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EFFECT OF FOLIAR SPRAY OF ZnO-NPs ON THE PHYSIOLOGICAL PARAMETERS AND ANTIOXIDANT SYSTEMS OF *LYCOPERSICON ESCULENTUM*

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Key words: catalase, *Lycopersicon esculentum*, zinc oxide nanoparticles, physiological parameters, superoxide dismutase.

Abstract

The nature of nanoparticles and their effective application has been given considerable attention by researchers in various fields, mainly agriculture. The present investigation examined the foliar effect of zinc oxide nanoparticles (ZnO-NPs) on plant growth profiling, photosynthetic machinery and associated biochemical changes in tomato (*Lycopersicon esculentum*) following growth in various concentrations (10, 50, 100 and 200 ppm ZnO-NPs). After 15 days of transplantation, ZnO-NPs sprayed to the foliage of tomato plant for five days (35-39 DAS). Treated plants at days 45 and 60 (pre-flowering stage), registered an increase in growth and biomass over their respective control. Among different concentrations of ZnO-NPs [0 (control), 10, 50, 100 and 200 ppm], 50 ppm proved to be the optimum foliar spray treatment and increase the SPAD chlorophyll (27% and 32%), net photosynthetic rate (31% and 35%), leaf protein content (17% and 22%), catalase (CAT, 55% and 61%), peroxidase (POX, 68% and 75%) and superoxide dismutase (SOD, 50% and 55%) activity. Interestingly, significant increases in lycopene (23%), β-carotene (25%) content followed by a decrease in the content of ascorbic acid (38%) in response to above treatments. Number of fruits and fruit yield in the treated plants were also higher (21% and 28%) as compare to respective controls. These results suggest that ZnO-NPs interact with meristematic cells triggering biochemical pathways conductive to an enhancement of growth attribute. Further studies are needed to investigate the mechanisms and the side effects of ZnO-NPs on tomato plants.

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Introduction

In the recent years, NPs has been attractive area of research due to their exclusive properties, such as better electrical conductivity, plasticity, roughness, and formability of ceramics, escalating the hardness and potency of metals and alloys, and by increasing the radiant effectiveness of semiconductors (RITTNER and ABRAHAM 1998). Alongthis, some other properties, such as small size, high surface-to-volume ratio and unique physio-chemical properties, leads to the use of NPs in industries and wide range of consumer products (STAMPOULIS et al. 2009).

The demand of agriculture production is increasing day by day due to uncontrolled growth of world population. Therefore, it is necessary to increase the productivity of the crop by using modern technologies. Nanotechnology is one of the most promising areas which can be exploited to achieve the goal. It has been studied by various scientists, that NPs has the potential to increase the productivity of the crop (Scott and CHEN 2002, BATSMANOVA et al. 2013). In plants, NPs either harmful or beneficial, it mainly depends upon the given manner and used nanomaterials (MONICA and CREMONINI 2009). Limited reports are available on nanomaterials effect on flora (BERNHARDT et al. 2010). NPs Study in flora have recognized that NPs may be taken-up (KUREPA et al. 2010, SCHWAB et al. 2015), transported, (WANG et al. 2012, ZHAO et al. 2012) and concentrated in vacuoles, nuclei and plasmodesmata (KUREPA et al. 2010, SCHWAB et al. 2015), which modify physiological processes of plant as well as growth and development (BURKLEW et al. 2012, GARCIA-SANCHEZ et al. 2015). In plants, reactive oxygen species (ROS) is formed as a natural by-product of the normal metabolism of O_2 and have very promising roles in cell signalling and homeostasis (RAY et al. 2012). Imbalance in ROS causes oxidative stress, higher formation of ROS damage to DNA, proteins and lipid and finally cell death (TRIPATHY and OELMULLER 2012). To overcome the toxic effect of oxidative stress plant activates enzymatic (CAT, POX and SOD) and non-enzymatic (proline) antioxidants (TRIPATHY and OELMULLER 2012, SEWELAM et al. 2016). These enzymes are the key elements in the defence mechanism (ANDRE et al. 2010). ZnO-NPs included as a third most widely used NPs with an estimation of total global production about 550 and 33,400 tons per annum (BONDARENKO et al. 2013, CONNOLLY et al. 2016, PENG et al. 2017). ZnO-NPs included as a bio-safe materials that give their impacts on the biological and chemical species from the photo-oxidizing and photo-catalysis (SIRELKHATIM et al. 2015).

The data present a new approach to evaluate the impact of ZnO-NPs on the performance of *Lycopersicon esculentum* in respect of growth param-

eters, photosynthetic attributes, biochemical parameters and yield characteristics. The hypothesis of this study is that the effect of ZnO-NPs on plant production is directly related to the photosynthesis process and indirectly in context with the defence system of the plant.

Material and Methods

Plant material and treatment

Seeds of *Lycopersicon esculentum* var. PKM-1 procured from Department of Horticulture, Indian Agricultural Research Institute, (New Delhi), were surface sterilized with 1% sodium hypochlorite solution for 10 minutes, followed by repeated washings with double distilled water (DDW). The experiment was arranged in a completely randomized design in the net house of the Department of Botany of Aligarh Muslim University, Aligarh, India, under natural environmental condition. In earthen pots, sterilized seeds were sown to make nursery. At the stage of 20 days, tomato seedlings were transplanted to the maintained pots, filled with soil and farmyard manure (6:1). The plants were treated with DDW (control) and 10, 50, 100 and 200 ppm of ZnO-NPs as foliar spray at 35-39 DAS at evening. Each plant was sprayed thrice at a time. The nozzle of the sprayer was adjusted in such a way that it pumped out about 1 cm^3 of the solution in a single spray. Therefore, each plant received about 3 cm^3 of DDW or ZnO-NPs solution. Each treatment was replicated five times with three plants per replicate and plants were sampled at 45 and 60 DAS to assess various growth, photosynthetic parameters, biochemical characteristics as well as the yield.

