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Impact of Zinc Excess on Germination, Growth Parameters and Oxidative Stress of Sweet Basil (*Ocimum basilicum* L.)

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Abstract

In the present study, the effects of elevated zinc concentrations on germination, physiological and biochemical parameters were investigated in basil (*Ocimum basilicum* L.). Results indicate that zinc excess (1–5 mM $ZnSO_4$) did not affect germination process, but it drastically reduced vigor index and radicle elongation, and induced oxidative stress. Exposure of basil plants to 400 and 800 µM Zn decreased aerial parts and roots dry biomass, root length and leaf number. Under these conditions, the reduction of plant growth was associated with the formation of branched and abnormally shaped brown roots. Translocation factor < 1 and bioconcentration factor > 1 was observed for 100 µM Zn suggested the possible use of basil as a phytostabiliser. Excess of Zn supply (> 100 µM) decreased chlorophyll content, total phenol and total flavonoid contents. Additionally, an increased TBARS levels reflecting an oxidative burst was observed in Zn-treated plants. These findings suggest that excess Zn adversely affects plant growth, photosynthetic pigments, phenolic and flavonoid contents, and enhances oxidative stress in basil plants.

Keywords *Ocimum basilicum* L. · Zinc · Germination · Antioxidant activity · Secondary metabolites

Zinc (Zn) is an essential micronutrient required for the plant growth and development. It is a key constituent of more than 1200 proteins and metalloenzymes or a cofactor for several enzymes such as anhydrases, dehydrogenases, oxidases and peroxidases (McCall et al. 2000; Hänsch and Mendel 2009). Zinc plays important roles in regulating nitrogen metabolism, cell multiplication, photosynthesis, proteins biosynthesis, and auxin production, among others (Kosesakal and Unal 2009). Like other trace metals, high Zn concentrations can lead to Zn toxicity that detrimentally affects the majority of vital processes at different plant organizational levels (Marichali et al. 2016). Zn toxicity is usually caused by geochemical and anthropogenic (i.e. industrial activities, mining, smelting, fertilization, and sewage sludge) activities (Siedlecka et al. 2001). Common symptoms of Zn toxicity include growth inhibition, repression of root elongation owing to inhibition of cell proliferation, alteration of water and nutrient uptake, loss of membrane integrity, disruption of redox homeostasis, reduction of chlorophyll content and subsequent photosynthesis, generation of reactive oxygen species and installation of oxidative stress (Subba et al. 2014; Balafrej et al. 2020).

To cope with the deleterious effects of elevated concentration of trace metals, plants have developed adaptive strategies including avoidance, sequestration inside the cells, and/or efflux outside cytosol (Di Baccio et al. 2005). The induction of antioxidant system has also been described as putative strategy against trace metal toxicity. Accumulating evidences indicate increased activity of antioxidant enzymes

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namely catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (GPX), and ascorbate peroxidase (APX) in Zn-exposed plants (Nowak et al. 2018). The induction of non-enzymatic antioxidants such as glutathione, ascorbate, and phenolics has also been reported as a part of the defense mechanism of plants against Zn toxicity (DalCorso et al. 2014; Marichali et al. 2016). The morpho-physiological and biochemical responses to Zn excess have been widely studied in the so-called Zn hyperaccumulator species like *Arabidopsis halleri, Sedium alfredii, Thlapsi caerulescens, Paulownia tomentosa*, and *Populus* spp. (Di Baccio et al. 2005; Azzarello et al. 2012), but little is known about the response of aromatic and medicinal plants to Zn toxicity (Marichali et al. 2014, 2016) although their wide use to remediate heavy metal polluted soils (Youssef 2020). Basil (*Ocimum basilicum* L.) is an annual plant belonging to the Lamiaceae family widely cultivated in the Mediterranean regions. It has high culinary and medicinal values owing to its chemical composition particularly rich in bioactive ingredients (Hossain et al. 2010). Earlier pharmacological studies showed that basil had a wide array of biological activities including antioxidant, antimicrobial, insecticide, anti-inflammatory, analgesic, and anticancer properties (Pandey et al. 2014). Recent compositional analyses showed that phenolic compounds and essential oils are the main bioactive compounds responsible for the mentioned activities (Kiferli et al. 2019; Mahmoudi et al. 2020). However, the content of these bioactive ingredients is particularly prone to environmental constraints. In this context, a previous pot experiment revealed that application of high dose of Cd, Pb, and Ni adversely affect shoot, root yields, and the content of essential oils (Prasad et al. 2010). Later, it has been that basil seedlings cultivated in soils contaminated with Al and Cd exhibited high antioxidant activity which was correlated with increased content of total phenol and total flavonoid contents (Đogić et al. 2017). Increased cellular damages and induction of antioxidant enzymes have been reported as typical responses of basil cultivated in soils contaminated with Ni, Cu, and Zn (Georgiadou et al. 2018). More recently, it has been found that basil cultivated in the Cd and Pb contaminated soils exhibited a marked decrease in fresh and dry weight, while the production of essential oils was significantly increased (Youssef 2020).

