were kept for 20 min at room temperature for maximum color development. The OD of the content was recorded at 540 nm. Activity of NR was expressed as μM NO_2 g^{-1} leaf FW h^{-1}.

Leaf chlorophyll (Chl) content: Total Chl content in the leaves was estimated using the method of Lichtenthaler and Buschmann (2001). The fresh tissue from the interveinal area of leaf was grounded with 100% acetone using a mortar and pestle. Optical Density (OD) of the pigment solution was recorded at 662 and 645 nm to determine chlorophyll a and chlorophyll b content, respectively, using a spectrophotometer. Total chlorophyll content was assessed by totaling chlorophyll a and b contents. The photosynthetic pigments, thus measured was expressed as mg g^{-1} leaf FW.

Determination of Leaf Relative Water Content (LRWC): The LRWC was measured by adopting the method of Yamasaki and Dillenburg (1999). For each treatment 10 pieces of leaves were taken. To obtain their Fresh Mass (FM), the leaves were weighed just after 24 h of treatments. In order to determine Turgid Mass (TM), the leaves were kept in DDW inside a covered petri dish for 4 h. After gently wiping the water from the leaf surface with tissue paper, the leaves were weighed. To determine Dry Mass (DM), the leaf samples were dried at 80°C for 24 h. Values for FM, TM and DM were used to calculate LRWC using the equation below.

$$\text{LRWC (\%)} = \frac{\text{FM-DM}}{\text{TM-DM}} \times 100$$

Determination of proline (Pro) and Glycine Betaine (GB) content: The Pro content was determined spectrophotometrically adopting the ninhydrin method of Bates *et al.* (1973). First homogenized 300 mg of leaf samples in sulphosalicylic acid and then 2 mL each of acid ninhydrin and glacial acetic acid was added. The samples were heated at 100°C. The mixture was extracted with toluene and the free toluene was quantified spectrophotometrically at 528 nm using L-proline as a standard.

The GB content was estimated by the method of Grieve and Grattan (1983). Leaves were weighed and oven-dried at 80°C, the dried leaves were finely ground with deionized water at 100°C for 60 min. The GB concentration was determined at 365 nm, using aqueous extracts of dry-ground leaf material after reaction with KI_2-I_2.

Determination of lipid peroxidation: Lipid peroxidation was estimated by the content of thiobarbituric acid reactive substances (TBARS) as described by Cakmak and Horst (1991). The TBARS were extracted from 0.5 g chopped leaves, ground with 5 mL of 0.1% (w/v) trichloroacetic acid (TCA). Following the centrifugation at 12,000 g for 5 min, an aliquot of 1 mL of the supernatant was added to 4 mL of 0.5% (w/v) TBA in 20% (w/v) TCA. Samples were incubated at 90°C for 30 min. Thereafter, the reaction was stopped using an ice bath. Centrifugation was performed at 10,000 g for 5 min and the absorbance of the supernatant was recorded at 532 nm with the help of a spectrophotometer at 600 nm. The TBARS content were expressed as nmol g^{-1} leaf FW.

Assay of antioxidant enzymes: Leaf tissues were homogenized with three volumes (w/v) of an ice-cold extraction buffer (50 mM tris-HCl, pH 7.8, 1 mM EDTA, 1 mM $MgCl_2$ and 1.5% (w/w) polyvinyl pyrrolidone). The homogenate was centrifuged at 15,000 g for 20 min at 4°C. The supernatant was used as the crude extract for the assay of enzyme activities.

Activity of superoxide dismutase (SOD: E.C. 1.15.1.1) was determined according to Beauchamp and Fridovich (1971) by following the photo-reduction of Nitro Blue Tetrazolium (NBT). The reaction mixture contained: The 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 µM NBT, 2 µM riboflavin and 100 µL of the supernatant. Riboflavin was added as the last component and the reaction was initiated by placing the tubes under fluorescent lamps. The reaction was terminated after 10 min by removing the reaction tubes from the light source. Non-illuminated and illuminated reaction without supernatant served as calibration standards. The absorbance of the solution was measured at 560 nm.

