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PHYSIOLOGICAL ROLE OF EXOGENOUS NITRIC OXIDE IN IMPROVING PERFORMANCE, YIELD AND SOME BIOCHEMICAL ASPECTS OF SUNFLOWER PLANT UNDER ZINC STRESS

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The present study was undertaken to examine the possible roles of sodium nitroprusside in protection against oxidative damage due to zinc toxicity in sunflower plants. Physiochemical parameters in sunflower plants exposed to Zn^{2+} (100, 200 and 300 mg/kg soil) alone or combined with SNP were measured. The results showed that excess of Zn decreased plant growth, seed yield components and photosynthetic pigments content. On the other hand, Zn stress increased the level of non-enzymatic antioxidants (ascorbic acid and reduced glutathione) and enzymatic antioxidants (superoxide dismutase, ascrobate peroxidase and glutathione reductase), coupled with the appearance of novel protein bands. Furthermore, Zn stress increased Zn content in roots and shoots. The amounts of Zn in roots were higher than shoots. A marked increase in total saturated fatty acids accompanied by a decrease in total unsaturated fatty acids was observed. Exogenously application of SNP (20 μM) increased growth parameters, photosynthetic pigments content, ascorbic acid and glutathione contents, antioxidant enzyme activities and the quality of the oil in favour of the increase of unsaturated fatty acids. Moreover, SNP application increased Zn concentration in roots and inhibited Zn accumulation in shoots. Therefore, it is concluded that SNP treatment can help reduce Zn toxicity in sunflower plants.

Keywords: Helianthus annuus – enzymatic antioxidants – fatty acids – non-enzymatic antioxidants – protein electrophoresis

INTRODUCTION

Zinc (Zn) is an essential micronutrient element which plays an important role in a number of physiological processes [20]. Zn is a component of a number of dehydrogenases, proteinases and peptidases; thus Zn has an influence on electron transfer reactions including those of the Krebs cycle and can be considered as a component of many proteins such as Zn finger-containing transcription factors, Cu/Zn superoxide dismutase, carbonic anhydrase and Zn-metal loproteases [7].

Zn is toxic to plants at higher concentrations and can retard plant growth and disrupt various essential physiological processes [8, 29]. The oxidative stress causes the generation of harmful reactive oxygen species (ROS) that have the capacity to damage biological molecules and membranes by lipid peroxidation and degrade

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proteins, lipids and nucleic acids [16]. Excess Zn may bind to proteins and lead to the displacement of other ions, such as Fe^{2+}, from protein–binding sites. Recent studies have shown that Zn toxicity affects the activity of antioxidative enzymes, such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in plants [17].

Attempts have been made by many researchers to alleviate Zn toxicity effects in plants through the application of mineral nutrients such as nitric oxide [37]. Nitric oxide (NO) is involved in the regulation of multiple responses to a variety of abiotic and biotic stresses [9, 35] and acts as a diffusible free radical to readily react with a variety of intracellular and extracellular targets [25]. NO can alleviate the oxidative stress by directly scavenging ROS, such as $O_2^{\cdot-}$ to form peroxynitrite ($ONOO^-$) which is less toxic than peroxides as well as by enhancing antioxidant enzymes [22, 37].

Sunflower (*Helianthus annuus* L.) is an important oilseed crop which ranks the third after soybean and peanut along with other oil seed crops like (canola and cotton) which contributes considerably to consumable oil in the world [31]. Sunflower occupies an important place in oil seed crops because of short duration as well as its ability to adapt wide range of climate and soil conditions [32]. This crop has an ideal place in the present cropping system but due to some constraints the average yield is much lower than world's average.

Therefore, the aim of this study was to examine the effects of toxic levels of Zn on growth, yield components, photosynthetic pigments, enzymatic and non-enzymatic antioxidants, protein patterns and fatty acid composition of harvested seeds and to explore the possible role of exogenous application of NO in the alleviation of Zn toxicity in sunflower plants.

