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NANO-ZINC OXIDE EFFECTS ON EGGPLANT (*SOLANUM MELONGENA* L.) TRANSPLANT QUALITY IN COMPARISON WITH CONVENTIONAL ZINC OXIDE

SAAD M. HOWLADAR

University of Jeddah, College of Science, Department of Biology, Jeddah, Saudi Arabia
Corresponding email: smhowladar@uj.edu.sa

Abstract

Eggplant (*Solanum melongena* L.) growth has been reported to be sensitive or moderately sensitive undesirable fluctuations in the environmental conditions resulting from climate changes. Consequently, producing strong eggplant transplants for open fields is needed. A nursery study was implemented in 2018 to evaluate the potential beneficial impacts of nano zinc oxide (n-ZnO) in comparison with conventional zinc oxide (c-ZnO) on growth characteristics, physiological parameters, nutrient contents, and antioxidative activities in eggplant transplants. Foliar application of 200 mg l^{-1} c-ZnO (c-ZnO_{200}) significantly increased growth characteristics, leaf relative content of water (RWC), membranes stability index (MSI), efficiency of photosynthesis (e.g., performance index; PI, Fv/Fm, and SPAD chlorophyll), nutrient (Zn, N, P, and K^+) contents, components of non-enzymatic antioxidants (soluble sugars, AsA, free proline, and GSH) and osmoprotectant contents, and antioxidative enzymes (CAT, APX, GR, and SOD) activities comparing with control. Application of 25 mg l^{-1} n-ZnO (n-ZnO_{25}) showed the same results of all investigated parameters obtained from c-ZnO_{200} application. Foliar spray of 50 mg l^{-1} n-ZnO (n-ZnO_{50}) significantly increased all abovementioned parameters compared to other treatments including 100 mg l^{-1} n-ZnO (showed toxic effect) and the control. Foliar application of n-ZnO_{50} is, therefore, the best treatment, recommended for producing healthy eggplant transplants for open fields.

Key words: Eggplant, Nano-ZnO, Growth, Physio-biochemical attributes, Antioxidant system.

Introduction

Economically, plants have low efficiency of absorption of some micro-nutrients, which causes large losses to farmers/producers, so, alternative methods, such as foliar spraying, are needed (Siavashi *et al*., 2004). Under suitable conditions, nutrients can be accessed for plants by foliar applications to achieve high plant performances. Zinc (Zn) is a pivotal micro-nutrient for all plant kinds, which is absorbed in divalent cations form. It has pivotal role in plant physiology as it participates in carbohydrates, proteins, lipids, and nucleic acids metabolisms. It is also implicated in biosynthesis of auxin and photosynthesis (Malakoti & Tehrani, 2001; Farahat *et al*., 2007). Zinc functions as a part of enzyme structure and/or it acts as a regulator cofactor for several enzymes. It is used in a group of enzyme building, may not be limited to its contributing in constructing of Cu-Zn superoxide dismutase (SOD), RNA polymerase, carbonic anhydrase, and alcohol dehydrogenase. Another group which need Zn for its activity, include aldolase, alcohol dehydrogenase, DNA and RNA polymerase, and trans-phosphorylase (Marschner, 1995). In plants that suffer from Zn deficiency, protein and indole acetic acid syntheses are reduced and ribosomes are also broken (Marschner, 1995). Zinc is absorbed by plants through two mechanisms; active and passive. Active mechanism (more affected by temperature and ventilation root) is a major Zn supplier compared to the passive one that occur by means of the adsorption of ions electrostatically on the root cell walls (Mohsenzadeh & Moosavian, 2017). Due to a slow Zn absorption from soil, it is better to provide plants by this micro-nutrient through foliar spraying (Siavashi *et al*., 2004). Foliar Zn application may affect the ability of maintaining high yield on soils having low availability of Zn. Under which several mechanisms may underlie Zn efficiency (Rengel, 2001). Although plants need zinc at low concentration (0.005–0.1 mg g^{-1}), the plant's lack of adequate zinc concentration shows its deficiency symptoms and plants suffer from physiological stress due to that multiple enzymatic systems in addition to other metabolic actions become ineffective with regard to Zn (Baybordi, 2006). Previous reports suggest that use of Zn in different plant growth stages could be affected plant performance in different ways (Alloway, 2008). It is functioned as a cofactor in activation of several enzymes for pathways of secondary metabolites biosynthesis (Baybordi, 2006) along with carbohydrates, proteins, and lipids metabolism, positively affecting plant growth and yield. It is also involved in K^+ maintenance in stomatal guard cells to regulate stomatal opening (Welch, 1995). In addition, Zn application has been reported to increase the chlorophyll fluorescence and photosynthetic rate and efficiency of physiological attributes (Munirah *et al*., 2015).

