J. Agr. Sci. Tech. (2018) Vol. 20: 787-802

Comparative Evaluation of Zinc Oxide Effects on Tobacco (*Nicotiana tabacum* L.) Grown in Different Media

M. Mazaheri Tirani[1], M. Madadkar Haghjou[1*], S. Sulieman[2], and A. Ismaili [3]

ABSTRACT

ZnO is extensively used in a wide range of industrial applications. Hence, considerable accumulation of this substance occurs in the environment. The objective of the present study was to compare and characterize the growth of model tobacco plant at different ZnO levels in various rooting media (i.e., water-based, perlite-based, and sand-based). In soilless medium (especially water-based), ZnO levels lower and/or higher than 1µM showed negative impacts on fresh weights, some of leaves indices, and number of flowers and fruits. In soil-based media, 250 and 500 mg ZnO kg^{-1} often scored the highest values for the mentioned indices. However, all evaluated indices were much higher in water-based than the other two media. Flavonoids, antocyanins and FRAP capacity increased at highest levels in soil-grown and perlite-grown media, but Zn content was the same for all concentrations in soil-grown plants. Photosynthetic pigments decreased at 1,000 mg kg^{-1} in soil-based media. Overall, the sensitivity to small changes in ZnO levels was much higher in water-based compared to the other two media, while ZnO supply resulted in improvement of some parameters in soil-based media. Soil and perlite possess certain experimental limitations (e.g., surface absorption, unfavorable pH, low gas exchange, limited spread of roots and insoluble Zn-complexes), while water-grown plants were comparatively better than the other media in terms of experimental control and handling. These results show different effects of ZnO levels in different media and also suggest the water-based medium as a possible alternate for future accurate investigations of Zn trials.

Keywords: Growth indices, Root growth media, Soilless system, ZnO.

INTRODUCTION

For thousands of years, heavy metals have been used extensively by humans for different uses. Elevated levels of heavy metals in contaminated soils and waters are widely recognized and many environmental concerns have been emphasized regarding the potential risks and harmful impacts to living cells such as animals, humans, fungal, and agricultural crops (Yoon *et al.,* 2006; Houshmandfar and Moraghebi, 2011). Hence, metal stress remains one of the major limiting factors for plant growth and productivity (Yoon *et al.,* 2006). On the other hand, heavy metals such as Zn, Cu, and Mn are well known to be essential microelements for the life of plants (Alloway, 2013).

Because of the low solubility of Zn in the alkaline as well as acidic soils, it is virtually impossible to avoid Zn deficiencies in these types of soils. Generally, the optimum pH for most plants to acquire Zn is within the range of 6.0–6.5 (Sagardoy *et al.,* 2009). In plants, Zn is actively involved in a wide range of metabolic processes including respiration, photosynthesis, and protein synthesis (Candan and Tarhan, 2003;

[1] Plant Physiology Scection, Department of Science, Faculty of Science, Lorestan University, Khoramabad, Islamic Republic of Iran.
*Corresponding author; e-mail: madadkar.m@lu.ac.ir
[2] Department of Agronomy, Faculty of Agriculture, University of Khartoum, Shambat, Sudan.
[3] Department of Agronomy and Plant breeding, Faculty of Agriculture, Lorestan University, Islamic Republic of Iran.

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

González *et al*., 2012). This was confirmed by the increased level of lipid peroxidation, decreased Chlorophyll (Chl) pigmentation, changes in ferric ion metabolism, modified enzymatic activates, as well as levels of phenolic compounds in stressed-plants in response to Zn supplementations (Rosen *et al.,* 1977; Candan and Tarhan, 2003; González *et al*., 2012). Other studies implicated that, unlike other metals, Zn can occur in very high concentrations that are extremely toxic or in some cases lethal to most growing plants (Sagardoy *et al.,* 2009). Not surprisingly, metal stress disturbs various plant physiological and metabolic processes, pending on the stress severity and/or duration. Zinc Oxide (ZnO) occurs naturally as the mineral Zincite, but most of it is produced synthetically and widely used as an additive in many products including plastics, rubbers, ceramics, cosmetic, paints, ointments, adhesives, batteries and other electronic products (Broadley *et al.,* 2007; Kołodziejczak-Radzimska and Jesionowski, 2014). Although some information about the effect of $ZnSO_4$ (Abdollahi *et al*., 2011; Farhad *et al*., 2014), or recently ZnO nanoparticles, has been described in the literature, very limited investigations have been directed to characterize the effects of bulk ZnO on plant growth and development (Haslett *et al.,* 2001). Of all the known species of flowering plants, *Nicotiana tabacum* (common tobacco) stands alone as the most extensively studied. Globally, the tobacco research activities have been increased enormously over the last decades. This allotetraploid plant has emerged as a major crop species and a model organism for studying the general biology of plants and for functional genomics and biotechnological applications. The focus to date has been mostly on its extreme utilization as a versatile model system for all aspects of cell and plant tissue research. This is due to its relatively short generation time, large leaves, small size of seed, and prolific seed production through self-pollination (Ganapathi *et al.,* 2004; Schaeffer *et al.,* 2012).