Source of NPs

Characterization and manufacturing of ZnO-NPs as KHAN et al. (2016) described. Material study and properties are included under the process of characterization. Widely separation, microscopy and spectroscopy used in this procedure (FABREGA et al. 2011). ZnO-NPs were procured from Sigma-Aldrich Chemicals Pvt. Ltd. India. A stock solution of 200 ppm was prepared by dissolving required amount of ZnO-NPs in 10 ml DDW in 100 ml volumetric flask, and make total volume 100 ml adding DDW. Other required concentrations were prepared by diluting the stock solution.

Plant growth analysis

The plants were detached from pots along attached soil and were dipped in a water filled bucket. Soil removed gently and the lengths of root and shoot were measured by using a meter scale. The plants were stored in oven and run 24 hours at 80°C, and weighed the dry plant weight. Through the meter of leaf-area (ADC Bio scientific, Hoddesdon, UK), deliberate leaf area.

Determination of chlorophyll content and photosynthetic attributes

The SPAD value of chlorophyll in newly leaf was calibrate through SPAD chlorophyll meter (SPAD-502; Konica, Minolta sensing, Inc., Japan). Net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and internal CO_2 concentration (C_i) at each selected stage, was measured in fully expanded leaves of the plants by using portable photosynthesis system (LI-COR 6400, LI-COR, Lincoln, NE, USA). The atmospheric conditions during measurement were photosynthetic active radiation, 1,016 µmol $m^{-2}s^{-1}$, air temperature, 25°C, relative humidity, 85%, CO_2 concentration, 600 ppm and photosynthetic photon flux density (PPFD), 800 µmol mol^{-2} s^{-1}, respectively. All the measurements were made between 11:00 to 12:00 h under the clear sun light.

Analysis of NR and CA activities

Activity of NR was compute by JAWORSKI (1971) procedure. A mixture of newly form leaf (0.1 g), phosphate buffer (pH 7.5), KNO_3, and isopropanol was store in incubator at 30°C for 2 h. Sulfanilamide and N-1-napthylethylenediamine hydrochloride mixture were added to the incubated mixture. At 540 nm read absorbance with a spectrophotometer (Spectronic 20D; Milton Roy, USA). CA action in leaves was measured through DWIVEDI and RANDHAWA (1974) procedure. Leaf was slash into minute pieces in a cysteine hydrochloride solution. They were blotted and conveyed in a test tube, pursue phosphate buffer addition (pH = 6.8), 0.2 M $NaHCO_3$, bromothymol blue, and red indicator of methyl. 0.5 N HCl used for titrating.

Protein content estimation

1 g newly formed leaf, homogenized in freeze buffer extraction, which is consisted of 40 mM tris-HCl (pH = 7.5), 0.07% β-mercaptoethanol, 2% polyvinylpyrrolidone, 0.5% Triton X-100 and 1 mM phenylmethane sulfo-

nyl floride (PMFS), 1 mM ethylenediaminetetraacetic acid (EDTA) by pestle and mortar. Centrifuged at 20,000 xg for 10 minutes, and supernatant was collected to valuation the protein by BRADFORD'S (1976) method.

Antioxidative enzymes assay

In antioxidant enzymes estimation, the leaf tissue (0.5 g) was homogenized in a 50 mM phosphate buffer (pH = 7.0) containing 1% polyvinylpyrrolidone. Centrifuged at 15,000 x *g* for 10 minutes at 4ºC, resulting supernatant used as a source of enzymes like CAT, POX and SOD.

For the estimation of POX activity, the enzyme extract (0.1 mL) was added in the reaction mixture of pyrogallol, phosphate buffer (pH = 6.8), and 1% H_2O_2. The change in the absorbance was read at every 20 seconds for 2 minutes at 420 nm (CHANCE and MAEHLY 1956). A control mixture was prepared by adding DDW instead of enzyme extract. The reaction mixture for CAT consisted of phosphate buffer (pH = 6.8), 0.1 M H_2O_2, and enzyme extract (0.10 mL). H_2SO_4 was added to the reaction mixture, after its incubation for 1 minute at 25ºC, and it was titrated against potassium permanganate solution (CHANCE and MAEHLY 1956). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium following the method of BEAUCHAMP and FRIDOVICH (1971). The reaction mixture consisted of 50 mM phosphate buffer (pH 7.8), 20 µM riboflavin, 75 mM nitroblue tetrazolium (NBT), 13 mM methionine, and 0.1 mM ethylenediaminetetraacetic acid (EDTA) were irradiated under two fluorescent light tubes (40 µmol m^{-1} s^{-1}) for 10 min. The absorbance was measured at 560 nm with a UV-visible spectrophotometer (KONO 1978) with slight modifications. Blanks and controls were also run in the same manner but without illumination and enzyme, respectively. The amount of SOD activity that gave half-maximal inhibition of NBT reduction was defined as one unit of SOD activity.

Determination of proline content

BATES et al. (1973), method was used for identification of proline amount in newly form leaves. Leaves extracted in sulfosalicylic acid, an equal volume of glacial acetic acid and ninhydrin solutions was added. The sample was heated at 100°C, to which 5 ml of toluene was added. The absorbance of the aspired layer was read at 528 nm on a spectrophotometer.

Determination of Lycopene, β-carotene and ascorbic acid content

In ripe fruits amount of lycopene measured by the method given by RANGANNA (1976). In this procedure, pigment was extracted in acetone and transferred to petroleum ether layer in a separating funnel. Absorbance of the petroleum ether layer was recorded on spectrophotometer at 503 nm. Petroleum ether is also used as a blank. Lycopene content in the sample was calculated by the formula-

$$\text{Lycopene [mg g}^{-1}\text{]} = \frac{31.20 \cdot \text{absorbance}}{\text{weight of sample [g]}}$$

β-carotene content in a fruit h described by method given by SADASIVAM and MANICKAM (1997). Pigment was extracted in acetone and hexane and absorbance was read at 436 nm with the help of spectrophotometer. The amount of carotene in the sample was calculated by using standard curve prepared from pure carotene and expressed in µg g^{-1} FM.