Based on these antecedents, Zn excess could improve the enzymatic antioxidant system and the production of basil secondary metabolites namely phenolic compounds was hypothesized under hydroponic conditions. Therefore, the main objective of this work was to investigate the effect of elevated Zn concentrations on germination and growth phases of sweet basil seedlings. The effects of Zn excess on plant growth, contents of chlorophylls, carotenoids, and phenolics, and antioxidant activities were explored in a controlled hydroponic culture. It is anticipated that the results of this study could provide insights on the plant response to Zn toxicity, thus establishing the potential of sweet basil plants for phytoremediation purposes.

Material and Methods

Seeds of the cultivar Genovese of basil (*O. basilicum* L.), purchased from a local horticulturist were surface sterilized in 0.5% sodium hypochlorite for 5 min, and then thoroughly rinsed with sterile distilled water. Thereafter, 25 seeds/replicate/treatment were placed in 10 cm Petri dishes fitted with double-layer filter paper moistened with 5 mL of treatment solution containing 0 (as control), 1, 2, 3, 4 or 5 mM Zn as $ZnSO_4 7H_2O$. The experiment was conducted in a germination cabinet at 25 ± 1°C in the dark. Germinated seeds were counted daily for up to 6 days and germination percentage was calculated using the following formula:

$$\text{Germination percentage (\%)} = \left[\text{(Number of germinated seeds)/(Number of total seeds)}\right] \times 100$$

Seedling vigor index was calculated as follows:

$$\text{Seedling vigor index} = \text{Germination percentage} \times \text{Seedling dry weight}$$

Sweet basil seeds were sown in plastic pots containing commercial peat, and irrigated with distilled water after sowing. After 7 days, young seedlings were irrigated with eight-fold diluted Hoagland's liquid solution (Hoagland and Arnon 1950) and placed in a culture chamber with a 16 h photoperiod (150 µmol m^{-2} s^{-1}), and temperature/relative humidity of 22°C/40% and 18°C/86% for day and night, respectively. Individual plants were grown in different treatments including: Control treatment (25% Hoagland's solution containing 5 µM Zn), and 25% Hoagland's solution supplemented with 100, 200, 400 or 800 µM Zn provided as $ZnSO_4$, $5H_2O$, respectively. After 45 days of treatment, plants were harvested for growth, physiological and biochemical analyses.

Fresh weights (FW) of roots and aerial parts were immediately recorded at harvest. Thereafter, samples of fresh roots and aerial parts were placed in a forced-oven air at 60°C for 3 days, and then the dry weights (DW) were determined. The percent of tissue water was calculated as follows: water content (%) $[(FW - DW)/FW] \times 100$. The remaining plants were frozen at − 80°C for biochemical analysis.

For the determination of Zn and Fe contents, dried plant samples (500 mg) were acid digested using HNO_3:$HClO_4$ (4/1, v/v) (Larsson and Asp Bornman 1998) and analyzed using a Perkin Elmer Aanalyst 300 atomic absorption spectrophotometer (IET, Mundelein, IL, USA). The Zn and Fe contents were expressed as µg g^{-1} DW. The recovery of

both metals exceeds 95% and detection limits for Zn and Fe were 0.04 and 0.11 µg/g, respectively. As part of quality control practices, calibration curves of reference standards were used in duplicate in each set of samples. The Zn translocation factor (TF) and bioconcentration factor (BCF) were calculated as follows: $TF = [Zn]_{shoot}/[Zn]_{root}$ and $BCF = [Zn]_{shoot\ or\ root}/[Zn]_{soil}$ (Shi and Cai 2009).

The extent of lipid peroxidation was determined in term of thiobarbituric acid-reactive substances (TBARS) as described by Hernandez and Almansa (2002). Frozen samples (200 mg) were homogenized in 2 mL mixture of 20% thiobarbituric acid (TBA) and 0.5% trichloroacetic acid (TCA). After incubation at 95°C for 20 min, the reaction was stopped in an ice water bath, then the mixture was centrifuged at 10,000×*g* for 5 min, and the absorbance of the supernatant was measured at 532 nm (Alonso-Álvarez et al. 2008)).

Concentrations of Chlorophylls (a, b and total) and carotenoids in fresh leaves were measured as described by Lichtenthaler and Wellburn (1983). Frozen leaf samples (0.5 g) were extracted with 80% acetone and the absorbance were measured at 663, 644, and 452.5 nm for chlorophyll a, chlorophyll b, and carotenoids, respectively.