Soil sustains life by supporting the roots and providing nutrients for plant growth. The nutrient availability affects not only cellular primary metabolism, but also the efficiency of various secondary metabolic pathways (Roca-Pérez *et al.,* 2004). Unfortunately, soil media might have great technical limitations that could restrict the applicability of appropriate experimental approaches and/or reduces the precision of results. Soil limiting factors such as salinity, limited gas exchange, unfavorable pH, soil borne pathogens or residual nitrate and pesticides might interfere with the imposed experimental conditions, thereby increasing the demand for suitable alternative approach without soil culture (Ghehsareh *et al.,* 2012; Marinou *et al.,* 2013; García-Gómez *et al*., 2017). Accordingly, hydroponics and soilless cultures, especially in controlled environmental conditions, have attracted the scientific interest and, therefore, are rapidly expanding throughout the world. The use of various organic and inorganic substrates allows plants to possess a better nutrient acquisition and sufficient growth for gas exchange and water holding optimizations (Ghehsareh and Kalbasi, 2012). Since perlite, pumice, and sand are chemically inert inorganic substrates, they are widely adopted to supply nutrients in a controlled pattern (Marinou *et al.,* 2013). For instance, perlite is widely used as an ideal substrate especially for vegetable cultivations in greenhouses, mainly due to its higher stability, lower bulk density, and electrochemical conductivity (Ghehsareh *et al.* 2012).

Therefore, the main aim of the present study was to evaluate the response of tobacco to ZnO at different rooting media (i.e., water-based, perlite-based and sand-based). The comparative approach was based on the determination of the most convenient and optimum procedure for future investigations of Zn-toxicity experiments.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of *Nicotiana tabacum* (L.) cv. "Isfahan" [courtesy from the Company of Isfahan, Iran], were surface-sterilized with 2.5% sodium hypochloride for 15 minutes and sown in 5 L plastic pots containing an autoclaved sterilized perlite as a growth medium. The plants were maintained in a controlled greenhouse with a 14/10 hour day/night cycle and an approximate range of 25-32°C temperature during the day and 20-23°C during the night. Photosynthetic active radiation was ~100 µmol m^{-2} s^{-1} measured by a

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

photon meter (Hansatech QSPAR, UK) at the top of the canopy. The seedlings were irrigated with tap water three-times a week and 1/10 strength modified Johnson's nutrient solution (Siddiqi *et al.,* 1989) once a week. The nutrient solution had the following composition: 0.001 g L^{-1} $CaCO_3$; 0.1 mM $MgSO_4$; 0.2 mM KH_2PO_4; 0.4 mM K_2SO_4; 0.05 mM $Ca(NO_3)_2$; 4 µM $CaSO_4$; 0.2µM H_3BO_3; 0.2 µM $MnSO_4$; 0.2 µM $ZnSO_4$; 0.05 µM $CuSO_4$; 0.05 µM Na_2MoO_4; 2 µM Fe-EDDHA (a highly stable chelate). The pH was buffered with $CaCO_3$ and adjusted to 5.6±0.2 with 1N HCl. The pH was measured by pH meter (Metrohm, Swiss). Eight weeks later (at 4-leaf stage), seedlings were transferred to soilless culture (water-grown and perlite-grown) with three plants per 1.5 L nutrient solution. Two days later, ZnO (Sigma-Aldrich, MO, USA) was applied at different levels including 0, 0.2, 1, 5, and 25 µM, corresponding to the initial level into the nutrient solution (0.2 µM) and up to 125 times higher (25 µM) in accordance to the suggested range for Zn supplementation (Ingram, 2014). For this purpose, ZnO was dissolved in deionized water with sonication (20 min) two times, by using a sonicator instrument (Ultrasonic cleaner 2200 MH, Soltec, Italy) (Hwang *et al.,* 2007). The nutrient solution was continuously aerated by flow of normal air of about 0.5 L min^{-1} and the solution was changed once a week. Each treatment was carried out in three replicates. Three weeks later, when plants had developed 6-7 leaves, the 6th leaf of each single plant was harvested, frozen in liquid nitrogen, and stored (-20ºC) for subsequent biochemical analyses. The Chl concentration was measured on fresh basis. For soil-grown plants, Zn treatments were added as powder to soil in concentrations (dry mass/dry mass basis) of 0, 250, 500, and 1,000 mg kg^{-1} for ZnO. The soil was slowly mixed thoroughly with the solution using a glass rod and left to equilibrate for one day. General-purpose plastic pots were filled with 500 g of the treated soil which was classified as sand-soil mixture (1:2) in a greenhouse with the above mentioned conditions. The chemical characteristics of the soil are presented in Table 1. Three seeds were grown in each pot as one replication, and 45 days after germination, when plants had totally 6-7 leaves, the 4th leaf of each plant was harvested, frozen in liquid nitrogen, and stored at -20ºC until subsequent biochemical analyses. Based on the published reports, both the 4th and 6th leaves are representative and convenient for such kind of analyses (Feng *et al.,* 2009; Bellasio *et al.,* 2012). Moreover, numerous parameters related to the growth of plants were also recorded.