In the mature fruit content of ascorbic acid was determined through the procedure given by RAGHURAMULA et al. (1983). The samples were homogenized with 4% oxalic acid and then centrifuged. Supernatant was titrated against 2, 6-dichlorophenol indophenol dye. The amount of dye used was placed in the following formula for calculating ascorbic acid content

$$\text{Amount of ascorbic acid [µg g}^{-1}\text{ FM]} = \frac{0.5\text{ mg}}{V_1\,\text{cm}^3} \cdot \frac{V_2}{5\text{ cm}^3} \cdot \frac{100\text{ cm}^3}{\text{Wt of sample}} \cdot 100$$

where:
V_1 – liter value of working standard
V_2 is the liter value of the sample
Wt – the weight of the sample.

Results were expressed as milligrams per gram on fresh mass basis.

Yield characteristics

At the stage of harvesting (180 DAS or post flowering stage), 9 plants (3 plant from each pot) representing each treatments were randomly sampled and counted for the number of fruits and per plant and weight to assess fruit yield per plant.

Statistical analysis

The experiment was conducted according to simple randomized block design. Each treatment was replicated five times. Data were statistically

analysed for analysis of variance (*ANOVA*) using *SPSS*, *17.0 for Windows* (*SPSS*, Chicago, IL, USA). Least significant difference (LSD) was calculated to separate the means.

Results

Growth biomarkers

ZnO-NPs treated plants showed an obvious increased in the growth of the plant, and the increased in the growth were positively related with the concentrations of ZnO-NPs applied upto certain concentrations (Figures 1a–*f*, Figure 2*a*). The maximum increase in shoot length (30.1%), shoot fresh mass (27.7%), shoot dry mass (29.0%), root length (28.7%), root fresh mass (26.1%), root dry mass (24.6%) and leaf area (24.1%) at 60 DAS was recorded in the plants treated with 50 ppm of ZnO-NPs over their control. The maximum decrease was reported in the plant sprayed with 200 ppm of ZnO-NPs.

SPAD value

With the progress of time from 45 to 60 days stage, SPAD chlorophyll content increased and also increased in the presence of ZnO-NPs in a concentration dependent manner (Figure 2*b*). Moreover, the maximum increase (32.1%) in SPAD chlorophyll values was found in the plants sprayed with 50 ppm of ZnO-NPs over their control.

Leaf gas-exchange traits

The foliar application of 50 ppm of ZnO-NPs proved best and increased the values of P_N (35.1%), gs (29.1%), Ci (31.2%) and E (32.4%) over their non-treated control plants at 60 DAS (Figures 2 *c–f*). The pattern of response of plants for photosynthesis and related attributes are 50 ppm > 100 ppm > 10 ppm > 0 ppm > 200 ppm respectively.

Activities of CA and NR

Plants raised from foliar application of ZnO-NPs had significantly higher activity of CA and NR over their respective controls. Out of 2 stages of analysis (45 and 60 DAS), the maximum CA (25.9%) and NR (28.5%) activities were noted at the stage of 60 days (Figures 3*a–b*). 50 ppm ZnO-NPs demonstrated to be best.