For the extraction and determination of the activities of antioxidant enzymes, frozen tissue (0.5 g) was homogenized with liquid nitrogen and extracted with 5 mL of the extraction buffer containing 50 mM potassium phosphate buffer (pH 7.6), and 0.1 mM sodium Na-EDTA. The homogenate was centrifuged at 15,000×*g* for 15 min and the supernatant was used for enzyme analysis. The activities of antioxidant enzymes were determined using the procedure of Cakmak and Marschner (1992) for catalase (CAT, EC 1.11.1.6) and Srinivas et al. (1999) for guaiacol peroxidase (GPX, EC 1.11.1.7). Total protein content was determined spectrophotometrically according Bradford (1976).

For the preparation of extract, dry tissue powder (1 g) was extracted by stirring with 10 mL of 80% methanol for 30 min at room temperature. The extracts were kept in the dark for 24 h at 4°C, then filtered and stored at 4 °C until analysis (Mau et al. 2001). Total phenolic contents were determined using the Folin–Ciocalteu colorimetric assay (Kaur and Kapoor 2002) and total flavonoid contents were measured according to Zhishen et al. (1999). Calibration curves of gallic acid and quercetin standards were linear in the range of 0–120 µg/mL and 0–100 µg/mL with coefficient of determination of 0.999 (intercept = 0.033; slope = 0.010) and 0.996 (intercept = 0.025; slope = 0.009) for gallic acid and quercetin, respectively. The TAA was determined according to Prieto et al. (1999).

Total soluble sugar and starch were determined using the previously described method (Yemm and Willis 1954). Total soluble sugar was calculated using a calibration curve of glucose over the range of 0–100 µg/mL (intercept = 0.089; slope = 0.0012; $R^2 = 0.9969$).

Data are presented as means ± SD of at least three replications. Comparison between means were done using one-way analysis of variance (ANOVA), followed by Duncan's multi range post-hoc test at $p < 0.05$. All analyses were performed using SPSS 18.0 statistical software package (SPSS Inc., Chicago, IL, USA).

Results and Discussion

The germination percentage of basil seeds remained unchanged, but the germination rate and the seedling vigor index were markedly reduced at elevated zinc concentrations (Fig. 1). A significant ($p < 0.05$) reduction in radicle and hypocotyls lengths as well as their dry weight was observed in Zn-stressed seedlings in comparison with the control (Fig. 2a, Table 1). In contrast, the dry weight of cotyledons was virtually unaffected by excessive Zn concentrations.

These results indicate that radicle elongation is more sensitive to Zn excess than seed germination. The inhibition of radicle growth in response to Zn excess could be the due to the inhibition of mitotic activity in the root tips and the reduction of cell elongation, and viability. These manifestations could be exacerbated by the extended root lignification in response to elevated Zn concentrations (Li et al. 2012).

In contrast, the resistance of the germination to Zn excess suggests that the water uptake was sufficient to ensure normal imbibition inducing thereby the germination process (Levèbre et al. 2009). Additionally, the presence of seed coat

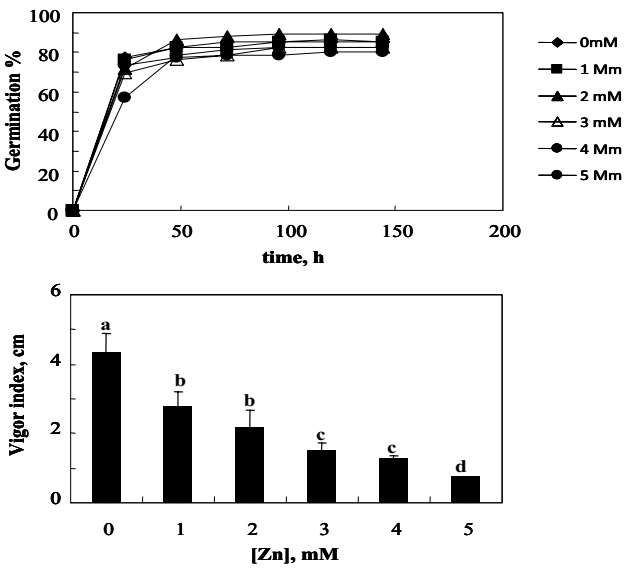

Fig. 1 Effect of zinc on germination percentage (%) and vigor index of sweet basil seeds. Means followed by different letters are significantly different ($p \leq 0.05$)

could limit the entry of this trace metal which confers a better protection of the embryo from the toxic Zn concentrations. These results are in agreement with previous studies (Marichali et al. 2014, 2016) and underpins that Zn excess did not affect the germination of coriander (*Coriandrum sativum* L.) and black cumin (*Nigella sativa* L.) seeds.