MATERIALS AND METHODS

Experimental procedures

The seeds of sunflower (*Helianthus annuus* L. cv. Sakha53) were obtained from the Agricultural Research Center (ARC), Cairo, Egypt. Before potting, the experimental soil was mixed homogeneously with Zn at a rate of 100, 200 and 300 mg/kg soil as $ZnSO_4 \cdot 7H_2O$. The pots were divided into 4 groups. Each group contains five pots as replication for each treatment. The pot (25 cm diameter) was filled with 4 kg of sand and loam soil. Ten seeds were sown in each pot. The first group (5 pots) represented the plants grown in Zn untreated soil and irrigated with tap water to serve as control. The second group (15 pots) was subdivided into three sets (5 pots for each set) representing the plants grown in soil mixed with test levels of Zn at concentrations of 100, 200 and 300 mg/kg (Zn1, Zn2 and Zn3), respectively. The third group (5 pots) represented the plants grown in Zn-untreated soil and sprayed with sodium nitroprusside (SNP) solution, used as an exogenous NO donor, at a rate of 20 μM with 20 ml/plant at the flowering stage in the 9th week. The fourth group (15 pots) was subdivided into three sets including plants grown in soil mixed with different concentra-

tions of Zn and sprayed with the same concentration of SNP at the flowering stage. Plants were kept under the natural conditions (day length 12–14 h, at 20–22 °C and 70% humidity) and were watered daily.

Plant sampling

When the developed plants were 70 days old, 5 plants were carefully up-rooted from the soil of each treatment where samples of roots, shoots or leaves were detached and analyzed for the biochemical analyses. Moreover, after 120 days (final harvest) 5 heads were selected for the determination of different yield components such as plant height, head diameter, number of grains per head, 1000-grain weight and grain yield.

Biochemical analyses

Determination of photosynthetic pigments

Chlorophyll a, chlorophyll b and carotenoids were determined in young leaves tissues of sunflower plants. The spectrophotometric method recommended by Vernon and Seely [33] was used. The pigment contents were calculated as mg g^{-1} fresh weight of leaves.

Determination of non-enzymatic antioxidant

Ascorbic acid content was determined as described by Mukherjee and Choudhuri [25]. The absorbance was recorded at 525 nm by spectrophotometer.

Glutathione content The GSH concentrations were estimated fluorimetrically following a previously described method by Hissin and Hilf [15] with some modifications. Fluorescence intensity was recorded at 420 nm after excitation at 350 nm using a fluorescence spectrophotometer.

Determination of enzymatic antioxidant

The leaves were grinded in sodium phosphate buffer at pH 6.5 for SOD, APX and GR. The supernatant was used to measure the activity of the following enzymes.

Superoxide dismutase (SOD: EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium chloride (NBT). Absorbance was read at 560 nm according to Beauchamp and Fridovich [6].

Ascorbate peroxidase (APX: EC 1.11.1.11) activity was estimated according to the method of Nakano and Asada [27]. Enzyme activity was determined by the decrease of absorbance of ascorbate at 290 nm.

Glutathione reductase (GR: EC 1.6.4.2) activity was determined based on the decrease of absorbance at 340 nm due to the oxidation of NADPH to NADP, according to the method of Foyer and Halliwell [10].

Determination of Zn concentration

Harvested plant materials were thoroughly washed in distilled water and separated into leaves, stem and roots (after Na-EDTA treatment) and were oven dried at 65 °C and digested in a 10:1 mixture of nitric acid (HNO_3): perchloric acid ($HClO_4$) at 160 °C. Digested material was diluted with deionized water and Zn concentration was determined using an Inductively Coupled Plasma Mass Spectrometer (ICP/MS, Agilent 7500a, Wilmington, DE, USA) [2].

Determination of fatty acids

The oil content of the seeds was determined according to the A.O.A.C. [1]. The quality of the oil depends on the proportion of different fatty acids; their composition was determined quantitatively by Gas Liquid Chromatography according to the method described by Harborne [14].

Protein electrophoresis

SDS-PAGE was carried out with gel slabs according to the method of Laemmli [20]. Protein subunit bands were stained with Coomassie blue R-250 using standard techniques. The gel was scanned using Gel pro-Analyzer. The molecular weights were calculated by Gel-Pro analyzer program according to the molecular weight of marker.