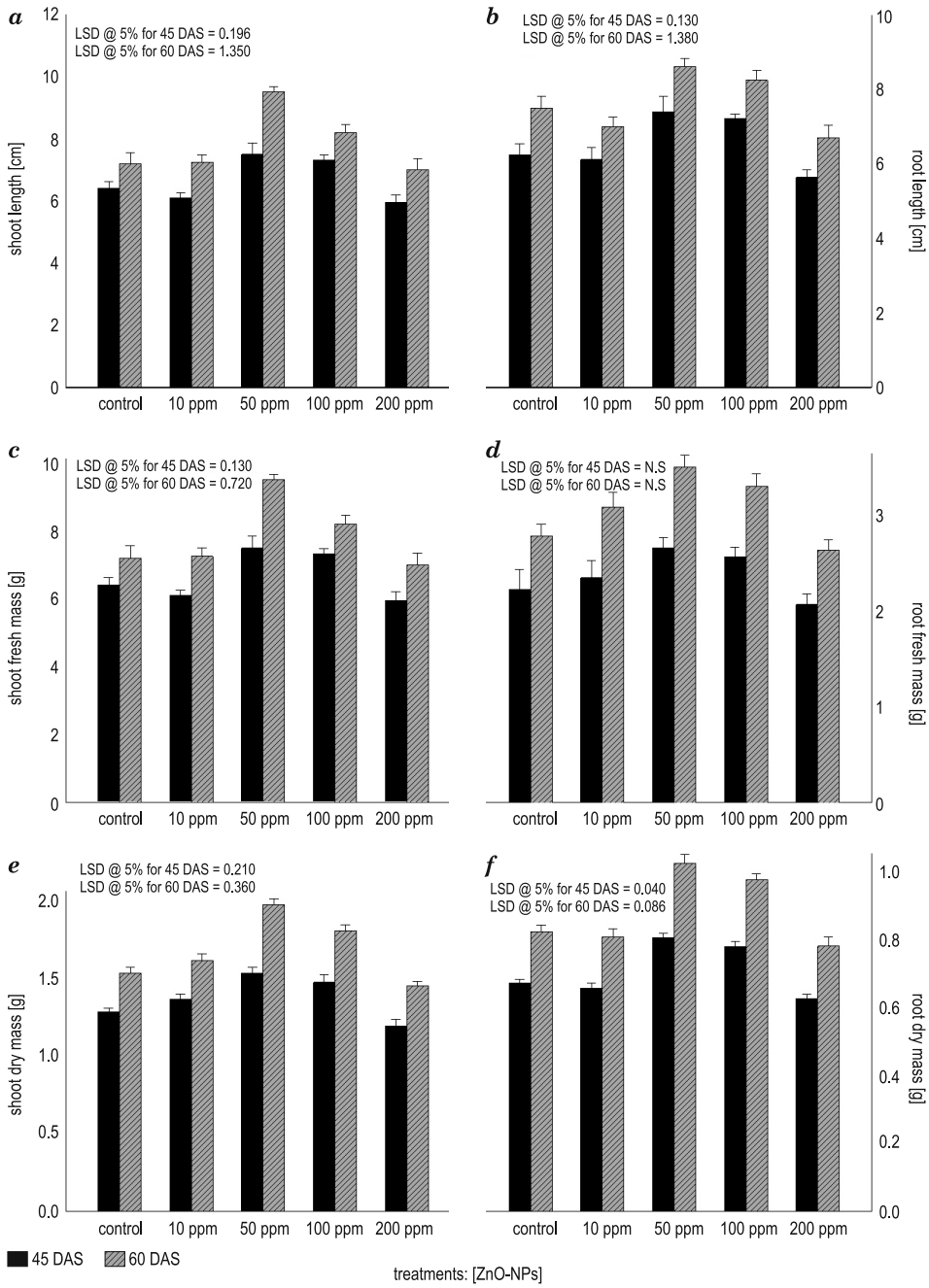

Fig. 1. Effect of different concentrations (0, 10, 50, 100 or 200 ppm) of zinc oxide nanoparticles (ZnO-NPs) on (*a*) shoot length, (*b*) root length, (*c*) shoot fresh mass, (*d*) root fresh mass, (*e*) shoot dry mass, and (*f*) root dry mass in the leaves of *Lycopersicon esculentum* seedlings at 45 and 60 DAS. Vertical bars indicate standard errors between the replicates

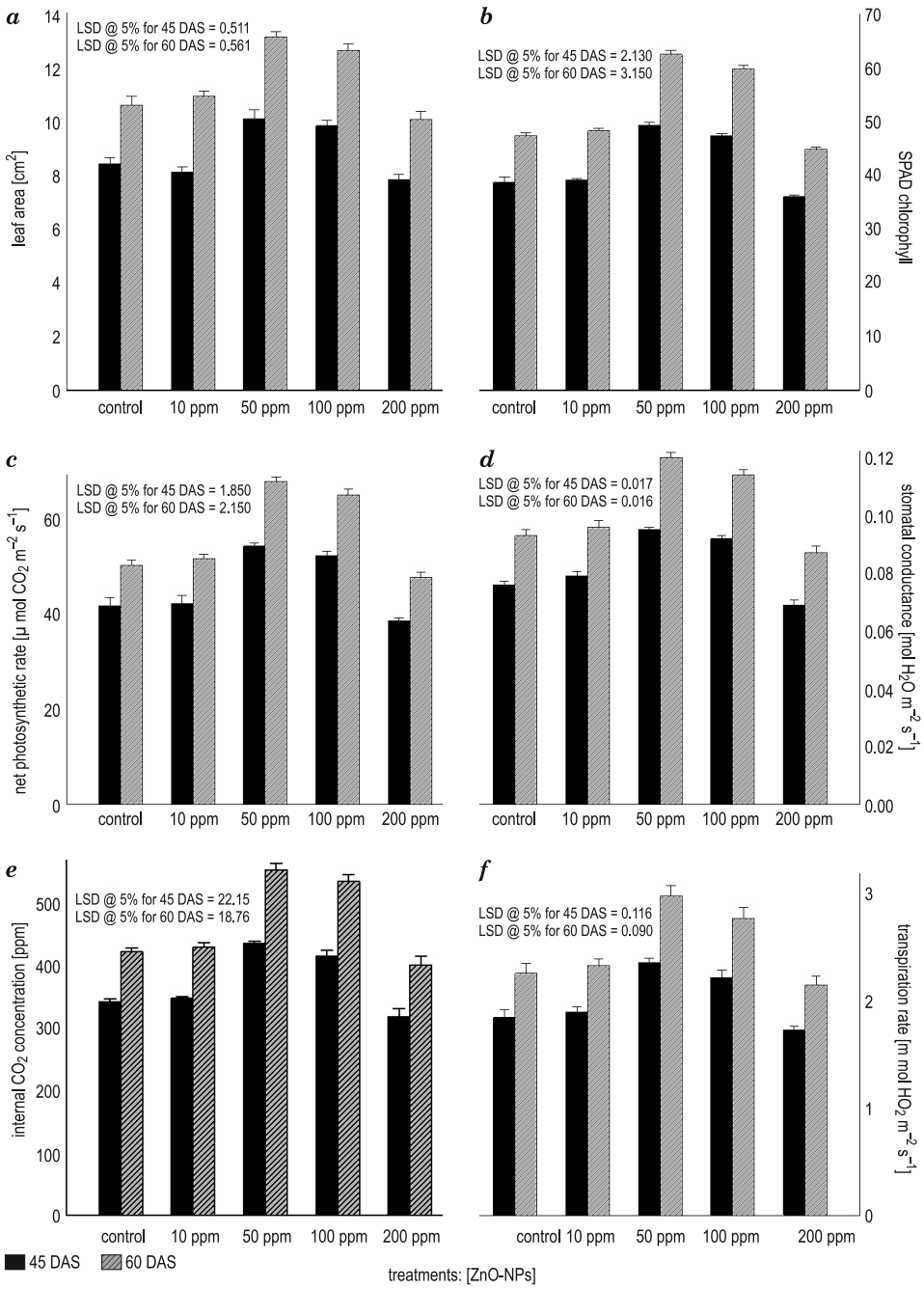

Fig. 2. Effect of different concentrations (0, 10, 50, 100 or 200 ppm) of zinc oxide nanoparticles (ZnO-NPs) on (*a*) leaf area, (*b*) SPAD chlorophyll, (*c*) photosynthetic rate, (d) stomatal conductance, (*e*) internal CO_2 concentration, and (*f*) transpiration rate in the leaves of *Lycopersicon esculentum* seedlings at 45 and 60 DAS. Vertical bars indicate standard errors between the replicates

Protein content

It is induced from the Figure 4*a* that protein content in the leaves increased with the advancement of age irrespective of treatment. The maximum value of protein content was recorded at 60 DAS in the plant treated with 50 ppm of ZnO-NPs as foliar application. The pattern of response of plants for ZnO-NPs is 50 ppm > 100 ppm > 10 ppm > 0 ppm > 200 ppm respectively.