Activity of peroxidase (POX: E.C. 1.11.1.7) was assayed by the method of Upadhyaya *et al.* (1985). The reaction mixture contained 2.5 mL of 50 mM potassium phosphate buffer (pH 6.1), 1 mL of 1% hydrogen peroxide, 1 mL of 1% guaiacol and 10-20 µL of enzyme extract. The increase in absorbance at 420 nm was read.

Determination of yield and quality attributes: Yield attributes were studied in terms of fruit number per plant, fruit fresh and dry weight and fruit yield per plant and quality attributes were studied by measuring lycopene and phenolics content and antioxidant capacity of tomato fruits.

Lycopene content: Lycopene was estimated spectrophotometrically according to the method of Fish *et al.* (2002). Approximately 0.3-0.6 g samples were added to 5 mL of 0.05% (w/v) BHT in acetone, 5 mL of ethanol and 10 mL of hexane. The recipient was introduced in ice and stirred on a magnetic stirring plate for 15 min. After shaking, 3 mL of deionised water was added and the samples were shaken for 5 min on ice. Samples were then left at room temperature for 5 min to allow the separation of both phases. The absorbance of the hexane layer (upper layer) was measured at 503 nm blanked with hexane.

Total phenolics: Total phenolic contents of tomato fruits were determined using the modified Folin-ciocalteu reagent (McDonald *et al.*, 2001). An aliquot of plant extract was added to 1.58 mL of distilled water and 100 µL of Folin-ciocalteu reagent. The reaction mixture was shaken and allowed to stand for 5 min before the addition of 300 µL of 20% Na_2CO_3. After 20 min at 40°C, the absorbance was measured at 765 nm against each blank. The content of phenol was calculated from the standard curve obtained from different concentrations of gallic acid and expressed as mg g^{-1} FW.

Determination of antioxidant capacity: The capacity of the sample extracts to capture free radicals was measured. Antioxidant capacity was determined according to the Ferric Reducing Antioxidant Power (FRAP) method of Benzie and Strain (1996). The working FRAP reagent was prepared daily by mixing 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride and 10 mM 2,4,6-tripyridyl-S-triazine in 40 mM HCl in the ratio of 10:1:1 (v/v/v). The total phenolics extracted samples (300 µL) was added to 2.7 mL of the FRAP working solution incubated at 37°C and vortexed. The absorbance was recorded at 593 nm using a UV-vis spectrophotometer after the mixture had been incubated at 37°C for 10 min. The FRAP values was calculated from $FeSO_4$-$7H_2O$ standard curves and expressed as µmol g^{-1} DW.

Statistical analysis: Each pot was considered as one replicate and all the treatments were repeated five times. The data were analyzed statistically with SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). When F value was found to be significant at 5% level of probability, Least Significant Difference (LSD) was calculated. Values were expressed as Means±Standard Deviation (SD).

Table 1: Effect of nano-TiO_2 on growth attributes of tomato (*Lycopersicon esculentum* Mill.) grown under NaCl

	Parameters						
Treatments	Plant height (cm)	No. of leaves	Leaf area per plant (cm^2)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
T0	79.3±2.15	17±1.31	126.49±1.61	5.89±1.02	1.86±0.053	1.77±0.020	0.85±0.010
T1	73.6±1.87	9±1.17	89.74±1.35	3.61±1.14	1.47±0.061	1.15±0.018	0.61±0.016
T2	75.5±1.26	11±1.02	97.93±2.10	4.16±0.89	1.51±0.049	1.19±0.051	0.49±0.021
T3	76.3±1.11	13±1.39	101.51±1.71	4.75±1.12	1.69±0.051	1.46±0.062	0.62±0.035
T4	76.4±1.04	12±0.96	108.36±2.73	5.76±0.88	1.83±0.029	1.66±0.039	0.77±0,051
T5	74.8±1.18	16±1.10	114.35±1.86	4.21±1.28	1.78±0.045	1.53±0.044	0.65±0.039
LSD at 5%	NS	2.15	3.27	1.27	0.05	0.09	0.03

T0: DDW (control), T1: 200 mM NaCl, T2: 200 mM NaCl+5 mg L^{-1} TiO_2, T3: 200 mM NaCl+10 mg L^{-1} TiO_2, T4: 200 mM NaCl+20 mg L^{-1} TiO_2 and T5: 200 mM NaCl+40 mg L^{-1} TiO_2. Average of five determinations is presented with LSD at 5%±SD