Nanotechnology is inquisitive and size controlling of a useful substance at an accurate range of 1 to 100 nm. Nutrient particles are considered as a nano-nutrient if they are included in this range (Klaine *et al*., 2008). Nanoparticles (NPs) use in agricultural sector has received significant attention, especially with the beginning of the third millennium because of their unique and distinguished properties that are intermediate to those of singular molecules and bulk matter (Rajput *et al*., 2018). In 2014, it is rated globally that 225 thousand tons of NPs were consumed (Yadav *et al*., 2014). In 2019, this consumed rate is expected to increase to 585 thousand tons (Anon., 2014). However, real figure of the global production of NPs to the researchers' knowledge is not avalilable. In recent years, nano-nutrient in the oxidized form like NPs of zinc oxide (n-ZnO) is used in various conditions (Handy *et al*., 2008). Application of n-ZnO is reported to clearly improve plant growth and yield, physiological attributes, photosynthetic efficiency, and antioxidant (enzymatic and non-enzymatic) defensive systems compared to conventional ZnO (c-ZnO) or no Zn

application (Burman *et al*., 2013; Mohsenzadeh and Moosavian, 2017; Faizan *et al*., 2018). However, compared to conventional nutrients, nano-nutrients have higher toxicity due to their easier penetration through the cell membranes and release nutrient ions inside the cell (Wu *et al*., 2010). Hence, crop producers are advised to carefully use the nano-nutrients in small concentrations compared to the conventional nutrients.

For human nutrition, eggplant (*Solanum melongena* L.) is a crucial traditional vegetable crop, especially in many tropical and subtropical countries, as well as in Mediterranean ones. Eggplant growth has been reported to be sensitive or moderately sensitive to any undesirable fluctuations in the environmental conditions resulting from climate changes (Lesk *et al*., 2016). So, producing vigor eggplant transplants for transplanting in the open field is needed to cope well with these undesirable fluctuations.

Therefore, the main objective of the present investigation was to assess the beneficial effects of n-ZnO in comparison to the c-ZnO treatment on the growth, photosynthetic efficiency, physiological attributes, macro- and micro-nutrients, and non-enzymatic and enzymatic antioxidant defensive systems in eggplant transplants before their transplantation in the open field.

Materials and Methods

Eggplant material, growing conditions and experimental layout: Sterilized seeds (cv. Soma F1 hybrid) of *Solanum melongena* eggplant were obtained from the Agricultural Research Center (ARC), Giza governorate, Egypt. Seeds were sown (one seed per cell) in Styrofoam flats (2.6 cm × 2.6 cm × 7.0 cm = 25 cm^3 per inverted pyramid cell, 209-pyramid cell). Each Styrofoam flat was contained a growth medium consisted of peat moss (organic component), and perlite and vermiculite (inorganic components). The growing medium was mixed with a compound fertilizer consisted of 415 mg NH_4NO_3 per liter, 500 mg calcium superphosphate ($H_6CaO_9P_2$) per liter, 333 mg potassium sulfate (K_2SO_4) per liter, 833 mg magnesium sulfate ($MgSO_4$) per liter, 333 mg Fe^{2+} per liter, 333 mg Zn per liter, 333 mg Mn per liter, 1.25 g $CaCO_3$ (to modify pH value of peat-containing medium) per liter, and 125 mg Moncut SC [a 25% (w/w) active flutolanil-containing, wettable powder, fungicide; Central Glass Co. Ltd, Tokyo, Japan] per liter. After seed planting and flats irrigation, twenty Styrofoam flats (for five treatments) were exposed to an average day temperature of 24º ± 3ºC and night temperature of 16º ± 2ºC, and relative humidity range of 62.0–65.1%, and a range of 11–12 h for natural day-length.

Trays were put on rails in a controlled greenhouse and each tray was planned as one replicate. Daily, eggplant transplants were overhead-irrigated, a day with tap water and the following day with a nutrient solution free from Zn. The nutrient solution was prepared to contain 1.75 g N L^{-1}, 0.875 g P L^{-1}, 1.75 g K L^{-1}, 17.5 mg Fe^{2+} L^{-1}, 8.75 mg Mn L^{-1}, 0.875 mg B L^{-1}, 0.875 mg Cu L^{-1}, and 0.35 mg Mo L^{-1}. Within the block, trays were rotated to avoid any favoritism for a position. The experiment was consisted of 5 treatments; 1) control (without Zn foliar spray), 2) foliar spray 2 times with conventional ZnO at 200 mg l^{-1} as an optimum concentration obtained from our preliminary experiment (data not shown) and designated a the second control, 3) foliar spray 2 times with nano-zinc (n-ZnO) at 25 mg l^{-1}, 4) foliar spray 2 times with nano-zinc (n-ZnO) at 50 mg l^{-1}, and 5) foliar spray 2 times with nano-zinc (n-ZnO) at 100 mg l^{-1}. Foliar spray solutions were received 1% tween-20 as a surfactant, and sprays were conducted at early morning. The flats (n = 20) were arranged in a completely randomized design (CRD) with four replications and 6-week-old transplants were collected for various measurements of morphological characters, physio-biochemical attributes, and antioxidant defense systems.