Activity of antioxidant enzymes

Activity of antioxidant enzymes (CAT, POX and SOD) increased with age of the plant. Effect of ZnO-NPs varies from concentration to concentration in all the antioxidant enzymes. The maximum activity of CAT, POX and SOD (60%, 74% and 55%) was recorded in the plant sprayed with 50 ppm of ZnO-NPs at 60 DAS (Figures 3*c–e*).

Proline content

In tomato leaves content of proline was significantly elevated with application of ZnO-NPs. The leaves of plants exposed to the 50 ppm ZnO-NPs possessed the higher concentration of proline as compare to the non-treated (control) plants at 60 DAS which was 54 % higher (Figure 3*f*).

Lycopene, *β*-carotene and ascorbic acid content

Fruits grown from ZnO-NPs treated plants had higher content of lycopene and *β*-carotene as compared to control plants. Maximum value of lycopene and *β*-carotene was noted in plants whose leaf exposed with 50 ppm of ZnO-NPs. The respective increase was 22.6% and 24.9% over their controls at 180 DAS (Figures 4*d–e*). Ascorbic acid content decreased in the fruits in proportion to the treatment of ZnO-NPs (Figure 4*f*). Foliar application of ZnO-NPs had a negative impact on ascorbic acid content, and decreased the ascorbic acid content as compared to that of control plants.

Number of fruits and fruit yield

Graph 4*b* and *c* clearly revealed that the plants treated with ZnO-NPs had significantly higher fruits number and yield at harvest. ZnO-NPs (50 ppm) greatly increased fruit numbers per plant (21.1%) and fruit yield (19.4%) over their respective controls.