Growth Parameters

For evaluation of growth parameters, plants were fractioned into shoots (leaf blades+petioles) and roots. The Fresh Weight (FW) of different fractions was recorded, while leaf characteristics (length, area and dry weight) were separately estimated. Shoot/root ratios were calculated and leaf samples were oven-dried at 70°C (72 hours) for the determination of dry matter. Leaf length was estimated excluding the petiole segment. Leaf area was measured following the procedure of Pandey and Sing (2011). Dried plant material was ground to a fine powder and its Zn contents were measured. For this purpose, about 0.5 g of plant material was extracted with 2 mL HNO_3 (65%) according to Kalra (1997). Zinc contents were estimated by an atomic absorption spectrophotometer (Chromophor, AASpect 203) and data was then recorded on a dry weight basis.

Estimation of Photosynthetic Pigments

The photosynthetic pigments [(Chl *a*, *b*, total Chl, and Carotenoids (Car)] were determined following the procedure described by Lichtenthaler and Buschmann (2001). The pigment extract was measured against a blank of

Table 1. Chemical characteristics of the soil used in the experiment.

Texture	EC (dS m^{-1})	pH	Total N (%)	OC [a] (%)	P_{ava} [b]	K_{ava}	Cu_{ava}	Zn_{ava}	Mn_{ava}	Fe_{ava}
					(mg kg^{-1})					
Soil	4.05	7.8	0.10	0.97	37.7	331	4.8	3.64	13.42	9

[a] Organic Carbon, [b] Available.

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

pure methanol at wavelengths of 665.2 and 652.4 nm for Chl assays and 470 nm for Car assays using spectrophotometer UV-1601 (Rayleigh, China).

Determination of Anthocyanin Content

For anthocyanin determination, frozen tissue samples (50 mg) were immediately soaked in 5 mL of acidified methanol [Pure methanol: HCl 37%= 99:1 (v/v)]. Tissues were carefully crushed using a glass pestle and kept for one day (25ºC) in full dark conditions. Thereafter, the extract was centrifuged at 4,000×g for 10 minutes at room temperature and absorption rate was read by a UV-VIS spectrophotometer (Rayleigh, China) at 550 nm. Anthocyanin content was calculated using an extinction coefficient of 33,000 mol^{-1} cm^{-1} (Wagner, 1979).

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing power of plant extract was measured based on the FRAP as described by Wojdyło *et al.* (2007). Frozen tissue samples (50 mg) were finely ground using a liquid-nitrogen cooled system. Subsequently, 2 mL of 50 mM phosphate buffer (pH= 6) were added and the extracts were centrifuged at 12,000×g at 4°C for 10 minutes. The reducing capacity was performed using TPTZ (2, 4, 6-tri (2-pyridyl)-1, 3, 5-triazine). At low pH, reducing Fe^{+3} to Fe^{+2} by reducing agents of the extract causes an intense blue color after the formation of TPTZ-Fe^{+2} complex, with a maximum absorption peak at 593 nm. The FRAP reagent was prepared by mixing acetate buffer (pH= 3.6), a solution of 10 µM TPTZ in 40 µM HCl, and 20 µM $FeCl_3$ at 10:1:1 (v/v/v). The reagent (900 µL) and sample extractions (100 µL) were added to each and mixed thoroughly. The standard curve was prepared by using different concentrations of $FeSO_4$.