Table 2: Effect of nano-TiO_2 on the activities of Carbonic Anhydrae (CA), NR and leaf chlorophyll content and Leaf Relative Water Content (LRWC) of tomato (*Lycopersicon esculentum* Mill.) grown under NaCl

	Parameters			
Treatments	CA activity (μM CO_2 kg^{-1} FW sec^{-1})	NR activity (μM NO^{-2} g^{-1} FW h^{-1})	Chlorophyll content (mg g^{-1} FW)	LRWC (%)
T0	331.81±1.38	0.68±0.021	1.671±0.057	86.39±1.59
T1	254.31±2.30	0.57±0.035	1.352±0.049	58.41±2.31
T2	268.37±1.25	0.59±0.036	1.392±0.031	67.27±1.82
T3	293.49±1.61	0.60±0.051	1.533±0.009	76.55±1.66
T4	329.58±1.49	0.63±0.042	1.686±0.031	84.52±1.58
T5	311.09±1.44	0.62±0.049	1.614±0.049	71.53±2.22
LSD at 5%	4.87	0.031	0.071	2.39

T0: DDW (control), T1: 200 mM NaCl, T2: 200 mM NaCl+5 mg L^{-1} TiO_2, T3: 200 mM NaCl+10 mg L^{-1} TiO_2, T4: 200 mM NaCl+20 mg L^{-1} TiO_2 and T5: 200 mM NaCl+40 mg L^{-1} TiO_2. Average of five determinations is presented with LSD at 5%±SD

RESULTS

The present investigation was carried out to evaluate the effect of various levels of nano-TiO_2 on growth, physiological, biochemical, yield and quality characteristics of tomato grown under 200 mM NaCl and to find out the most effective dose of nano-TiO_2 against salt stress.

Effect of nano-TiO_2 on growth attributes of tomato grown under NaCl stress: The results show shat application of 200 mM NaCl significantly reduced all the growth attributes studied except plant height, which showed no effect of salt stress (Table 1). Salt stress reduced leaf number, leaf area, shoot fresh and dry weight and root fresh and dry weight by 88.9, 41.0, 63.2, 26.5, 53.9 and 39.3%, respectively, compared with their respective controls. However, application of nano-TiO_2 improved all the growth attributes with no effect on plant height. Among the five levels of nano-TiO_2 applied, 20 mg L^{-1} nano-TiO_2 (T4) alleviated salt stress more effectively than the other levels. Foliar spray of nano-TiO_2 at the rate of 20 mg L^{-1} improved leaf number, leaf area, shoot fresh and dry weight and root fresh and dry weight by 33.3, 20.7, 59.6, 24.5, 44.3 and 26.2%, respectively, compared to the plants treated with NaCl only. However, 40 mg L^{-1} nano-TiO_2 (T5) caused a reduction in these growth parameters (Table 1).

Effect of nano-TiO_2 on physiological and biochemical parameters of tomato grown under NaCl stress

Activities of CA and NR enzymes: Perusal of data show that presence of NaCl in growth medium suppressed the activities of CA and NR enzymes by 30.5 and 19.3%, respectively (Table 2). On the other hand, foliar spray of nano-TiO_2 considerably enhanced the activities of these enzymes, which show a concomitant increase with increasing concentration of nano-TiO_2 up to 20 mg L^{-1} nano-TiO_2. However, further increase in the concentration of nano-TiO_2 (T5) did not improve the enzyme activities. Nano-TiO_2 at the rate of 20 mg L^{-1} enhanced the activities of CA and NR by 29.6 and 10.5%, respectively, compared to the plants grown with NaCl only (Table 2).

Leaf Chl content and LRWC: It is evident from Table 2 that salt stress significantly reduced Chl and LRWC. However, application of nano-TiO_2 overcame the adverse effect of salinity and improved Chl and LRWC. These parameters show a concomitant increase with increasing concentration of nano-TiO_2 up to the level of 20 mg L^{-1} nano-TiO_2, however, a further increase in the concentration of nano-TiO_2 could not make any improvement in Chl and LRWC. Nano-TiO_2 at the rate of 20 mg L^{-1} improved Chl and LRWC by 24.7 and 44.7%, respectively, compared with NaCl-suffered plants (Table 2).