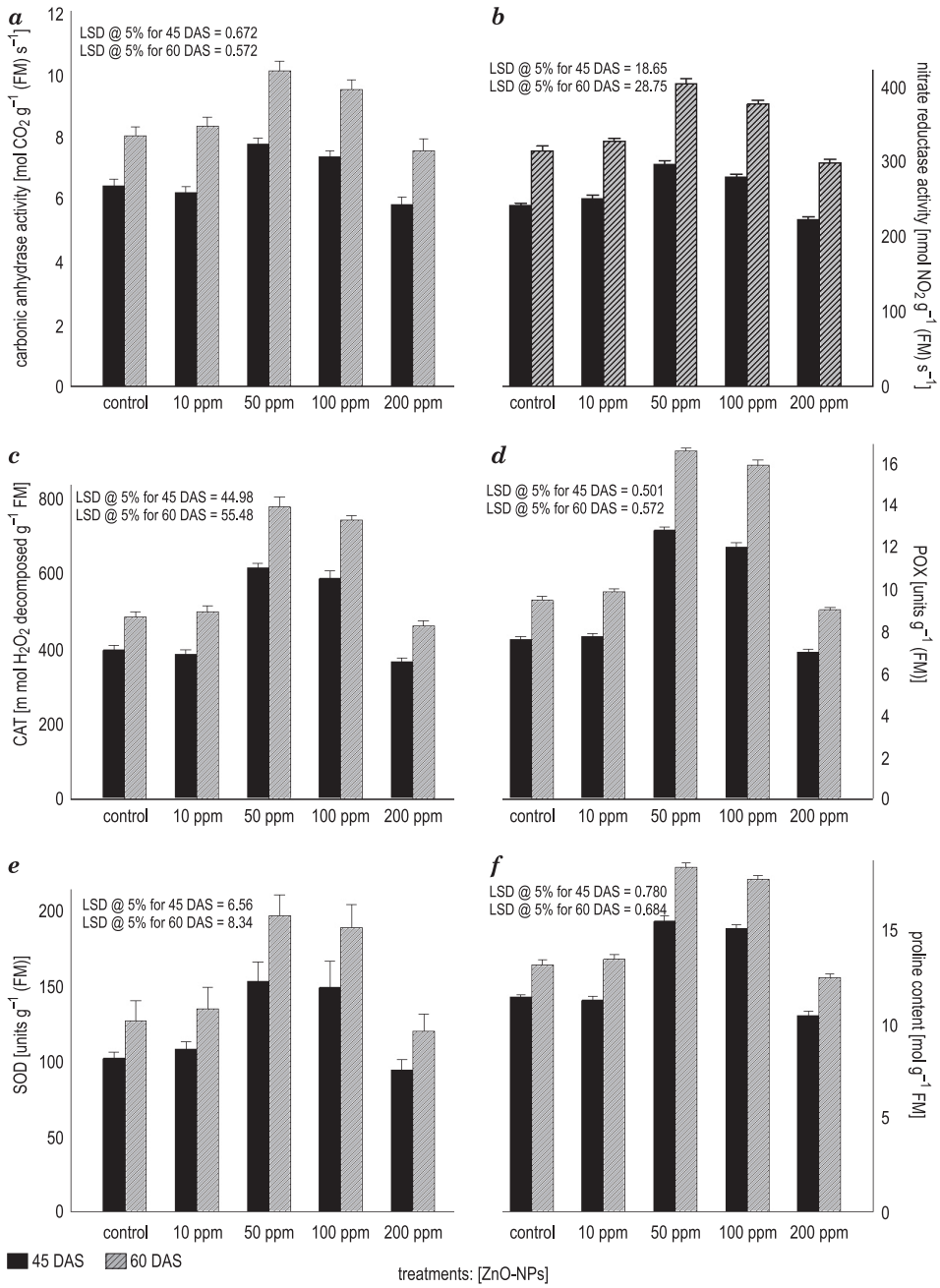

Fig. 3. Effect of different concentrations (0, 10, 50, 100 or 200 ppm) of zinc oxide nanoparticles (ZnO-NPs) on (*a*) CA activity, (*b*) NR activity, (*c*) catalase, (*d*) peroxidase, (*e*) superoxide dismutase, and (*f*) proline content in the leaves of *Lycopersicon esculentum* seedlings at 45 and 60 DAS. Vertical bars indicate standard errors between the replicates

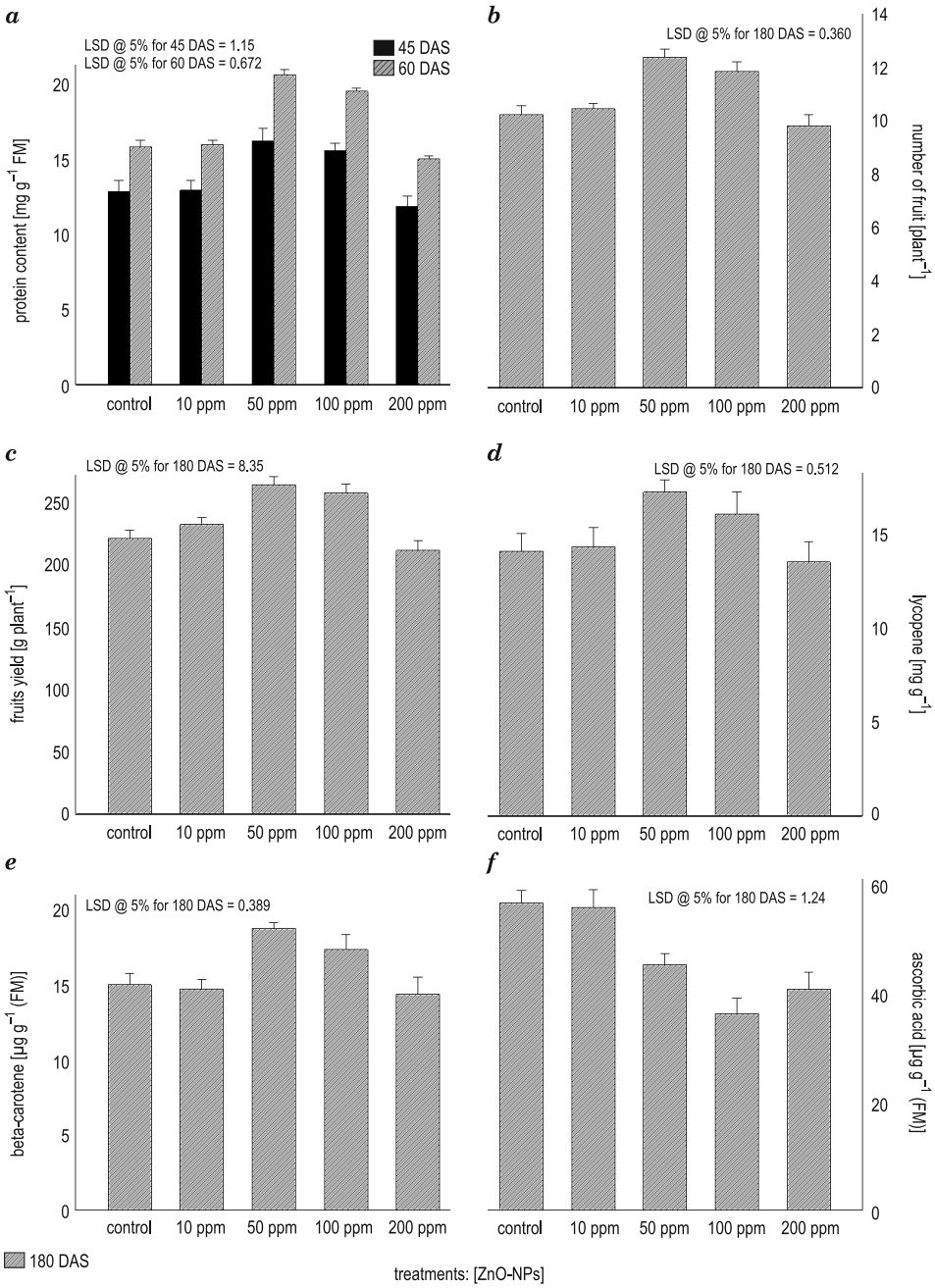

Fig. 4. Effect of different concentrations (0, 10, 50, 100 or 200 ppm) of zinc oxide nanoparticles (ZnO-NPs) on (*a*) protein content in the leaves of *Lycopersicon esculentum* seedlings at 45 and 60 DAS, (*b*) number of fruits, (*c*) fruit yield, (*d*) lycopene content, (*e*) *β*-carotene, (*f*) ascorbic acid in the leaves of *Lycopersicon esculentum* seedlings at 180 DAS. Vertical bars indicate standard errors between the replicates

Discussions

Nanotechnology is a one of the new discipline and NPs have become a centre of attraction for researchers due to its exclusive physico-chemical properties compared to their large particles (MONICA and CREMONINI 2009). Zn used in various functions like protein synthesis, membrane activity, elongation of cell etc. (CAKMAK 2000). In recent years scientist work in the field of nanotechnology and try to increase the crop productivity through changes in physiological and biochemical parameters. In plants, NPs increased crop productivity by alteration in photosynthetic machinery, biochemical and growth parameters. This increment mainly based on the concentration of NPs and its size (KHODAKOVSKAYA et al. 2012). Therefore, the present study was undertaken.