Statistical analysis

All data were subjected to statistical analysis following the procedure described by Gomez and Gomez [12]. Duncan's multiple range test was applied to assess the significance of the treatment, at the 5% level of probability ($P \leq 0.05$).

RESULTS AND DISCUSSION

Changes in growth parameters

Changes in growth parameters of sunflower plants are presented in Table 1. The obtained data revealed that the lowest concentration of Zn (Zn1) significantly increased the growth of sunflower plants, while the higher concentrations of Zn (Zn2, Zn3) had adverse effects on growth and yield parameters (plant height, head diameter,

Table 1
Effect of different concentrations of Zn and/or SNP application on the growth, yield criteria and total photosynthetic pigments of sunflower plants

Treatments	Plant height (cm)	Head diameter (cm)	No. of seeds/ head	1000-seed wt. (g)	Seed yield/ plant	Total pigments mg/g F.wt
Control	105.8^{d}	8.4^{f}	420.7^{e}	22.4^{d}	19.4^{e}	53.8^{d}
Zn1	109.7^{c}	9.0^{e}	440.8^{d}	23.7^{c}	20.8^{d}	54.8^{d}
Zn2	95.7^{e}	8.1^{f}	390.4^{f}	19.3^{e}	17.6^{f}	51.6^{e}
Zn3	87.3^{f}	7.6^{g}	340.9^{g}	17.2^{f}	15.2^{g}	43.0^{e}
SNP	118.3^{a}	11.6^{b}	530.3^{b}	29.5^{a}	26.7^{b}	71.7^{b}
SNP+Zn1	119.5^{a}	12.1^{a}	576.5^{a}	30.6^{a}	28.5^{a}	79.6^{a}
SNP+Zn2	115.4^{b}	10.8^{c}	522.3^{b}	27.8^{b}	22.3^{c}	68.8^{c}
SNP+Zn3	111.6^{c}	9.7^{d}	480.7^{c}	24.6^{c}	21.5^{c}	67.3^{c}

†Mean values (n = 5) in each column followed by the same lower-case letters are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

number of seeds/head, weight of 1000 seeds and seed yield/plant) as compared to the control. The highest concentration of Zn (Zn3) was proven to be most inhibitory to plant growth. These results are in agreement with previous reports showing that excess Zn has inhibitory effects on growth in various plant species and disrupt various essential physiological processes [21, 29, 34]. Inhibition of growth in sunflower plants might be the result of Zn which caused alteration of fundamental metabolic processes such as the change in the rate of net photosynthesis that reduces the supply of carbohydrates or proteins and consequently decreases the growth of the plant.

Foliar spraying of plants with SNP significantly enhanced all of the above growth parameters in Zn treated and untreated plants as compared to the control. The alleviated effect of SNP was much more pronounced under low Zn supply (Zn1) which was reflected by the increase in head diameter, number of seeds/head and weight of 1000 seeds. These results are consistent with those of Mohamed et al. [23] who found that the addition of SNP had best effect on promoting growth of *Vicia faba* plants under arsenic stress.

Changes in photosynthetic pigments

Photosynthesis is one of the most important metabolic processes for energy transformation in plants. The obtained results in this study demonstrated that the exposure of sunflower plants to low concentration of zinc (Zn1) induced non-significant effect in total photosynthetic pigments content (Table 1). On the other hand, high concentrations of Zn (Zn2, Zn3) significantly reduced the amount of total photosynthetic pigments content as compared to the control. The decline in chlorophyll content under Zn stress is believed to be due to the inhibition of enzymes associated with chloro-

phyll biosynthesis such as δ-aminolaevulinic acid dehydratase and protochlorophyllide reductase [26] and the inhibition of uptake and transportation of other metal elements such as Mn, Zn and Fe by antagonistic effects [37].