Data from Table 2 showed that the level of TBARS increased by 8.3%, 158.33%, 325%, 391.66%, and 500% in the presence of 1, 2, 3, 4, and 5 mM Zn, respectively, over control. These results indicate that Zn-treated seedlings experienced oxidative stress and extended lipid peroxidation leading to oxidative damages to membranes that dramatically hampered the seedling growth. These manifestations were associated with increased activity of GPX versus a decreased activity of CAT in Zn-stressed seedlings. These results indicate that GPX take the large part in the defense against Zn-induced oxidative stress. In contrast, the reduced activity of CAT could be due to structural modification leading to the loss of its functionality in response to Zn application (Torasa et al. 2019). Regarding non-enzymatic antioxidants, Zn supply at concentration higher than 1 mM resulted in a drastic decrease of TPC and TFC, while a concentration of 1 mM Zn induced the accumulation of TFC. At this point, it seems that Zn supply at 1 mM could be beneficial for the production of bioactive flavonoids in basil seedlings.

Morphological examination showed that basil plants exposed to Zn excess were short with a reduced number of cholorotic leaves (Fig. 2b). In Zn-stressed plants (400 and 800 µM Zn), the root system was remarkably reduced and showed branched and abnormally brown lateral roots. In contrast, treatment with 100 µM Zn had a stimulatory effect on growth and leaf number suggesting that the optimum growth of basil plants could be achieved at 100 µM Zn.

The reduction of growth at elevated Zn concentration (> 100 µM) was associated with a remarkable decrease of all biometric (DW, length of the aerial and root parts, and the leaf number) and physiological (water and chlorophyll

Fig. 2 Photos of untreated and treated seedlings (1, 2, 3, 4 and 5 mM) (**a**) and plants growing under control, 100, 200, 400 or 800 µM zinc (**b**)

Table 1 Effect of different zinc concentrations (0, 1, 2, 3, 4 and 5 mM) in DW (mg) and length (L, cm), in radicle, hypocotyls and cotyledons of sweet basil

Zn, mM	Germination (%)	Radicle		Hypocotyls		Cotyledons
		DW	L	DW	L	DW
0	85 ± 2.6a	1.48 ± 0.21a	2.53 ± 0.41a	2.07 ± 0.31a	2.60 ± 0.44a	1.88 ± 0.25a
1	85 ± 2.6a	1.10 ± 0.01b	1.20 ± 0.10b	1.25 ± 0.05b	2.18 ± 0.29a	1.98 ± 0.14a
2	89 ± 6.9a	0.47 ± 0.10c	0.73 ± 0.10c	1.13 ± 0.05c	1.70 ± 0.56a	1.48 ± 0.27a
3	83 ± 5.2a	0.42 ± 0.03c	0.20 ± 0.00d	1.15 ± 0.22c	1.56 ± 0.22a	1.65 ± 0.05a
4	83 ± 10.5a	0.40 ± 0.00c	0.08 ± 0.03e	0.80 ± 0.01d	1.45 ± 0.10a	1.63 ± 0.13a
5	80 ± 9.1a	0.35 ± 0.05c	0.10 ± 0.00e	0.60 ± 0.01e	0.90 ± 0.19b	1.10 ± 0.18a

Means followed by different letters are significantly different ($p \leq 0.05$)

Table 2 Effect of different zinc concentrations (0, 1, 2, 3, 4 and 5 mM) on TBARS (µmol g^{-1}FW) content, GPX and CAT (Unit mg^{-1} P) activities and TPC (mgEGA g^{-1} DW) and TFC (mgCE g^{-1} DW) contents on basil seedlings in response of zinc treatment

Zn, mM	TBARS	GPX	CAT	TPC	TFC
0	1.2 ± 0.3d	167.68 ± 35.06b	12.26 ± 2.08a	22.66 ± 4.81a	9.78 ± 0.34b
1	1.3 ± 0.3d	238.95 ± 73.64b	9.19 ± 1.61a	22.24 ± 2.79a	13.84 ± 2.10a
2	3.1 ± 0.7c	544.364 ± 59.72a	1.72 ± 0.22b	11.96 ± 1.01b	6.33 ± 1.02c
3	5.1 ± 0.7b	468.21 ± 82.29a	1.43 ± 0.37b	9.20 ± 1.70b	3.71 ± 0.80d
4	5.9 ± 0.5b	446.22 ± 32.64a	1.46 ± 0.18b	12.62 ± 1.39b	1.74 ± 0.24e
5	7.2 ± 0.5a	469.98 ± 22.45a	1.12 ± 0.09c	8.76 ± 0.74b	1.29 ± 0.24e

Means followed by different letters are significantly different ($p \leq 0.05$)

contents) parameters (Table 3). The reduction of these parameters is regarded as a common response to Zn excess (Jain et al. 2010).