In the present study the value of growth biomarkers (root & shoot length, leaf area, fresh and dry masses of plants and leaf area) increased significantly when exposed to the ZnO-NPs. Application of ZnO-NPs could enhance the rate of photosynthesis of the plants, which leads the increased cell division and ultimately enhanced the biomass of the plant (ALLOWAY 2004). However, this study is further corroborated by the study of MARSCHNER (1993), where ZnO-NPs increased the values of shoot length, root length, shoot and root biomass in Cyamopsis tetragonoloba plant. Alongthis various other researchers also confirmed the present observations (PANDEY et al. 2010, PRASAD et al. 2012, SEDGHI et al. 2013, MUKHERJEE et al. 2014, RAMESH and TARAFDAR 2014, RASKAR and LAWARE 2014, FAIZAN et al. 2018) on various other crops. The chlorophyll content in leaves was measured as an indicator of the plants photosynthetic performance. ZnO-NPs (50 ppm), treated plant leaves showed maximum increase in chlorophyll content (SPAD value; Figure 2b). It is hypothesized that ZnO-NPs involved in the enhancement of transcription and/or translation, this leads the increase chlorophyll content in plants, and more efficiently for the synthesis of photosynthetic pigments which increased the rate of photosynthesis (Figure 5). This statement is corroborated by the study of AN et al. (2008), where NPs treatment increased the chlorophyll content in Asparagus plant. In sustainable agriculture routine, NPs arbitrate changing mechanism, need to additional investigation.

The photosynthetic machinery is the back bone of plant system; therefore if any enhancement in photosynthesis occurs due to ZnO-NPs, the related attributes such as gs, E and Ci will also increases. In this observation, photosynthetic machinery shows irregularity in contrast with different treatments of ZnO-NPs. Application of ZnO-NPs increased the formation of chlorophyll pigment and encourages Ribulose 1,5-bisphosphate

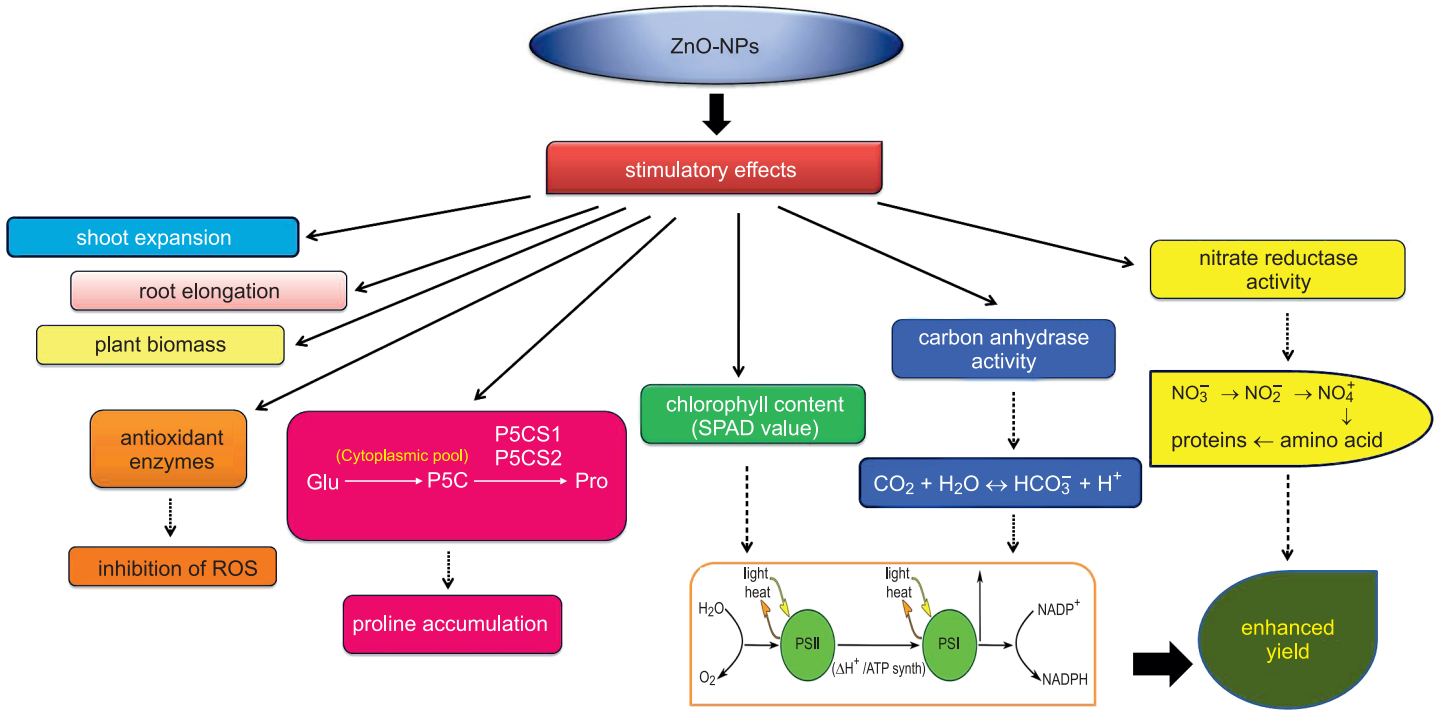

Fig. 5. Effect of ZnO-NPs on the shoot expansion, root elongation, plant biomass, carbonic anhydrase activity, nitrate reductase activity, antioxidant enzymes activity, content of proline, chlorophyll content, photosynthetic rate and yield: CO_2 – carbon dioxide; H_2O – water; HCO^{3-} – carbonic acid; O_2 – oxygen; PSI – photosystem 1st; PSII – photosystem 2nd; $NADP^+$ – Nicotinamide adenine dinucleotide phosphate; Glu – glutamate; Pro – proline; P5C – 1-Pyrroline-5-carboxylic acid; P5CS1 and 2 – delta-1-pyrroline-5-carboxylate synthase 1 and 2; NO_2^{-} – nitrite; NO_3^{-} – nirate; NO_4^{+} – ammonia