Compared with Zn treatments, addition of SNP increased chlorophyll contents especially at Zn1. The increase in total photosynthetic pigments content of sunflower leaves may be attributed to NO effect on protection of chloroplast enzymes and thus increase the biosynthesis of photosynthetic pigments [8]. Also, Mohamed et al. [23] reported that NO mediated rebalance of ion content which involved in the alleviation of photosynthetic inhibition in As stressed *Vicia faba*. Thus, it can be inferred that adding appropriate SNP could really alleviate Zn toxicity though improving the plant photosynthesis.

Changes in antioxidant enzymes

Data presented in Table 2 show that Zn application significantly increased the activities of antioxidant enzymes including SOD, APX and GR in the shoots of sunflower plants as compared to the control. Zn stress can induce ROS accumulation in plants and increase cell death. However, the obtained results demonstrate that exogenous NO application stimulated the activities of antioxidant enzymes as compared to Zn treated and non-treated plants, and suggest that NO application enhances the antioxidant response in sunflower plants to resist oxidative stress [24]. Many studies reported that NO protects the plant from oxidation damage by regulating general mechanisms for cellular redox homeostasis and promoting the transformation of $O_2^{\bullet -}$ to H_2O and O_2. APX and GR play an important role in combating oxidative stress [5]. APX and GR activity was increased by NO in sunflower plants under Zn stress, thereby

Table 2
Effect of different concentrations of Zn and/or SNP application on enzymatic and non-enzymatic antioxidants in sunflower plants

Treatments	Superoxide dismutase (SOD) (unit/min/g FW)	Ascorbate peroxidase (APX) (unit/min/g FW)	Glutathione reductase (GR) (unit/min/g FW)	Ascorbic acid µg/g FW	Reduced glutathione (GSH) µg/g FW
Control	0.39^{f}	1.46^{f}	3.37^{f}	5.43^{f}	0.51^{f}
Zn1	0.43^{d}	1.89^{d}	4.65^{d}	9.56^{b}	0.75^{b}
Zn2	0.48^{b}	1.67^{e}	4.30^{d}	7.90^{d}	0.67^{c}
Zn3	0.41^{e}	1.58^{e}	4.00^{e}	6.38^{e}	0.61^{d}
SNP	0.47^{c}	1.94^{d}	5.31^{c}	6.06^{e}	0.55^{e}
SNP+Zn1	0.40^{e}	3.00^{a}	7.80^{a}	11.95^{a}	0.88^{a}
SNP+Zn2	0.53^{a}	2.44^{b}	7.53^{a}	8.97^{c}	0.74^{b}
SNP+Zn3	0.49^{b}	2.24^{c}	6.81^{b}	7.70^{d}	0.67^{c}

†Mean values (n = 5) in each column followed by the same lower-case letters are not significantly different at $P \leq 0.05$ by Duncan's multiple range test.

strengthening the capabilities of the defense system to scavenge free radicals. The increased antioxidant enzyme activity might be ascribed to the role of NO in stimulating the expression of their genes and improve biosynthesis of these enzymes [22, 24].

Changes in ascorbic acid (AsA) and glutathione (GSH) contents

Data presented in Table 2 indicat that AsA and GSH contents in the leaves of sunflower plants were significantly affected by various concentrations of Zn treatments. Increasing levels of Zn from 100 to 300 mg/kg markedly increased AsA and GSH contents in contrast to control. These results are in accordance with those of Jin et al. [18] who reported that Zn supplement significantly elevated GSH contents in leaves of *Sedum alfredii*. The endogenous non-enzymatic antioxidants such as AsA and GSH are effective free radical scavengers. GSH is a substrate for several reductive enzymes including enzymes that reduce peroxides and act as a key redox buffer, forming a barrier between protein Cys groups and ROS [11]. AsA and GSH contents increased sharply when NO was applied in combination with Zn treatments as compared to those treated with Zn alone. Plants possess several antioxidant defense systems consisting of enzymatic and non-enzymatic components that normally keep ROS in balance within the cell. For instance, AsA is the major redox regulatory antioxidant and is able to detoxify ROS by direct scavenging, acting as a substrate in the ascorbate glutathione cycle [5].