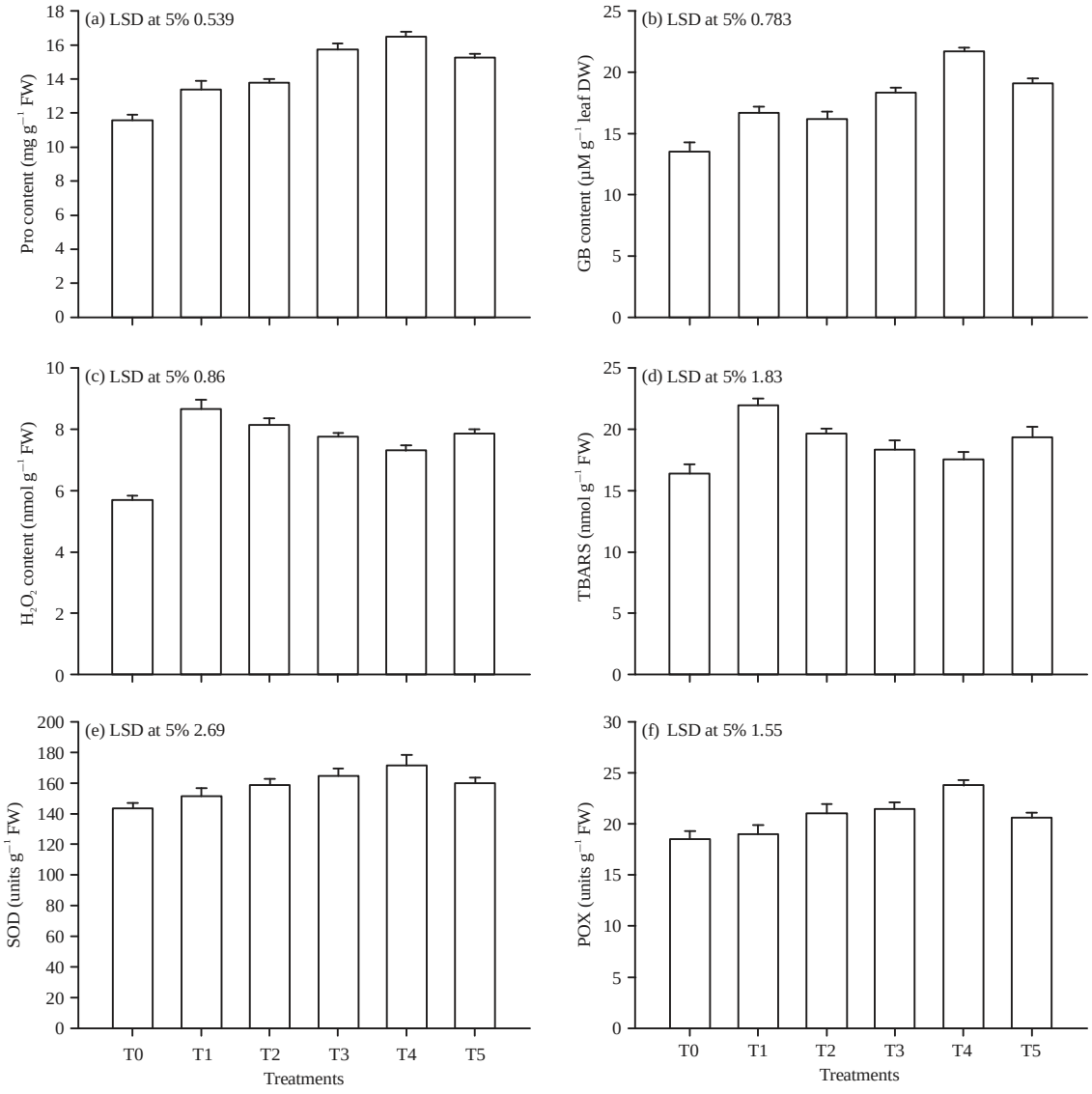

Fig. 1(a-f): Effect of (a) nano-TiO_2 on proline, (b) glycine betaine, (c) H_2O_2 content, (d) TBARS, (e) activity of SOD and (f) activity of POX of tomato (*Lycopersicon esculentum* Mill.) grown under NaCl. (To) DDW (control), (T1) 200 mM NaCl, (T2) 200 mM NaCl+5 mg L^{-1} TiO_2, (T3) 200 mM NaCl+10 mg L^{-1} TiO_2, (T4) 200 mM NaCl+20 mg L^{-1} TiO_2 and (T5) 200 mM NaCl+40 mg L^{-1} TiO_2. Average of five determinations is presented with LSD at 5%; T bars indicating SD Pro: Proline, GB: Glycine betaine, H_2O_2: Hydrogen peroxide, TBARS: Thiobarbituric acid reactive substances, SOD: Superoxide dismutase and POX: Peroxidase

Pro and GB contents: Accumulation of osmolytes under stress conditions is a defence strategy of plants to counter osmotic stress. Figure 1a and b show that osmolytes Pro and GB were enhanced by NaCl stress, moreover, foliar spray of nano-TiO_2 further enhanced Pro and GB levels. Nano-TiO_2 at the rate of 20 mg L^{-1} nano-TiO_2 gave maximum values for Pro and GB contents. The data show that NaCl treated plant gave 15.6 and 23.1% higher values of Pro and GB contents, respectively compared with controls. However, 20 mg L^{-1} nano-TiO_2 improved these compatible solutes by 23.4 and 29.7%, respectively compared to the plants treated with NaCl only (Fig. 1a and b).

H_2O_2 content and TBARS: Effect of salt stress on oxidative stress and lipid peroxidation was assessed in terms of H_2O_2 content and TBARS. The data show that salt stress generated oxidative stress and also caused peroxidation of membrane lipids as reflected by increased concentration of H_2O_2 content