Estimation of Flavonoid Content

To determine flavonoid content, frozen leaf tissue samples (50 mg) were soaked immediately in 5 mL of acidified ethanol [Ethanol 96%: Acetic acid, 99:1 (v/v)] and the samples were gently boiled for 10 minutes in a water bath at 80ºC and brought up to volume. The extracts were then centrifuged at 4,000×g for 5 minutes at room temperature, and the absorbance at three wavelengths (270, 300, and 330 nm) was determined with UV-VIS spectrophotometer (Rayleigh, China) (Krizek *et al.*, 1998).

Statistical Analysis

The experiment was arranged in completely randomized design with three replicates. Data were subjected to combined Analysis Of Variance (ANOVA), and means were separated by using the Tukey's test followed by the Least Significant Difference (LSD), for double-checks. All statistical analyses were conducted using statistical tools imbedded in the SPSS Software package 18.0.

RESULTS AND DISCUSSION

Plant Biomass Production

Previously published reports have observed that there were remarkable differences in plant responses to heavy metals depending on the growing conditions that affect the perception of heavy metals (Sobrino-Plata *et al.*, 2009). Based on such evidence, we decided to study the interaction of ZnO and kind of root zone on plant growth in three different substrate media, namely, water-based, perlite-based, and soil-based. To achieve this, the effects of different levels of ZnO were studied using tobacco as a test plant.

Combined analysis of variance for different treatments showed a significant ($P< 0.01$) response for the evaluated indices (Table 2). Table 3 shows the effect of Zn treatments on the shoot, root, and leaf FW of tobacco grown on the three types of substrate in question. With exception of 1 µM, ZnO supplementation in the water and perlite growth media resulted in significant reductions in shoot FW. Compared to shoot, root FW was less sensitive to Zn supply in water-based medium. Increasing the Zn supply

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

Table 2. Combined analysis of variance of different treatments (different media substrate and concentration) for studied traits/characteristics.

Source of variation	*df*	Shoot FW	Root FW	Shoot Root	Leaf FW	Zn in plant	Leaf length	Leaf area	Leaf DW	Leaf No	Flower No	Fruit No	Chl *a*	Chl *b*	Total Chl	Car	Fla 270	Fla 300	Fla 330	Antho cyanin	FRAP
Treatment	13	23.1**	5.4**	4.9**	0.47**	0.0002**	86.8**	7169.8**	49.4**	37.9**	208.9**	18.1**	0.54**	0.56**	2.14**	0.22**	0.04**	0.09**	0.07**	0.013**	0.36**
Error	28	0.17	0.03	0.03	0.00	0.000	0.49	126.9	32.1	0.62	0.70	0.08	0.014	0.007	0.03	0.002	0.001	0.001	0.001	0.000	0.001

**: Significant at 1 percent probability.

Table 3. Effect of ZnO supply on shoot and root FWs (gr), shoot/root FW, leaf fresh weight (gr), Zn content (ppm gr^{-1} DW) of tobacco plants grown in media over a period of 21 days and soil-based medium over a period of 45 days with supplementation with the indicated level of ZnO.[a]

	Water-based					Perlite-based				
ZnO (µM)	Shoot FW (g)	Root FW (g)	Shoot Root	Leaf FW (g)	Zn in plant (µg g^{-1} DW)	Shoot FW (g)	Root FW (g)	Shoot Root	Leaf FW (g)	Zn in plant (µg g^{-1} DW)
Control	10 ±0.2^{a}	3.9±0.2^{b}	2.5±0.06^{a}	0.57±0.03^{b}	0.018±0.0007^{c}	5.7±0.5^{a}	3.5±0.3^{a}	1.6±0.09^{d}	0.34±0.02^{ab}	0.009±0.0007^{bc}
0.2	7.2±0.6^{c}	3.1±0.2^{c}	2.1±0.08^{b}	0.33±0.02^{c}	0.018±0.0032^{c}	4.4±0.4^{b}	2.3±0.2^{b}	1.9±0.07^{cd}	0.24±0.03^{c}	0.006±0.0007^{c}
1	11.1±0.4^{a}	4.7±0.3^{a}	2.4±0.07^{ab}	0.70±0.02^{a}	0.020±0.0007^{bc}	6.8±0.4^{a}	3.4±0.2^{a}	2.0±0.04^{c}	0.38±0.02^{a}	0.011±0.0024^{ab}
5	8.5±0.6^{b}	3.4±0.3^{bc}	2.5±0.07^{ab}	0.35±0.02^{c}	0.024±0.0010^{ab}	4.4±0.6^{b}	1.9±0.2^{b}	2.3±0.17^{b}	0.37±0.01^{a}	0.010±0.0007^{b}
25	8.2±0.3^{bc}	3.5±0.2^{bc}	2.4±0.05^{b}	0.27±0.01^{d}	0.028±0.0010^{a}	3.9±0.5^{b}	1.2±0.1^{c}	3.4±0.14^{a}	0.32±0.01^{b}	0.015±0.0008^{a}
ZnO (mg Kg^{-1})	Soil-based									
Control	1.7±0.2^{c}	0.6±0.0^{c}	3.1±0.12^{b}	0.21±0.02^{c}	0.003±0.0006^{b}					
250	4.3±0.4^{a}	0.6±0.1^{c}	6.8±0.58^{a}	0.38±0.02^{b}	0.006±0.0006^{a}					
500	3.5±0.1^{b}	1.6±0.0^{a}	2.1±0.09^{c}	0.41±0.02^{a}	0.009±0.0016^{a}					
1000	3.1±0.2^{b}	0.9±0.1^{b}	3.3±0.20^{b}	0.35±0.01^{b}	0.009±0.0014^{a}					
LSD (0.05)	0.726	0.316	0.330	0.034	0.0024					