The manifest physiological alterations interfere with nutrient balance as indicated in Table 3. So, the data presented showed that plants supplied with excessive Zn (> 100 µM) accumulated high Zn concentrations in the leaves *versus* a drastic decrease of the Fe content (Table 3). Accumulation of Zn in leaves could induce Zn toxicity that impaired the photosynthetic apparatus, a fact that will be amplified by the reduction of Fe content. In roots however, it appears that the reduction of their length and DW was associated with Zn toxicity that resulted in disintegration of cell organelles, disruption of membranes, condensation of chromatin and increased nuclei number in root tips leading to alteration of root physiology and consequently, inhibition of their elongation (Rout and Das 2003). Translocation factor (TF) is a measure of the ability of plants to transfer accumulated metals from the roots to the shoots. The results show that the TF decreased with increasing Zn concentrations in the growth medium. This factor was always < 1 which suggest a low translocation of Zn from roots to shoots likely to protect photosynthetic apparatus from Zn deleterious effects. The BCF_{shoot} and BCF_{root} decreased with increasing Zn concentration in the growth medium indicating the limitation in Zn soil-root transport at elevated Zn concentrations. Besides, BCFroot > 1 and TF < 1 suggest that basil plant have the potential for phytostabilization which decrease with increasing Zn concentration in the growth medium (Subhashini et al. 2013).

Being a required micronutrient for chlorophyll biosynthesis, the reduction of Fe in Zn-stressed plants decreased the concentration of chlorophyll leading ultimately to growth suppression. In confirmation of these results, data from Table 3 showed that Zn excess dose dependently reduced the concentration of chlorophyll a, b and total chlorophyll, whereas it improved the synthesis/accumulation of carotenoids (Table 3). The Zn-mediated reduction of the concentration of chlorophyll suggests that Zn ions interfere with chlorophyll biosynthesis either by substitution of the Mg/Fe ions, inhibition of key biosynthetic enzymes mainly δ-aminolevulinic acid dehydratase and protochlorophillide reductase, and/or through the oxidative damages of chloroplasts (Marichali et al. 2016). The Zn-induced activity of chlorophyllase is suggested too. In contrast, the increase in carotenoid (known for their antioxidant properties) content could be regarded as adaptive response to Zn-induced oxidative stress through their protective role of the photosynthetic machinery and their ROS detoxifying ability. Similar results have been reported in the hybrid *Salix purpurea* × *triandra* × *viminalis* 2 (Borowiak et al. 2015), basil (Georgiadou et al. 2018), *Chenopodium murale* (Zoufan et al. 2018), and

Table 3 Effect of zinc on morpho-physiological parameters (dry weight (g), water content (mg g^{-1} DW), aerial parts and root length (cm), leaf number, number of leaf stage, Zn^{2+} and Fe^{2+} contents (µmol gDW), on shoot and root Zn translocation factor (TF) and Bio-concentration Factor (BCF), on chlorophyll (a, b and total; mg^{-1} chl g^{-1} FW tissue) contents and total carotenoids (µg g^{-1} FW tissue) of sweet basil plants cultivated in the presence of 0, 100, 200, 400 or 800 µM zinc for 45 days