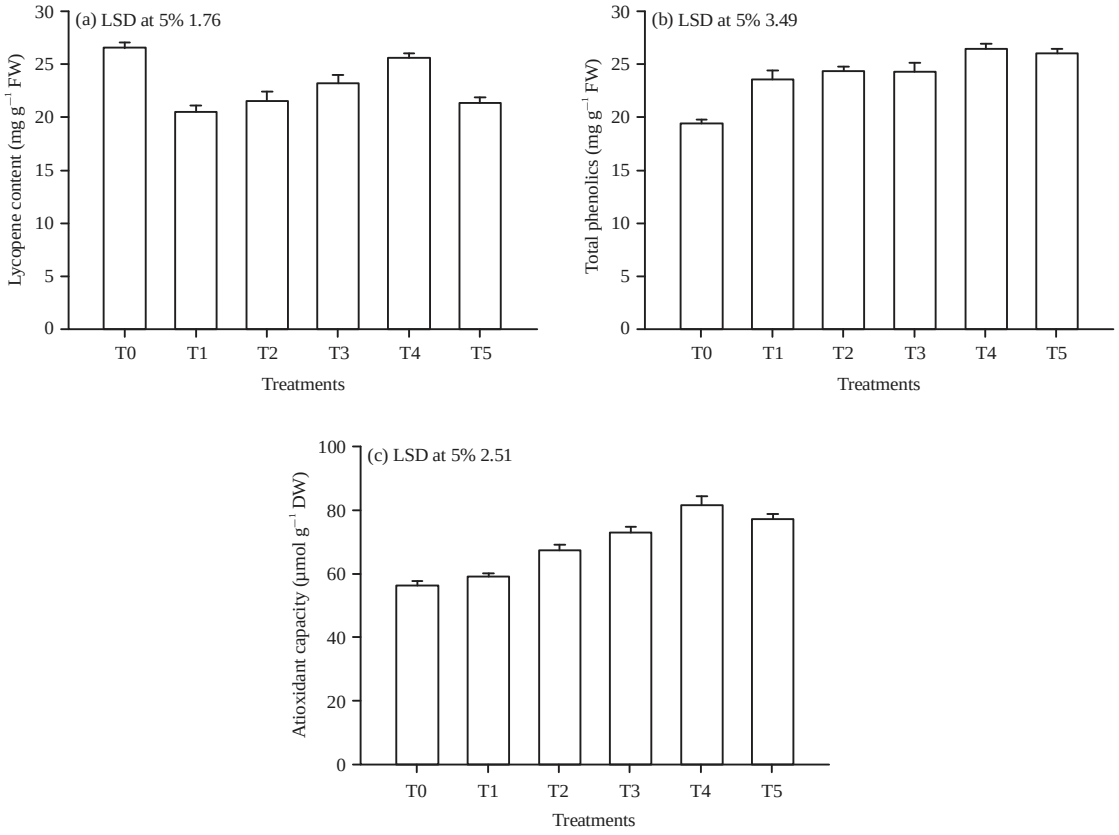

Fig. 2(a-c): Effect of (a) nano-TiO_2 on lycopene conten, (b) total phenolics and (c) antioxidant capacity of tomato (*Lycopersicon esculentum* Mill.) grown under NaCl. (T0) DDW (control), (T1) 200 mM NaCl, (T2) 200 mM NaCl+5 mg L^{-1} TiO_2, (T3) 200 mM NaCl+10 mg L^{-1} TiO_2 , (T4) 200 mM NaCl+20 mg L^{-1} TiO_2 and (T5) 200 mM NaCl+40 mg L^{-1} TiO_2. Average of five determinations is presented with LSD at 5%, T bars indicating SD

and TBARS. Presence of 200 mM NaCl in growth medium accumulated 51.5 and 33.9% more H_2O_2 content and TBARS as compared with the control plants which received DDW only (Fig. 1c and d). On contrary, foliar spray of nano-TiO_2 to salt-stressed plants reduced the level of oxidative stress and lipid peroxidation which was witnessed by a decrease in H_2O_2 content and TBARS. Nano-TiO_2 at the rate of 20 mg L^{-1} proved best and reduced H_2O_2 content and TBARS by 15.4 and 20.25% respectively (Fig. 1c and d). However, an increase in these two parameters was noticed when concentration of nano-TiO_2 was increased to 40 mg L^{-1} (Fig. 1c and d).

Activities of antioxidant enzymes: Effect of NaCl and nano-TiO_2 on antioxidative enzymes was assessed in terms of the activities of SOD and POX (Fig. 1e and f). The results exhibit that salt-stressed tomato plants protected themselves by enhancing the activities of SOD and POX. However, application of nano-TiO_2 to salt-suffered plants further enhanced the activities of SOD and POX. Highest enzymes activities were recorded in salt-suffered plants supplied with 20 mg L^{-1} nano-TiO_2, which increased the activities by 19.9 and 27.9% respectively compared with the plants supplemented with DDW only. On the other hand, a further increase in the level of nano-TiO_2 could not increase the enzyme activities further (Fig. 1e and f).