Growth measurements: Nine of 42-day-old transplants (n = 9) were selected, randomly, from each of the 5 treatments to record growth attributes. Leaf number of each transplant was counted. A meter scale was used to measure shoot length, the relationship of leaf area-leaf weight method was used to assess leaf area according to Semida *et al.*, (2017), and the Vernier Caliper (with least 0.1 mm count) was used to determine stem diameter. Seedling dry weight (DW) was recorded using a digital balance after drying the seedlings in an electric oven at 70°C for 48 h or until reaching constant weights.

Assessments of leaf relative water content (RWC) and stability index of cell membranes (MSI): The method described in Osman & Rady (2014) was used to assess RWC. At first, the midribs of leaves were removed and 20 of 2 cm-diameter discs were weighed to score their fresh weight. For 24 h in dark, discs were immersed into double-distilled water to saturate them. The adhering water droplets were softly removed from the surface of the saturated discs and then weighed to record their turgid weight. For 48 h at 70°C, discs dehydration was then implemented for dry weight. Percentage of RWC was calculated as follows:

$$\text{RWC (\%)} = \frac{\text{(Fresh weight – Dry weight)}}{\text{(Turgid weight – Dry weight)}} \text{ x } 100$$

The MSI determination method of Rady (2011) was followed using midribs-excluded leaves. A weight of 0.2 g was put in test-tube containing double-distilled water (10 ml). Using a water-bath with 40°C, tubes were kept for 30 min for recording the electrical conductivity (EC_1) of the solution. Another weight of 0.2 g was boiled for 10 min at 100 °C. The EC_2 was also recorded. Percentage of MSI was calculated as follows:

$$\text{MSI (\%)} = [1 - (EC_1/EC_2)] \times 100$$

Photosynthetic efficiency assessments: The two upper leaves of transplants were used to determine chlorophyll content by using chlorophyll (SPAD-502, Minolta, Japan) meter.

Photosynthesis performance index (PI_{ABS}) and PSII Fv/Fm maximum quantum yield were obtained according to the procedures of Clark *et al.*, (2000) and Maxwell & Johnson (2000), respectively. These determinations were performed on two different sunny days.

Nutrients determinations: To assess the macro-nutrients (i.e., N, P, and K^+) and micro-nutrients (i.e., Zn, Fe, Mn, and Cu) contents, leaves of transplants were dried and powdered. Content of N was determined using micro-Kjeldahl (Ningbo Medical Instruments Co., Ningbo, China) apparatus (Anon., 1995). To assess the P content, blue color method of Jackson (1967) was used. The standard reagents such as H_2MoO_7S, Mo blue, diluted H_2MoO_7S, and 8% (w/v) $NaHSO_3$-H_2SO_4 were used. Content of K^+ was assessed by a Perkin-Elmer Model 52-A (Glenbrook, Stamford, CT, USA) flame photometer as detailed in Page *et al.*, (1982) procedures. Contents of micro-nutrients were assessed by an Atomic Absorption (Perkin-Elmer, Model 3300) Spectrophotometer as outlined in Chapman and Pratt (1961).

Osmoprotectants and non-enzymatic antioxidants determinations: The Bradford (1976) procedure was utilized to evaluate the content of leaf protein. After extraction using 96% (v/v) ethanol, leaf soluble sugars content was assessed as detailed in Irigoyen *et al.*, (1992). The extract was reacted with an anthrone reagent, and the obtained mixture was boiled for 10 min. The Spectronic (a Bausch and Lomb-2000) Spectrophotometer was used to read the cooled samples at 625 nm.

The procedure detailed in Bates *et al.*, (1973) was followed to assess leaf proline content. After extraction of the leaf sample (0.5 g) by using sulphosalicylic acid (3%, v/v) and centrifugation of the extract (at 10,000 × g for 10 min), supernatant (2 ml) was mixed in acid ninhydrin (2 ml) freshly prepared solution. Mixtures were incubated at 90°C for 30 min using a water bath. Reaction in each mixture was terminated in an ice-bath and mixtures were then extracted again by mixing with 5 ml toluene. At room temperature, mixtures were separated in dark for 20 min. Toluene phases were collected and their absorbance readings were taken at 520 nm.

Following the procedure of Kampfenkel *et al.*, (1995) leaf ascorbic acid (AsA) was extracted and its content was determined. To determine AsA content, a weight of 1.0 g leaf sample was homogenized and extracted using liquid N_2, 5% (w/v) TCA (trichloroacetic acid). Centrifugation process (15,600 ×*g*, 4°C, 5 min) was performed. Assay of AsA was conducted using a clean reaction vessel contained 1.0 ml of reaction mixture (i.e., supernatant, 0.5% Nethylmaleimide, 10 mM DTT, 10% TCA, 4% 2,2′-dipyridyl, 42% H_3PO_4, 3% ferric chloride, in addition to 0.2 M P-buffer with pH 7.4).