Zinc, µM	0	100	200	400	800
DW, aerial parts	0.76 ± 0.15 a	0.76 ± 0.14 a	0.29 ± 0.08 b	0.21 ± 0.05 b	0.07 ± 0.01 c
DW, roots	0.07 ± 0.02 a	0.08 ± 0.02 ab	0.05 ± 0.02 b	0.03 ± 0.01 c	0.01 ± 0.00 d
Water content, aerial parts	10,66 ± 0,77 a	9,93 ± 0,92 a	10,98 ± 0.44 a	11.02 ± 2.95 a	11.10 ± 1.82 a
Water content, roots	13,55 ± 1,87 a	13,90 ± 0,85 a	14.05 ± 1.64 a	11.02 ± 2.95 a	9.62 ± 2.22 b
Aerial parts length	26.90 ± 3.07 a	28.10 ± 1.76 a	13.60 ± 2.06 b	10.50 ± 0.74 c	5.80 ± 1.09 d
Roots length	12.90 ± 1.31 a	11.80 ± 0.88 ab	10.70 ± 1.02 b	8.40 ± 1.46 c	4.80 ± 1.09 d
Leaf number	10.40 ± 0.94 b	20.40 ± 2.74 a	10.00 ± 0.00 b	8.80 ± 1.15 b	6.00 ± 0.00 c
Number of leaf stage	5.60 ± 0.57 ab	6.00 ± 0.00 a	5.20 ± 0.47 b	5.40 ± 0.57 ab	3.80 ± 0.47 c
Leaf Zn^{2+} content	0.94 ± 0.01 e	3.29 ± 0.07 d	3.70 ± 0.29 c	4.83 ± 0.24 b	7.91 ± 0.06 a
Roots Zn^{2+} content	1.39 ± 0.01 d	18.27 ± 1.0 c	17.31 ± 0.78 c	38.38 ± 1.08 b	55.63 ± 0.78 a
TF	0.86 ± 0.06a	0.29 ± 0.05b	0.27 ± 0.02b	0.15 ± 0.01c	0.12 ± 0.00d
BCF_{shoot}	42.4 ± 3.8a	0.9 ± 0.1b	0.3 ± 0.0c	0.3 ± 0.0c	0.2 ± 0.0d
BCF_{root}	49.2 ± 1.0a	3.0 ± 0.3b	1.3 ± 0.1d	1.7 ± 0.1c	2.0 ± 0.2c
Leaf Fe^{2+} content	9.43 ± 1.41 a	5.39 ± 0.28 b	4.84 ± 0.27 b	4.44 ± 0.51 b	4.00 ± 0.12 b
Roots Fe^{2+} content	16.72 ± 0.40 a	11.32 ± 1.22a	9.47 ± 0.26 b	8.63 ± 0.12 c	5.79 ± 0.05 d
Chorophyll a	1.29 ± 0.04 a	0.87 ± 0.12 b	0.41 ± 0.14 c	0.45 ± 0.17 c	0.32 ± 0.05 c
Chlorophyll b	0.63 ± 0.20 a	0.63 ± 0.11 a	0.16 ± 0.05 b	0.16 ± 0.07 b	0.14 ± 0.02 b
Total chlorophyll	1.92 ± 0.18 a	1.50 ± 0.10 b	0.57 ± 0.19 c	0.61 ± 0.24 c	0.45 ± 0.07 c
Total carotenoids	3.17 ± 0.16 b	2.15 ± 0.32 c	4.51 ± 0.72 a	4.65 ± 0.95 a	4.04 ± 0.67 a

Means followed by different letters are significantly different ($p \leq 0.05$)

coffee under excessive Zn concentrations (dos Santos et al. 2019).

Data from Fig. 3 showed that Zn application enhanced the formation of TBARS in both roots and aerial parts. The highest TBARS content was observed in roots exposed to 800 µM Zn. The extended lipid peroxidation in roots could be due to their direct contact with the trace metal in the nutrient solution. In general, Zn-stressed plants experienced severe oxidative stress as a result of increased production of free radicals responsible for membranes damages, resulting for the loss of membrane integrity, depletion of the photosynthetic activity, and eventually growth inhibition (Jain et al. 2010; Zoufan et al. 2018).

To cope with the deleterious effects of free radicals and prevent oxidative damage, plants generally responded by enhancing the activities of antioxidant enzymes and the production of non-enzymatic antioxidants. In the present study, the activities of GPX and CAT were evaluated under control and excessive Zn supplies (Fig. 3).

In aerial parts, the GPX activity increased gradually with the application of Zn with the highest activity being observed under 800 µM Zn. In roots however, the reciprocal trend was observed. The response of the GPX was not

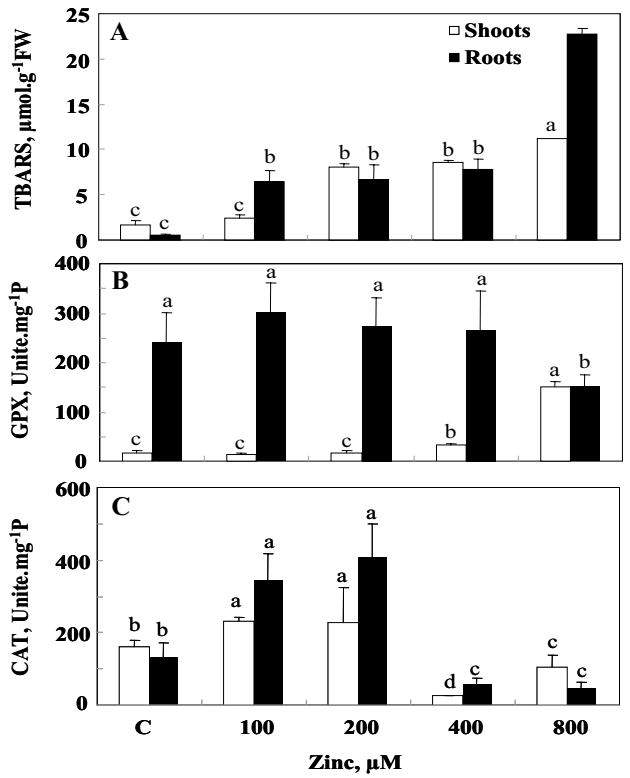

Fig. 3 Effect of Zinc on TBARS (**a**), gaiacol peroxidase (**b**) and catalase (**c**), on shoots and roots of basil plants cultivated in the absence (control, **c**) or in the presence of 100, 200, 400 and 800 µM Zn for 45 days. Means followed by different letters are significantly different ($p \leq 0.05$)