Effect of nano-TiO_2 on yield attributes of tomato grown under NaCl: Yield attributes were assessed in terms of number of fruits per plant, fruit fresh and dry weight and total yield per plant. Table 3 shows that presence of 200 mM NaCl in growth medium significantly inhibited all the yield attributes studied except number of fruits, which show a non-significant effect of NaCl. Salt stress reduced fruit fresh weight, dry weight and total yield by 11.2, 21.6 and 19.4%, respectively compared with the control. On the other hand all the yield attributes show a parallel increase with increasing concentration of nano-TiO_2. However, application of 20 mg L^{-1} TiO_2 to salt stressed plants proved best and improved these yield attributes by 7.9, 6.2

Table 3: Effect of nano-TiO_2 on yield attributes of tomato (*Lycopersicon esculentum* Mill.) grown under NaCl

	Parameters			
Treatments	No. of fruits per plant	Fruit fresh weight (g)	Fruit dry weight (g)	Total yield (kg per plant)
T0	16.00±1.04	73.6±1.52	3.15±0.404	1.152±0.0843
T1	15.67±0.93	66.2±1.37	2.19±0.337	0.965±0.0631
T2	13.67±0.65	66.5±1.41	2.11±0.371	0.959±0.0417
T3	15.33±0.59	68.9±1.29	2.28±0.260	0.983±0.0371
T4	15.00±0.62	71.4±1.51	2.71±0.518	1.071±0.0372
T5	14.67±0.48	69.7±1.28	2.64±0.410	1.048±0.0961
LSD at 5%	NS	2.58	0.863	0.135

T0: DDW (control), T1: 200 mM NaCl, T2: 200 mM NaCl+5 mg L^{-1} TiO_2, T3: 200 mM NaCl+10 mg L^{-1} TiO_2, T4: 200 mM NaCl+20 mg L^{-1} TiO_2 and T5: 200 mM NaCl+40 mg L^{-1} TiO_2. Average of five determinations is presented with LSD at 5%±SD

and 11.0%, respectively, compared to the plants which received only 200 mM NaCl (Table 3).