The Griffith (1980) method was used to assess leaf content of glutathione (GSH). Homogenization of leave samples was performed using metaphosphoric acid (2%, v/v) and centrifugation was then practiced (17,000 × g, 10 min). Using 10% (w/v) sodium citrate, supernatant was neutralized. Assessments were done 3 times and each assay consisted of NADPH (700 µl, 0.3 mM), 5,5′-dithio-bis-2-nitrobenzoic acid (100 µl, 6 mM), distilled water (100 µl) in addition to the extract (100 µl). Thereafter, stabilization was done at 25°C for 3–4 min. At 412 nm, absorbance readings were recorded after addition of 10 µl of 50 GSH reductase Units ml^{-1}.

Assays of enzymatic antioxidants: In an ice bath, pulverization for 0.5 g of fresh tissue (leaf) was conducted in 10 ml of 50 mM buffer of K-phosphate (K_2HPO_4 + KH_2PO_4, Merck, Germany, pH 7.8). Mixture centrifugation was performed at 10,000 ×g under 4°C for 15 min. Determination of extract protein concentration was performed as described in the Bradford (1976) method.

Superoxide dismutase (EC 1.15.1.1) activity was assayed as detailed in the Kono (1978) method. The Na_2CO_3 (a buffer) and nitro-blue tetrazolium (a substrate) were used, and at 540 nm using a Spectrophotometer the inhibition rate in the NBT reduction was taken.

Catalase (EC 1.11.1.6) activity was assayed as detailed in the Aebi (1984) method. K-phosphate (KH_2PO_4; a buffer) and hydrogen peroxide (H_2O_2; a substrate) were used, and at 240 nm using a Spectrophotometer the changes in the absorbance read were observed.

Ascorbate peroxidase (EC 1.11.1.11) activity was assayed based on the Rao *et al.*, (1996) method and at 290 nm using a Spectrophotometer the absorbance value was recorded.

Glutathione reductase (EC 1.6.4.1) activity was assayed according to the Rao *et al.*, (1996) method and the NADPH oxidation was monitored for 3 absorbance readings recorded at 340 nm.

Statistical analysis

The CRD was the work layout and data obtained were analyzed statistically using one-way ANOVA. Data analysis was followed by Tukey's HSD test (SPSS 14.0; SPSS Chicago, IL, USA). Significant differences were separated based on $p \leq 0.05$ among three means in each treatment.

Results

Except for some fluctuations (i.e., number of leaves $transplant^{-1}$ and transplant stem diameter, and Cu content were not affected, and Fe and Mn contents were significantly decreased), foliar application of conventional zinc at a level of 200 mg L^{-1} (c-ZnO_{200}; an optimum Zn level for eggplant transplants obtained from our preliminary study (data not shown) significantly increased growth characteristics (shoot length by 26%, leaf area $transplant^{-1}$ by 13%, and transplant dry weight by 12%; Table 1), relative water content (RWC by 6%; Table 2), membrane stability index (MSI by 9%; Table 2), photosynthetic efficiency (SPAD chlorophyll by 15%, Fv/Fm by 5%, and PI by 10%; Table 2), macro- and micro-nutrients contents (N by 9%, P by 23%, K^+ by 10%, and Zn by 22%; Tables 3 and 4), contents of osmoprotectants and non-enzymatic antioxidants (soluble protein by 7%, soluble sugars by 41%, free proline by 36%, AsA by 102%, and GSH by 135%; Table 5), and enzymatic antioxidants activities (SOD by 66%, CAT by 7%, APX by 23%, and GR by 33%; Table 6) compared to the control.

Foliar nano zinc oxide application at a level of 25 mg L^{-1} (n-ZnO_{25}) conferred the same results of all abovementioned parameters, which were obtained from c-ZnO_{200} foliar application.

Foliar spray of nano zinc at a level of 50 mg L^{-1} (n-ZnO_{50}) significantly increased growth characteristics (Table 1), RWC% (Table 2), MSI% (Table 2), photosynthetic efficiency (Table 2), contents of macro- and micro-nutrients (Tables 3 and 4), non-enzymatic antioxidants and osmoprotectants (Table 5), and activities of enzymatic antioxidants (Table 6) compared to all other treatments including the control. Foliar application of n-ZnO_{50} is, therefore, the best treatment. It significantly increased shoot length by 45%, average number of leaves on each transplant by 23%, average leaf area per transplant by 35%, transplant dry weight by 36%, RWC% by 13%, MSI% by 20%, SPAD chlorophyll by 26%, Fv/Fm by 11%, PI by 28%, N content by 26%, P content by 58%, K^+ content by 28%, Zn content by 56%, soluble protein content by 16%, total soluble sugars content by 84%, proline content by 91%, AsA content by 187%, GSH content by 273, SOD activity by 98%, CAT activity by 33%, APX activity by 63%, and GR activity by 70% compared to the control.

On contrast of other ZnO treatments, foliar spray application of nano zinc at a level of 100 mg L^{-1} (n-ZnO_{100}) had toxic effect on eggplant transplants and strongly decreased all aforementioned parameters compared to all other treatments including the control.