significant up to 400 µM while the GPX activity dropped by 38% at 800 µM Zn supply (Fig. 3). The comparison between the above and below ground parts showed that irrespective to Zn treatment, the highest GPX activity was observed in roots which exhibited the highest TBARS contents. For CAT activity, an irregular trend was observed in roots and aerial parts with the highest activity being recorded at 100 and 200 µM Zn supplies (Fig. 3). However, a reduction of 84% and 34% in the aerial parts and 56% and 64% in the roots is noted in plants grown in the presence of 400 and 800 µM Zn, respectively suggesting the impairment of CAT activity at elevated Zn concentration. This trend indicates that CAT is not required for the ROS detoxifying process in basil plants treated with Zn concentrations higher than 200 µM.

It is worthy to note that the activities of GPX and CAT in plants grown under 100 and 200 µM Zn suggest a cooperative action between both enzymes to cope with the Zn-induced oxidative stress. The concerted action between GPX and CAT for the detoxification of ROS namely H_2O_2 was previously reported in *Brassica juncea* exposed to toxic levels of Zn (Prasad et al. 1999). For much higher Zn concentrations (400 and 800 µM), GPX represents the key antioxidant enzyme responsible for the removal of ROS in basil plants. These results were consistent with those described for *Chenopodium murale*, where it has been found that GPX displays a more important role as compared with CAT to remove H_2O_2 generated by toxic Zn levels (Zoufan et al. 2018). Apart from its detoxifying properties, the increased GPX activity at excessive Zn concentrations (400 and 800 µM) could be associated with the lignification of cell walls conferring thus better resistance to Zn toxicity. In this way, earlier transcriptomic studies in rapeseed pointed that the up-regulation of GPX genes was highly correlated with the oxidative polymerization of phenolic monomers and proteins in the presence of H_2O_2 leading to lignification of cell walls (Wang et al. 2009).

The accumulation of organic compounds including soluble sugar and proteins is reported as a general response to cope with the deleterious effects of excess zinc, which will eventually lead to restoration of cellular homeostasis, detoxification, and mitigation of metal-induced lipid peroxidation and, therefore, survival under stress (Desoky et al. 2009). In this study, the contents of TSS, and total proteins were determined in aerial parts and roots of untreated control and Zn-treated plants (Fig. 4). A gradual increase of TSS contents was observed with increasing Zn concentrations. The content of TSS was markedly higher in aerial parts than in roots, suggesting possible protecting effects against Zn-induced oxidative damage, and their ability to provide adequate carbon and reducing power (NADPH) supply under stressful conditions. Similar results on TSS accumulation have already been described in wheat (Li et al. 2013) and black cumin

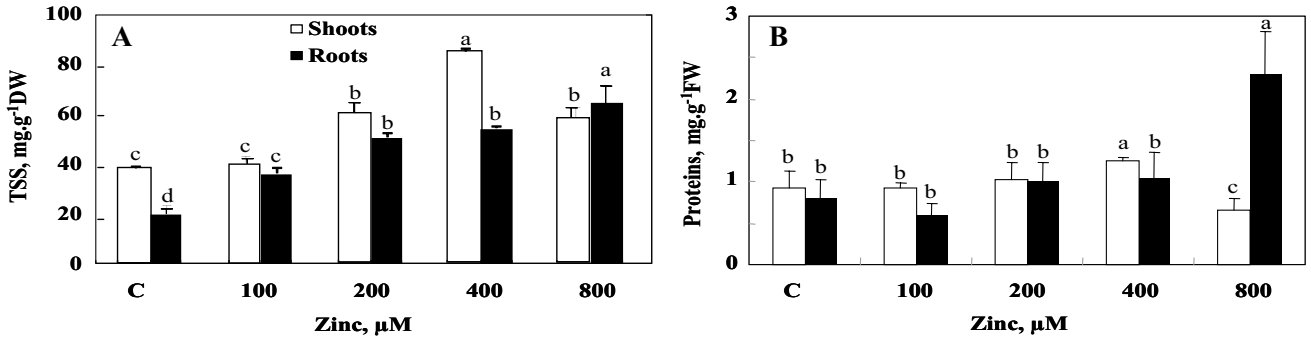

Fig. 4 Effect of Zinc on Total Soluble Sugar (TSS, **a**) and protein content (**b**) on shoots and roots of basil plants cultivated in the absence (control, **c**) or in the presence of 100, 200, 400 and 800 µM Zn for 45 days. Means followed by different letters are significantly different ($p \leq 0.05$)

(Marichali et al. 2016). Concerning proteins, it appears that their content was not affected by excess zinc in the aerial parts. However, a threefold increase of protein content was observed in roots of plants treated with 800 µM Zn. The remarkable increase of protein content at 800 µM Zn could be associated with the induction of protein synthesis especially those evolved in Zn homeostasis such as Zn transporters and Zn-chelating proteins which are endowed with a protective role against Zn-induced oxidative stress (Fukao et al. 2011; Li et al. 2013).