Changes in Zn concentration

As shown in Figure 1 Zn concentration in roots and shoots of sunflower increased significantly after treatment with Zn as compared to the control and the most Zn was

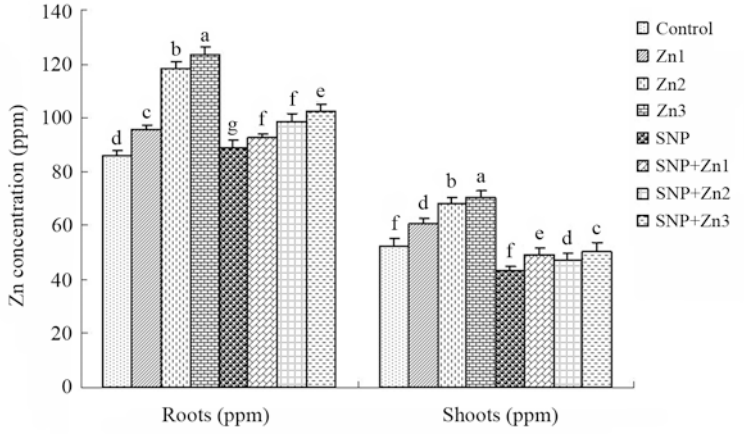

Fig. 1. Effect of different concentrations of of Zn and/or SNP application on Zn contents in roots and shoots of sunflower plants. Error bars represent the standard error (SE; $n = 3$)

Table 3
Effect of different concentrations of Zn and/or SNP application on fatty acid composition of the yielded sunflower oil

Fatty acids	Control	Zn1	Zn2	Zn3	SNP	SNP+Zn1	SNP+Zn2	SNP+Zn3
Palmitic (C16:0)	5.78	6.32	8.72	9.84	4.16	3.40	4.85	5.37
Stearic (18:0)	12.26	13.89	18.64	21.89	9.32	8.51	9.64	10.05
Oleic (C18:1)	42.45	42.29	39.43	37.40	49.42	57.44	47.33	46.05
Linoleic (C18:2)	21.00	21.33	18.52	17.64	29.18	31.98	26.85	23.14
Linolenic (C18:3)	0.78	0.90	0.62	0.54	1.07	1.60	1.00	0.98
Arachidic (C20:0)	0.66	0.69	0.72	0.79	0.33	0.25	0.42	0.56
Behenic (C22:0)	1.05	1.07	1.20	1.63	0.50	0.42	0.64	0.78
Ts	19.75	21.97	29.28	34.15	14.31	12.58	15.55	16.76
Tu	64.23	64.52	58.57	55.58	79.67	91.02	75.18	70.17
Total	83.98	86.49	87.85	89.73	93.95	103.60	90.73	86.93
Tu/Ts	3.25	2.94	2.00	1.63	5.57	7.24	4.84	4.19

Ts – Total saturated; Tu – Total unsaturated.

located in roots. However, the addition of SNP either separately or accompanied by Zn significantly inhibited the uptake of Zn compared with Zn stressed plants particularly at Zn1. Foliar spraying of NO to non-stressed plants inhibited the uptake of Zn in shoots of *H. annuus* plants. The uptake of Zn is associated with metallothionein proteins which are the critical targets of NO [3]. In this study, a majority of Zn accumulated in roots as compared to shoots. Addition of NO increased Zn accumulation in roots as compared to shoots, indicating that NO inhibited excess Zn transferred to shoots. Xiong et al. [35] pointed out that low accumulation of heavy metal in the aboveground parts of plants is the principal defense to counteract its toxicity.