Effect of nano-TiO_2 on quality characteristics of tomato grown under NaCl

Lycopene content and total phenolics: The results show that salt stress significantly suppressed lycopene content of tomato fruit. Presence of 200 mM NaCl in the growth medium reduced lycopene by 29.5% compared with the control (Fig. 2a). On contrary, tomato fruits collected from NaCl treated plants show an increase in the concentration of total phenolics. Plants grown with 200 mM NaCl produced the fruits with 21.4% higher levels of phenolics as compared with control (Fig. 2b). However, these parameters show a parallel increase with increasing concentration of nano-TiO_2 with 20 mg L^{-1} proved best and enhanced lycopene content by 24.7% and total phenolics by12.3% compared with the plants treated with NaCl only (Fig. 2a and b).

Antioxidant capacity: Perusal of the data show that fruits collected from the NaCl treated plants show a slight increase in antioxidant capacity as compared to those, received only DDW (Fig. 2c). The NaCl enhanced antioxidant capacity by 4.98% as compared with control. Moreover, foliar spray of nano-TiO_2 to NaCl-suffered plants further enhanced the antioxidant capacity. Among five levels of nano-TiO_2 applied, 20 mg L^{-1} nano-TiO_2 again proved best and improved antioxidant capacity by 38.0% compared with the plants treated with NaCl only (Fig. 2c).

DISCUSSION

The present investigation shows the effect of nano-TiO_2 on the performance of tomato plants grown under 200 mM NaCl stress.

The data show that salt stress significantly affected most of the growth characteristics studied. Presence of excessive amount of salts in growth medium caused lower hydration level of plants and over production of ROS as witnessed by lower LRWC and higher levels of H_2O_2 content. Excessive accumulation of H_2O_2 resulted in the disintegration of membrane lipids as shown by higher values of TBARS in NaCl treated plants which resulted in the generation of osmotic stress (Fig. 1d). All these damaging effects of salt stress definitely contributed to a reduction in growth attributes of tomato plants (Table 1). The present results are in agreement with the findings of Munns (2002), Munns and Tester (2008) and Giannakoula and Ilias (2013) who suggested that inhibition of growth is probably the most general response of plants to stress. Al Hassan *et al.* (2015) also studied the effect of salt stress on growth of cherry tomato; they reported that high NaCl concentration significantly inhibited plant growth.

However, application of nano-TiO_2 overcame adverse effect of salt stress on growth attributes of tomato plants. Plants treated with nano-TiO_2 also showed improved activities of antioxidant enzymes (SOD and POX) and enhanced accumulation of osmolytes (Pro and GB). It has been shown that SOD constitutes the first line of defence against ROS and salt stress tolerance of plants is correlated with increased activities of SOD and various peroxidases (Li *et al.*, 2014). Moreover, salt-induced osmotic stress was countered by additional accumulation of Pro and GB (Wutipraditkul *et al.*, 2015), which facilitated the plant cells with improved osmotic pressure leading to more water uptake, as witnessed by improved LRWC (Table 2). Nano-TiO_2 has been found to induce seed germination and plant growth (Zheng *et al.*, 2005; Feizi *et al.*, 2012). Results corroborate the findings of Dehkourdi and Mosavi (2013), Hatami *et al.* (2014) and Tumburu *et al.* (2015) who reported that nano-TiO_2 caused a significant increase in seed germination indices, root and shoot length, fresh weight, vigor index and chlorophyll content of seedlings. The TiO_2 nanoparticles have been shown to regulate the activities of enzymes involved in nitrogen metabolism that facilitate the conversion of inorganic nitrogen

to organic nitrogen in the form of protein and chlorophyll leading to increase in the fresh weight and dry weight of plant (Yang *et al*., 2006; Mishra *et al*., 2014). The results show that high concentration of TiO_2 nanoparticles show inhibitory effect on most of the growth attributes studied which indicates the level of toxicity of TiO_2 nanoparticles at higher concentration. These results are in agreement with the findings of Song *et al*. (2012).