The synthesis/accumulation of phenolic compounds as part of the non-enzymatic antioxidant system is a well-known biochemical response of trace metal-stressed plants (Marichali et al. 2014). In this study, the TPC content in aerial parts decreased gradually with increasing Zn concentration. In roots however, a remarkable stimulation of TPC was observed in plants treated with 100 µM Zn, followed by a drastic decrease at 200, 400 and 800 µM Zn (Fig. 5). These results confirm our previous conclusion regarding the optimal Zn concentration that ensure the highest growth rate and metabolite production. On the other hand, the decline in TPC under 200, 400 and 800 µM Zn could be due to its oxidation by GPX (peroxidase) in the lignification process (Wang et al. 2009).

A similar trend was observed for TFC in aerial parts, while in roots, a significant increase of TFC was noticed in plants treated with 100 and 200 µM Zn. In any case the TFC was higher in roots compared to aerial parts (Fig. 5). Such trend could be associated with their ability to chelate Zn ions particularly accumulated in roots inhibiting thereby the generation of ROS (Wei and Guo 2014). In connection with phenolic metabolites, total antioxidant activity (TAA) exhibited similar profile to TPC suggesting that the TAA was correlated with the TPC in basil plants. These results are in good agreement with those of Morina et al. (2008) who found a positive correlation ($R^2 = 0.99$) between TAA and TPC in common mullein (*Verbascum Thapsus* L.) roots exposed to excess Zn. In confirmation to our results, the

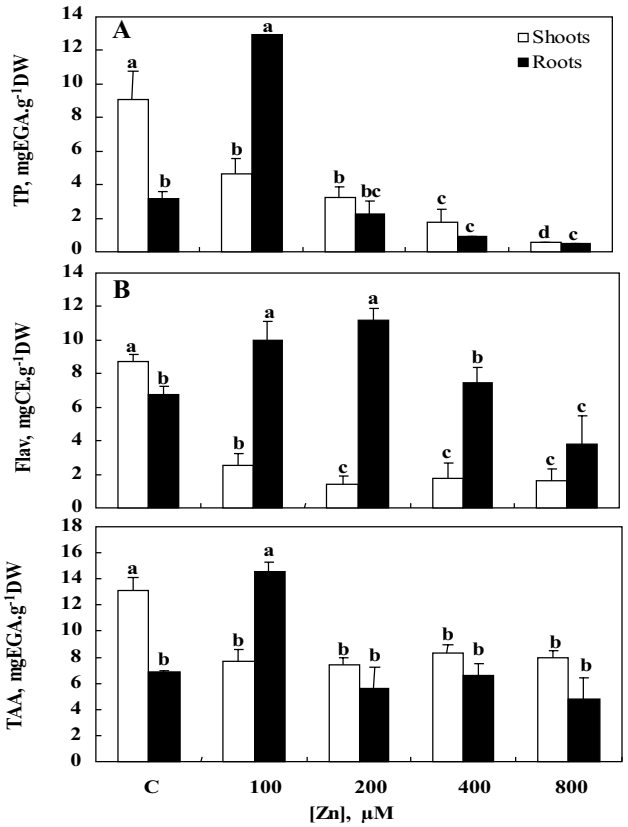

Fig. 5 Effect of Zinc on Total phenolics (**a**), Favonoids (**b**) and Total antioxidant activity (**c**) on shoots and roots of basil plants cultivated in the absence (control) or in the presence of 100, 200, 400 and 800 µM Zn for 45 days. Means followed by different letters are significantly different ($p \leq 0.05$)

study authors' also found an induction of GPX in roots indicating that TPC and GPX have a pivotal role in the defense system of basil against Zn toxicity.

Conclusion

In light of the above findings, it is concluded that basil is particularly sensitive to excess zinc as indicated by the sharp decrease of growth parameters, photosynthetic pigments, and phenolic compounds. Although that basil plants have the potential for phytostabilization (TF < 1 and BCFroot > 1) of Zn up to 100 µM, elevated zinc concentration (> 100 µM) induced severe oxidative stress as revealed by the increased production of TBARS as indicators of extended lipid peroxidation. The increased activities of CATand GPXas well as the enhanced production and /or accumulation of sugar did not however, overcome the adverse effects of zinc excess (> 100 µM) in basil plants. It can be concluded that basil could be used for the phytoremediation of Zn at concentrations lower than 100 µM.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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