Changes in fatty acids composition

The obtained data in Table 3 revealed that oils extracted from sunflower harvested seeds characterized by the presence of seven fatty acids, including four saturated fatty acids (palmitic, stearic, arachidic and behenic) and three unsaturated fatty acids (oleic, linoleic and linolenic). Zinc stress caused marked increase in total saturated fatty acids (Ts) accompanied by decrease in total unsaturated fatty acids (Tu) as compared with control plants. Thus, Tu/Ts also decreased. Palmitic (C16:0) and stearic (C18:0) acids were predominant saturated fatty acids while oleic acid (C18:1) was the major unsaturated fatty acid followed by linoleic acid (C18:2). Younis et al. [38] reported that oxidative stress resulted in increasing the saturated fatty acids and decreasing the unsaturated fatty acids in soybean. The effect of SNP was found to be contrary to that of Zn as marked increases were observed in unsaturated fatty acids. The highest increase in unsaturated fatty acids was detected under low Zn level (Zn1) with SNP treatment as well as at moderate Zn level (Zn2) with SNP. These results are in harmony with Kheir et al. [19] who found that the higher N-rate increased the percentage of unsaturated fatty acids and decreased saturated fatty acids of flax oil. Also, Sawan et al. [30] indicated that the higher N-rate, as well as the application of Zn resulted in an increase in total unsaturated fatty acids of cotton seed. The beneficial effect of applied SNP on Tu and Tu/Ts ratio can be due to the regulated effect of SNP which acts as an activator on enzymatic processes [13].

SDS-PAGE protein banding pattern

Three types of changes are observed in the protein patterns of sunflower leaves. Some protein bands disappeared, other proteins were selectively increased, and synthesis of a new set of proteins was induced. Some of these responses were observed under Zn stress, while others were induced by Zn concentrations and/or No (Table 4, Fig. 2). The molecular weights of the proteins ranged between 15 and 200 kDa. The total number of bands in leaves of control plants exhibited the existence of 13 protein bands having molecular weights ranging between 18 and 180 kDa. Furthermore, protein band with molecular weight of 89 kDa was present in all treatments which is

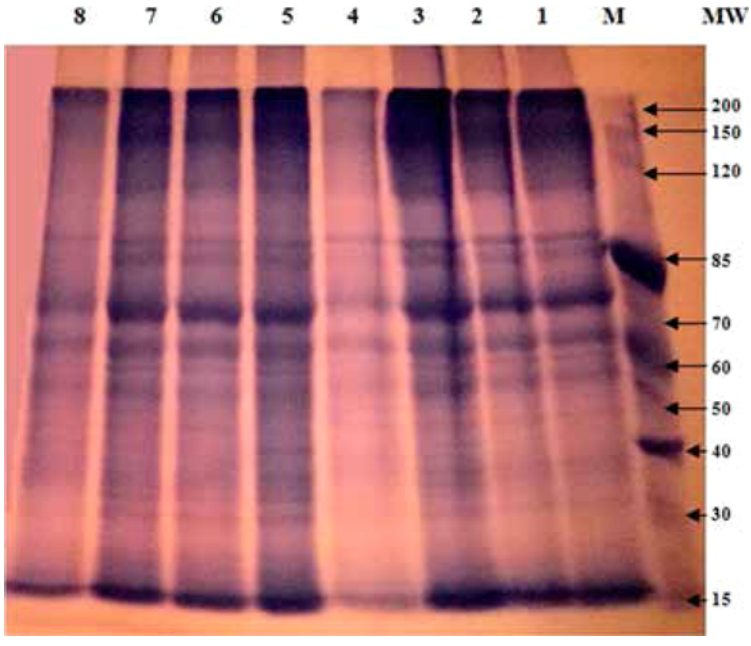

Fig. 2. Electrophoretic banding profiles of protein separated by SDS-PAGE of the leaves of sunflower plants as affected by different concentrations of zinc (Zn) and/or SNP application. MW: KDa. Lane (1): control. Lane (2): Zn1. Lane (3): Zn2. Lane (4): Zn3. Lane (5): SNP. Lane (6): SNP+Zn1. Lane (7): SNP+Zn2. Lane (8): SNP+Zn3

considered as common band. The total number of bands decreased with increasing Zn concentrations as compared to control plants. The lowest number of protein bands (11 bands) was recorded at Zn3 due to the disappearance of three bands having the molecular weights of 18, 106 and 180 kDa. These results are in accordance with those of Inbaraj and Krishnaswamy [17] who found a reduction in the total soluble protein of cowpea plants under Zn deficiency and Zn excess. The decrease in soluble protein content and the alteration in protein banding patterns and intensity of sunflower leaves may be attributed to the metal induced inhibition of amino acids involved in protein synthesis. Changes in protein synthesis under Zn stress can be due to changes in the efficiency of mRNA translation or the regulation of RNA transcription transport and stability. Also, Zn stress leads to difference in gene expressions where protein alterations could be the result of changes in the regulation of transcription, mRNA processing, or due to altered rates of protein degradation. The total amounts of protein increased in plants treated with Zn2 and plants treated with all concentrations of Zn in combination with NO as compared with control plants.