Table 1. Conventional (c-ZnO) or nano zinc (n-ZnO) impacts on growth characteristics of eggplant transplants.

Treatments	Characteristics				
	Length of shoot (cm)	Leaves No. transplant^{-1}	Leaves area transplant^{-1} (dm^2)	Stem diameter (mm)	Transplant DW (g)
Control (N)	22.3 ± 1.2c	4.4 ± 0.4b	0.92 ± 0.05c	4.2 ± 0.0a	3.32 ± 0.04c
c-ZnO	28.1 ± 1.6b	4.6 ± 0.6b	1.04 ± 0.09b	4.2 ± 0.0a	3.73 ± 0.04b
n-ZnO_{25}	27.9 ± 1.5b	4.5 ± 0.6b	1.05 ± 0.08b	4.1 ± 0.0a	3.68 ± 0.04b
n-ZnO_{50}	32.4 ± 1.8a	5.4 ± 0.6a	1.24 ± 0.10a	4.4 ± 0.0a	4.52 ± 0.06a
n-ZnO_{100}	15.1 ± 1.0d	3.0 ± 0.4c	0.52 ± 0.02d	3.2 ± 0.0b	2.02 ± 0.02d

Different letters after means ± SE in each column indicate significant differences based on the LSD test ($p \leq 0.05$)

Table 2. Conventional (c-ZnO) or nano zinc (n-ZnO) impacts on relative content of water (RWC), index of membrane stability (MSI), and photosynthetic efficiency (SPAD, Fv/Fm, and PI) of eggplant transplants.

Treatments	Parameters				
	RWC (%)	MSI (%)	SPAD	Fv/Fm	PI (%)
Control (N)	70.4 ± 1.2c	53.4 ± 1.0c	34.4 ± 1.0c	0.80 ± 0.01c	2.95 ± 0.17c
c-ZnO	74.6 ± 1.5b	58.0 ± 1.2b	39.7 ± 1.2b	0.84 ± 0.01b	3.24 ± 0.25b
n-ZnO_{25}	74.2 ± 1.4b	57.9 ± 0.9b	39.8 ± 1.4b	0.84 ± 0.01b	3.26 ± 0.26b
n-ZnO_{50}	79.8 ± 1.6a	64.3 ± 1.2a	43.2 ± 1.4a	0.89 ± 0.02a	3.78 ± 0.30a
n-ZnO_{100}	48.9 ± 0.8d	36.5 ± 0.7d	20.4 ± 0.8d	0.71 ± 0.00d	1.74 ± 0.11d

Different letters after means ± SE in each column indicate significant differences based on the LSD test ($p \leq 0.05$)

Table 3. Conventional (c-ZnO) or nano zinc (n-ZnO) impacts on the contents of macro-nutrients of eggplant transplants.

Treatments	Parameters		
	N	P	K^+
	mg/g leaf dry mass		
Control (N)	20.3 ±1.9c	2.82 ± 0.40c	24.1 ± 0.6c
c-ZnO	22.2 ± 2.1b	3.46 ± 0.61b	26.6 ± 0.7b
n-ZnO_{25}	22.3 ± 1.9b	3.44 ± 0.62b	26.8 ± 0.6b
n-ZnO_{50}	25.5 ± 1.4a	4.45 ± 0.78a	30.9 ± 0.9a
n-ZnO_{100}	16.4 ± 0.8d	1.83 ± 0.34d	15.4 ± 0.4d

Different letters after means ± SE in each column indicate significant differences based on the LSD test ($p \leq 0.05$).

Table 4. Conventional (c-ZnO) or nano zinc (n-ZnO) impacts on the contents of micro-nutrients of eggplant transplants.

Treatments	Parameters			
	Zn	Fe	Mn	Cu
	mg/g leaf dry mass			
Control (N)	0.27±0.00d	1.01±0.03a	0.68±0.02a	0.21±0.08a
c-ZnO	0.33±0.01c	0.88±0.02b	0.54±0.01b	0.20±0.08a
n-ZnO_{25}	0.34±0.01c	0.88±0.02b	0.54±0.01b	0.20±0.06a
n-ZnO_{50}	0.42±0.01b	0.86±0.02b	0.54±0.01b	0.21±0.09a
n-ZnO_{100}	0.72±0.00a	0.55±0.02c	0.32±0.01c	0.10±0.04b

Different letters after means ± SE in each column indicate significant differences based on the LSD test ($p \leq 0.05$)

Table 5. Conventional (c-ZnO) or nano zinc (n-ZnO) impacts on the contents of non-enzymatic antioxidants and osmoprotectants of eggplant transplants.