carboxylase (Rubisco) activity which stimulates the photosynthetic rate in plants. Moreover NOJI et al. (2011) suggested that silica nano particles obligated with PS II and induce constant performance of oxygen evolving reactions of photosynthetic machinery, signify the light directed transport of electron to quinine molecules from water. NOJI et al. (2011) also suggested that complexity of PS II have potential to form photosensors and imitated photosynthetic system. In this study, photosynthetic efficiency increased by the combined effect of all these altered processes (Figures 2*c–f*). Higher activity of photosynthetic system in plants would lead the higher yield of the plants (Figure 5).

In this study it was found that foliar application of different concentrations of ZnO-NPs treatment enhanced the photosynthesis and related attributes along with the CA activity (Figure 3*a*). There are several factors which determine the activity of CA, like hormonal signalling, light intensity, availability of Zn and regulation of genetic expression of the transcripts (TIWARI et al. 2005). Moreover, ZnO-NPs enhanced the assimilatory rate of CO_2, which are helpful to increase the CA activity. This can be supported by the result of Faizan et al. (2018) where ZnO-NPs treatment enhances CA activity in tomato. SiO_2-NPs also improve the photosynthetic rate by improving activity of CA (SIDDIQUI et al. 2014, XIE et al. 2012). Nitrate reductase is a primary enzyme in nitrate assimilation pathway and plays a role as a limiting factor of plant growth and development. NR activity provides an excellent estimation of N_2 amount in plant and is relevant to plant development and yield (SRIVASTAVA 1980). In above observation ZnO-NPs increased the activity of NR at certain concentration (Figure 3*b*). The total content of organic N_2 and head nitrogenous metabolites enhanced quantitatively during higher supply of NO_3^- in plants, this is reflected on increased plant growth and development (BOSE and SRIVASTAVA 2001). It is hypothesized that ZnO-NPs enhanced the activity of NR enzyme and NO_3^- uptake, this leads to higher growth and yield of the plant (Figure 5). Alongthis, the increase in chlorophyll content and CA activity by the application of ZnO-NPs enhanced the rate of photosynthesis, which finally increased the overall growth to the plants (Figure 5).

Plant yield depends on the ability of plant to adapt various types of environmental adversities, which mostly cause oxidative stress. Environmental stresses stimulate the formation of reactive oxygen species (ROS) in plant cell and cause dangerous oxidative damage in plants, which ultimately inhibit the growth and development of the plant (CAVERZAN et al. 2016). To overcome the production of ROS, plant produces several enzymes i.e. CAT, POX and SOD (CAVERZAN et al. 2016). However, application of ZnO-NPs considerably increased the formation of antioxidant

enzymes (Figure 3*c–e*) as compare to the non-treated plants. It is believed that increased activity of antioxidant enzymes is due to Zn, which maintain the protein and biomembranes stability which balance the production of scavenging ROS (KHAN et al. 1998). In stressed condition plant accumulate compatible solute i.e. proline. This solute is highly soluble and non-toxic. Proline provides protection against stress by maintaining cellular osmotic adjustment. ZnO-NPs increase the accumulation of proline in plant cell (Figure 3*f*) by conversion of glutamate into proline. It is hypothesized that ZnO-NPs increased the phosphorylation of glutamate, and this glutamate reduced into glutamic-5-semialdehyde (GSA) through the enzyme D1-pyrroline-5-carboxylate synthetase (P5CS), and randomly catalyzed into pyrroline-5-carboxylate (P5C). This P5C finally reduced into proline (Figure 5). Protein protects the plant cell from potential oxidative damage. Application of ZnO-NPs in tomato plants enhanced the content of protein through the activation of transcriptional and/or translational processes (Figure 4*a*). These results are lined with the earlier findings of RALIYA and TARAFDAR (2013) and MUKHERJEE et al. (2016), where treatment of ZnO-NPs increases the protein content in cluster bean and green pea respectively.

ZnO-NPs treated plants had higher level of lycopene and *β*-carotene in the mature fruits, over their respective controls (Figure 4*d–e*) and 50 ppm of ZnO-NPs proved best. Unlike lycopene and *β*-carotene the treatment (50 ppm) significantly decreased the ascorbic acid content in the fruits. It assumes that the increasement in lycopene and *β*-carotene is due to the ethylene-mediated alteration. A similar observation was found (KOLE et al. 2013) in bitter melon, in which they found an increase of 82% lycopene by carbon based fullerol NPs. Number of fruits and fruit yield elevated by the treatments of ZnO-NPs (Figure 4 *b–c*). The best concentration was noted to be the feeding of 50 ppm. The fruit bearing capacity of the plants is mainly determined by the density of the flowers retained in the plant (ZHAO et al. 1987). Therefore, higher yield of plants could be due to the better flowering and fruit development and improved photosynthetic rate (Figure 5).

Conclusion

The present study provides some important clues about the physiological role of ZnO-NPs in plants. NPs promote the photosynthetic rate and antioxident system of tomato plants at different concentrations. Out of the concentrations tested (0, 10, 50, 100 and 200 ppm); 50 ppm ZnO-NPs

increased the efficiency of photosynthesis and enhanced the antioxidant system of the tomato plants more significantly. Beyond this concentration, response was not very promising. The highest concentration tested (200 ppm ZnO-NPs) had an adverse effect on growth of tomato plants, and therefore should be considered as a toxic concentration. Consequently, it is concluded that 50 ppm ZnO-NPs is the preferred concentration to be used for enhancement of plant growth and development.

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