Plants grown under the regime of salinity, show suppressed activities of CA and NR enzymes and lower levels of chlorophyll content and LRWC (Table 2). However, an increase in H_2O_2 content, TBARS and in the activities of SOD and POX was recorded in salt-suffered plants (Fig. 1c-f). Sal-stressed plants accumulate higher Na^+ ions which cause reduction of the water status of leaves, it may be responsible for the higher levels of H_2O_2 and TBARS that hampered the activities of CA and NR which might have led to reduced leaf Chl content (Khan *et al*., 2012). Reduction of Chl content was probably due to the toxic effect of salinity on the instability of protein complexes and increase in the activity of chlorophyll-degrading enzyme chlorophyllase (Reddy and Vora, 1986) leading to destruction of chlorophyll. On the other hand, foliar application of nano-TiO_2 enhanced the activities of CA and NR enzymes and levels of chlorophyll content and LRWC and also further increased the activities of SOD and POX. Nano-TiO_2 increases NR activity (Akbari *et al*., 2014), light absorbance, hasten the transport and conversion of the light energy, protect chloroplasts from aging and prolong the photosynthetic time of the chloroplasts (Yang *et al*., 2006). Mohammadi *et al*. (2014) reported that TiO_2 nanoparticles cause stability of chlorophyll and carotenoid contents during cold stress. Nano-TiO_2 enhances the photosynthetic carbon assimilation by activating rubisco that could promote rubisco carboxylation, thereby increasing growth of plants (Gao *et al*., 2006). In plants treated with nano-TiO_2, increased level of SOD and POX was recorded which caused a decrease in H_2O_2 level coupled with a decrease in TBARS (Mohammadi *et al*., 2014; Laware and Raskar, 2014).

Impact of suppressed growth and physiological attributes due to salt stress was also reflected in yield and quality characteristic of tomato. Presence of 200 mM NaCl significantly suppressed fruit fresh and dry weight and total yield and lycopene content (Table 3 and Fig. 2a).

Decrease in total yield was probably due to decrease in average fruit weight not by fruit number because NaCl stress had no effect on number of fruits (Table 3). Our results are in agreement with the findings of Van Ieperen (1996) who reported a significant reduction in the average fruit weight but not in fruit number, even at low salinity levels. The data show that salt stress significantly decreased lycopene content which corroborates the findings of Ali and Ismail (2014). However, our results are not in agreement with the findings of Giannakoula and Ilias (2013). Perusal of data show that salt stress enhanced phenolic content of tomato fruits (Fig. 2b). Ali and Ismail (2014) also reported an increase in total phenolics of tomato grown under salt stress. Salt stress also caused a slight increase in antioxidant capacity of tomato fruits (Fig. 2c). It is well documented that plants possess a system of defense against various stresses, these defence systems are usually activated to counter oxidative stress as reflected by higher values of phenolics and antioxidant capacity of salt-stressed tomato fruits compared with the control.

Foliar application of nano-TiO_2 improved most of the yield and quality attributes. This can be explained on the basis of the results where nano-TiO_2 alleviated salt stress by enhancing activities antioxidative enzymes (SOD and POX) and accumulation of Pro and GB. All these collectively suppressed excessive generation of ROS and also countered osmotic stress which facilitated the plants with integrated cell membrane improved water status leading to improved yield and quality attributes. Nano-TiO_2 has been shown to enhance activity of NR, chlorophyll content and net photosynthetic rate by activating Rubisco carboxylase activity (Akbari *et al*., 2014), thereby increasing dry matter accumulation which ultimately culminated in improved yield of treated plants.

CONCLUSION

Present study shows that salt stress significantly affected most of the growth, physiological, biochemical, yield and quality attributes of tomato. However, increasing levels of foliar application of nano-TiO_2 considerably alleviated adverse effects of salt stress on tomato. Out of five levels of nano-TiO_2 applied, 20 mg L^{-1} nano-TiO_2 proved best and alleviated salt stress most efficiently. Therefore, in conclusion 20 mg L^{-1} nano-TiO_2 can be recommended for better cultivation of tomato.

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