Treatments	Parameters				
	Soluble protein	Soluble sugars	Free proline	AsA	GSH
	mg/g leaf dry mass			µmol/g leaf fresh weight	
Control (N)	77.2 ± 1.2c	10.1 ± 0.02c	0.11 ± 0.00c	0.82 ± 0.00c	0.46 ± 0.00c
c-ZnO	82.6 ± 1.4b	14.2 ± 0.11b	0.15 ± 0.00b	1.66 ± 0.02b	1.08 ± 0.01b
n-ZnO_{25}	82.8 ± 1.3b	13.9 ± 0.13b	0.15 ± 0.00b	1.64 ± 0.02b	1.09 ± 0.01b
n-ZnO_{50}	89.8 ± 1.8a	18.6 ± 0.20a	0.21 ± 0.00a	2.35 ± 0.03a	1.55 ± 0.02a
n-ZnO_{100}	42.4 ± 0.8d	7.3 ± 0.07d	0.07 ± 0.00d	0.63 ± 0.02d	0.32 ± 0.00d

Different letters after means ± SE in each column indicate significant differences based on the LSD test ($p \leq 0.05$)

Table 6. Conventional (c-ZnO) or nano zinc (n-ZnO) impacts on the activities of enzymatic antioxidants of eggplant transplants.

Treatments	Parameters			
	SOD	CAT	APX	GR
	µM/mg of protein			
Control (N)	0.284 ± 0.002c	0.182 ± 0.001c	0.148 ± 0.000c	0.198 ± 0.002c
c-ZnO	0.471 ± 0.005b	0.194 ± 0.002b	0.182 ± 0.002b	0.264 ± 0.004b
n-ZnO_{25}	0.474 ± 0.005b	0.193 ± 0.002b	0.184 ± 0.002b	0.269 ± 0.004b
n-ZnO_{50}	0.563 ± 0.007a	0.242 ± 0.003a	0.241 ± 0.002a	0.336 ± 0.005a
n-ZnO_{100}	0.182 ± 0.002d	0.126 ± 0.000d	0.106 ± 0.000d	0.139 ± 0.001d

Different letters after means ± SE in each column indicate significant differences based on the LSD test ($p \leq 0.05$)

Discussion

Nutrient-elements in the form of nano-particles (NPs) can contribute to nutrition of plants in two ways. The first one is to use nano-elements incorporated in a carrier complex, which may or may not be a nano-material such as nano-elements incorporated, by adsorption or absorption, in a matrix like clay, polyacrylic acid, chitosan, or zeolite (Golbashy *et al*., 2017). The second one is to utilize such element in a nano-form (i.e., in encapsulated or suspension) such as nano-Zn oxide (n-ZnO) for application to soil or by foliar spraying (Fedorenko *et al*., 2015). Both NPs contribution types have some specific advantages, including greater solubility with either less leaching or rapid absorption than conventional fertilizers. The first supplementation method is preferred due to its higher control providing over the timing and speed of release of the nutrient-element.

It has been observed that beneficial effects of nano zinc oxide particles (n-ZnO) in promoting plant growth, development, and yield were achieved at a lower n-ZnO concentration compared to a conventional Zn (c-ZnO). This may be attributed to the fact that n-ZnO is absorbed by plant roots (with soil addition) or leaves (with foliar spray) to a larger extent than c-ZnO (Prasad *et al*., 2012), therefore, n-ZnO should be used at lower levels than c-ZnO to avoid toxicity to plants. These results are in accordance with our data obtained, where the n-ZnO level of 25 mg L^{-1} application conferred results similar to those obtained with the application of c-ZnO level at 200 mg L^{-1}. The best n-ZnO level was 50 mg L^{-1} that showed the highest growth characteristics compared to all other treatments (Table 1). In contrast, the highest foliar sprayed level of n-ZnO (100 mg L^{-1}) shows toxic effects in eggplant transplants. This n-ZnO level strongly decreased transplant growth characteristics compared to all other treatments (Table 1). Reports generated from work on some plants such as soybean and *Lolium perenne* L. showed decreased height of plants treated with 500 mg kg^{-1} and 200 mg kg^{-1} of n-ZnO, respectively. Increased uptake of nutrients could explain the increased length of roots with n-ZnO treatment (Raliya *et al*., 2015). The increase in growth characteristics of eggplant seedlings with n-ZnO at a level of 50 mg L^{-1} might be attributed to that this level was suitable required Zn concentration in nano particles for strong seedling growth. In addition, the substantial roles of Zn for many functions in plants, including cell membrane function, cell elongation, maintained protection for stabilizing the cell membranes structure, protein synthesis, and increasing the tolerance to environmental stresses (Welch *et al*., 1982; Cakmak, 2000) explain the increased growth characteristics of eggplant seedling compared to the control (Table 1).