However, application of NO either alone or accompanied by different concentrations of Zn caused an increase in the total number of protein bands as a result of induction of 3 *de novo* protein bands having the molecular weights of 15, 58 and 94

kDa, respectively, which were absent in both Zn treated plants and the control. Plants treated with NO might be explained basing on the potentiality of NO to trigger the expression of specific genes along DNA molecule in the target cells, a process which appears to play a key role in regulating a cascade of biochemical reactions which might determine the ultimate appearance of growth patterns and yield. This suggestion is supported by the findings of Grün et al. [13].

Table 4
Effect of different concentrations of Zn and/or SNP application on the protein patterns separated by SDS-PAGE of the leaves of sunflower plants

Molecular weight (kDa)	Lane 1 Control	Lane 2 Zn1	Lane 3 Zn2	Lane 4 Zn3	Lane 5 SNP	Lane 6 SNP+Zn1	Lane 7 SNP+Zn2	Lane 8 SNP+Zn3
200	–	–	–	4.52	–	5.98	5.23	–
180	7.26	4.28	9.69	–	5.07	–	–	5.39
144	–	–	–	8.96	8.3	7.50	4.55	6.98
106	3.53	5.56	3.92	–	4.14	2.30	4.12	3.44
98	2.91	–	–	–	–	–	–	–
94	–	–	–	–	2.93	3.20	2.17	3.34
89	3.76	3.89	3.07	3.41	2.36	1.79	3.42	2.43
85	3.30	4.18	3.98	4.27	3.88	2.78	–	3.38
78	5.41	–	6.27	4.06	–	–	7.30	–
75	–	5.41	–	–	7.85	6.79	–	5.74
71	3.52	3.44	4.45	3.59	–	–	4.23	–
64	–	3.29	4.80	–	3.27	2.57	1.94	3.17
58	–	–	–	–	2.16	3.04	4.18	1.98
52	5.42	–	4.59	5.08	3.73	4.81	–	4.09
47	–	4.20	–	3.51	–	–	2.62	–
44	2.66	–	3.24	–	2.70	–	–	2.54
38	4.41	3.42	3.75	4.86	–	3.49	5.01	–
35	1.73	4.03	–	–	3.10	2.03	–	3.70
32	–	–	2.46	2.15	1.39	3.24	–	1.67
30	3.38	3.60	–	3.35	1.97	2.93	2.82	2.65
18	5.49	5.78	6.03	–	–	–	–	–
15	–	–	–	–	5.37	5.42	6.73	6.24
Total No. of bands	13	12	12	11	15	15	13	15
The amount of total protein	52.8	51.1	56.3	47.8	58.2	57.9	54.3	56.7
No. of new bands		3	2	4	7	8	7	7

CONCLUSIONS

It was shown that Zn becomes toxic at higher concentrations above 200 ppm. Zn toxicity is mostly determined by environmental pollution following industrial and agricultural activities, such as applications of Zn-containing fertilizers and pesticides. The results of this study demonstrated that Zn stress caused inhibition of plant growth and photosynthetic pigments but caused stimulation in enzymatic and non-enzymatic antioxidants. Also, Zn stress affect fatty acid composition and caused alteration in the protein pattern of leaves of sunflower plants. In addition, Zn stress caused accumulation in Zn content in roots and shoots. Foliar application with NO (20 μM) caused enhancement in all the above criteria. In summary, exogenous application of NO protects sunflower plants against the adverse effect of Zn and accumulated the excess Zn in roots and inhibits its transport to shoots.

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