Data of this study show that n-ZnO foliar application significantly increased SPAD chlorophyll and photosynthetic efficiency (Fv/Fm and PI) compared to c-ZnO foliar application, which in turn these attributes were increased, significantly, compared to the control (Table 2). Similarly, in safflower, Zn foliar spraying increased content of chlorophyll, showing the pivotal role of Zn in N metabolism and chlorophyll production (Movahhedi Dehnavi, 2004). Synthesis of chlorophyll occurs in the presence of Zn by its protection of –SH groups (Cakmak, 2000). In the presence of Zn, completion of chlorophyll formation is finally facilitated in indirectly manner, where it may affect N and Mg concentrations involved in the chlorophyll formation. In addition, activity of many enzymes implicated in chlorophyll biosynthesis needs Zn (Lebedev & Timco, 1998). It also prevents the destruction of chlorophyll under stress (Behtash *et al*., 2010) through the antagonistic effect. All of these positively affected the photosynthetic efficiency (Table 2). Like other heavy metals, Zn is considered as a heavy metal that in a large quantity (100 mg L^{-1} as n-ZnO; Table 2), is toxic to eggplant seedlings, and degradation of chlorophyll takes place. Application of n-ZnO at levels from 200 to 300 mg L^{-1} in the same *Arabidopsis* species reduced chlorophyll content and the rate of photosynthesis, resulting in less biomass (Wang *et al*., 2016).

Toxicity created along with preventing the essential elements (needed for chlorophyll biosynthesis; Fe, Mn, and Cu; Table 4) absorption, stimulated the activity of chlorophyll-degrading chlorophyllase enzyme against the maintained content of chlorophyll. All of these are negatively affected the photosynthetic efficiency (Table 2). Generally, nanoparticles of metals are strongly amplified the photosynthetic efficiency (Nadtochenko *et al*., 2008).

Data of the current study showed that n-ZnO foliar application significantly increased N, P, K, and Zn contents in eggplant seedlings compared to c-ZnO, which in turn significantly exceeded the control (Tables 3 and 4). For micro-nutrients, the toxic level of n-ZnO (100 mg L^{-1}) application significantly reduced Fe, Mn, and Cu contents, while the optimum level of n-ZnO (100 mg L^{-1}) sustained these micro-nutrients at the appropriate content in eggplant transplant tissues. In addition, the increased content of Zn in eggplant transplants could be explained on basis of n-ZnO which was absorbed and translocated in transplants at corresponding n-ZnO treatment.

Zn has favorable effects on the bio-availability of nutrients and enhances root cation-exchange capacity that enhances absorption of essential nutrients, particularly N that is responsible for protein synthesis (Mohsenzadeh & Moosavian, 2017). The reduction in Fe, Mn, and Cu contents in eggplant seedlings due to n-ZnO may be explained on the bases of Zn antagonistic effects. In addition, Zn decreased the uptake of toxic elements that might be due to its antagonistic effect as an indicator to the high competition between Zn and toxic elements for the same cell membrane-carriers (Aravind & Prasad, 2003).

Zinc plays pivotal role in carbohydrates and proteins metabolism and starch formation. It also controls plant growth hormone, i.e. IAA through its control on the activity of enzymes implicated in tryptophan biosynthesis. Zn is also an essential component of dehydrogenase, proteinase, and peptides enzymes. These facts indicate that the bio-availability of Zn to seedlings has very crucial physiological roles in seedling growth (Mohsenzadeh & Moosavian, 2017).

Data of this study showed increased content of total soluble sugars in seedlings because of the treatments of c-ZnO and n-ZnO with significant preference of n-ZnO (Table 5). This increase in the content of total soluble sugars in eggplant seedlings could be attributed to the increase in the movement of soluble sugars to the root system to maintain its pivotal functions, especially cell osmotic adjustment to absorb more water and nutrients (Mohsenzadeh & Moosavian, 2017). This was positively reflected in increasing the relative water content (RWC) by Zn application (Table 2). In addition, membrane stability index (MSI; Table 2) was also increased significantly by Zn application, especially n-ZnO due to the maintained protection of structural stability of tissue cell membranes occurred by Zn (Cakmak, 2000).

Free proline is most common stable amino acid in plants. It naturally accumulates in great amounts in response to the biotic and abiotic stresses (Mohsenzadeh & Moosavian, 2017). It is significantly increased by c-ZnO or n-ZnO foliar application with significant preference of n-ZnO (Table 5). Adding to its role as osmolyte/osmoprotectant, free proline plays a vital role in stabilizing micro-cellular structures including membranes, therefore, increasing the MSI and RWC (Table 2). It also plays a pivotal role in the stabilization of proteins and in destruction of free radicals (ROS) under stress situations. Free proline functions as a chemical chaperone, stabilizes the natural form of proteins and inhibits the disruption of the enzymes folding (Solomon & Beer, 1994). A correlation has been reported between free proline accumulation and plant tolerance to the environmental stresses (Ashraf & Foolad, 2007). Under stress event, the precursor of chlorophyll and proline syntheses is glutamate that goes into proline production. Four possible reasons have been suggested to explain the increased content of proline under stress. These proposed reasons are stimulating proline synthesis from glutamate, reducing its exports through phloem, preventing the oxidation under stress, and destructing and disordering the protein synthesis (Llamas *et al*., 2000).

It has also been observed that n-ZnO has tension effect on the activation of plant defense systems (Mohsenzadeh & Moosavian, 2017). To fight the excess generation of ROS, plants increase the activities of non-enzymatic and enzymatic antioxidants (Rady *et al*., 2019). Results obtained in this research revealed that the main reason for the high antioxidant activity in eggplant seedlings was the increased contents of the antioxidant compounds such as ascorbic acid (AsA), free proline, and glutathione (GSH), besides, the elevated activities of glutathione reductase (GR), ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) (Tables 5 and 6).

Zn may has a function in controlling the ROS and their related processes via its antioxidantive characteristic (Zago & Oteiza, 2001). It preferably binds to the –SH groups of the membrane moiety proteins, and conserves proteins and phospholipids from disulfide formation and thiol oxidation (Chvapil, 1973), via binding directly to a site near to the –SH group, or by ROS controlling to prevent the destruction of enzymes stabilization, proteins and lipid membranes (Sharma *et al*., 1994). The equilibrium of the ROS stable-state levels are controlled by the reaction between ROS production and ROS scavenging mechanisms (Polle, 2001). The enzymatic (SOD, CAT, APX and GR) and non-enzymatic (free proline, AsA, and GSH) antioxidants overcome ROS (Alzahrani *et al*., 2018; Rady *et al*., 2019; Semida *et al*., 2018). In the current study, the activities of enzymatic antioxidants SOD, GR, APX and CAT and non-enzymatic antioxidant as free proline, AsA, and GSH in eggplant seedling were increased with foliar spray of n-ZnO, especially at a level of 50 mg L^{-1} (Tables 5 and 6). This increased antioxidant activities might be due to the great synthesis of SOD. Zn is capable to participate in the Cu/Zn SOD structure isozyme, thus its treatment increased SOD activity (Asadi *et al*., 2012). The increased activities of SOD, APX, GR and CAT occurred in eggplant seedlings treated with n-ZnO are considered as an indicator to antioxidant enzymes efficiency in the presence of n-ZnO. Possibly, Zn is required indirectly for raising the enzymes activities, contributing to the detoxification of ROS (Cakmak, 2000). Zn has been observed in many systems, antagonizing the catalytic properties of the Fe and Cu redox-active transition regarding their ability to support the conversion of H_2O_2 and $O_2^{\bullet-}$ to OH^- (Powell, 2000).

There exist evidence that there is a positive relationship between the antioxidant compounds activities and a plant antioxidant power (Muret *et al*., 2007; Alzahrani *et al*., 2018), and this indicate from data of the current study that eggplant seedlings have a high antioxidant power. Antioxidant activities determined in this study are effective as H-donors and act as efficient antioxidants (Alzahrani *et al*., 2018; Rady *et al*., 2018; Semida *et al*., 2018). With increasing contents of AsA and GSH due to n-ZnO application, the possibility of hydrogen donation to ROS to increase the inhibition power of n-ZnO is existed. It can be explained that the antioxidant capacity in plant tissues has a closed relationship with the protective compounds activity such as free proline, AsA and GSH. The reduced activities of antioxidant enzymes assayed in this study such as SOD, CAT, APX, and GR are believed to remove the oxidative stress and, consequently, to increase seedling growth under a stress.

In other words n-ZnO stimulated eggplant seedling antioxidant defense systems to increase ROS scavenging capacity. Therefore, n-ZnO foliar supplementation in an appropriate concentration (50 mg L^{-1}) significantly improved the growth performance of eggplant transplants and generated strong transplants to cope with any fluctuations in the open fields. As we used Zn as foliar application, it was noted that water expeller potential of leaf surface acted as one of the limiting factors that could affect the uptake of Zn through spray application processes (Holder, 2007). Higher metal ion solubility in water might has some limitations for entering through the lipophilic cuticle, but lipophilic organic molecule permeability through cuticle increases with its mobility and solubility in the cuticles transport-limiting barricade. Consequently, n-ZnO has less hydrophilicity and more capacity of dispersion in lypophilic substances, therefore, it can penetrate rapidly through leaf surface and release ions across the cuticle compared to the water soluble ions (Da Silva *et al*., 2006). Prasad *et al.*, (2012) have noticed higher bioavailability of the n-ZnO in plants due to its nano size particles and lower solubility of water. These n-ZnO properties are responsible for giving higher growth performance. The Above facts regarding n-ZnO and possibility of its penetration in leaf cuticle can explain the positive role of n-ZnO on strong eggplant transplant growth.

Conclusions

The use of n-ZnO, especially at a level of 50 mg L^{-1} significantly increased transplant growth, photosynthetic efficiency, macro-nutrients, and enzymatic and non-enzymatic antioxidant activities. These results led to produce strong eggplant transplants, having strong antioxidant defense systems, for transplanting in the open fields to cope effectively any fluctuations in the environmental conditions. More studies are needed to exactly determine the beneficial effects and the appropriate, not toxic, level of n-ZnO used for strong growth of plants under normal or stress conditions.

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