[a] Means±*SD* followed by the same letter (s), in each column (each substrate-media) are not significantly different among treatments, as measured by Tukey's test (P≤ 0.05).

from the control level resulted in a significant increment in shoot FW of tobacco grown in soil-based medium, while root FW only showed a significant increase in Zn supply at higher dose i.e., 500 and 1,000 mg kg^{-1} (Table 3). The highest fresh matter of the shoot fractions was scored at 250 mg kg^{-1}.

The shoot/root ratio in water-based grown plants showed no significant difference among treatments (Table 3). In perlite-based plants, the highest ratio was observed at 25 µM ZnO, mainly due to the higher decrease in root FW compared to shoot FW. The obvious reduction in the root fraction could be due to the relatively higher negative impact of high ZnO concentration on the root growth and development. Regarding soil-based plants, some remarkable changes were also observed with the highest ratio scored at 250 ppm, presumably, a result of the high increase in shoot FW. Compared to the control, both 500 and 1,000 ppm caused an apparent increase in shoot and root FWs. However, there was a reduction in the root FW at 1,000 ppm compared to 500, which scored a higher ratio. As a whole, root FW seemed more sensitive to higher ZnO levels, especially for plants grown in perlite medium than the other media. With exception of 0.2 µM, plant Zn content showed a proportional increment at the highest concentrations in water and perlite media (Table 3). However, it showed similar contents for all ZnO levels in the soil media which were higher compared to the control.

Based on the published literature, sufficient level of Zn could increase K^+ content in soybean under salt stress and, consequently, improves water content and fresh weight of the plants (Weisany *et al*., 2014). Toxic concentrations of heavy metals (e.g., Zn) could negatively affect K^+ content of the cells, hydraulic permeability and conductivity and water content, thus causing a dramatic decline in the fresh weight of the organs, movement rate of assimilates from shoot to root, and shoot/root ratio (Barceló and Poschenrieder, 1990).

Based on the gene expression level, some scattered reports have shown that different kinds of Zn (e.g. Zn^{2+} from $ZnSO_4$, $ZnCl_2$ or ZnO NanoParticles, NPs) revealed different mode of action and response, pending on the medium used (Wang *et al*., 2013; Beutler *et al*., 2014; Wagner *et al*., 2016). For instance, soluble Zn ($ZnCl_2$) appeared to be more toxic than ZnO-NPs in solution cultures; however, both Zn substances showed similar responses in the soil culture (Wang *et al*., 2013). In another soil study, ZnO NPs and ionic Zn significantly reduced the root and shoot biomass of symbiotic alfalfa at 750 mg kg^{-1}, while bulk ZnO revealed a reversible response i.e., increased the shoot and root biomass (Bandyopadhyay *et al*., 2015). In the present study, increasing the bulk ZnO concentrations up to 500 mg kg^{-1} was proportionally followed by a concomitant increase in the amounts of FWs (and some of other indices), and decreased thereafter. Apart from here, a recorded 20 and 80% reductions in the growth of *Arabidopsis* was reported when 200 and 300 ppm ZnO NPs were added to the soil, respectively (Wang *et al*., 2016). Collectively, these and other similar studies point to the fact that the plant genotype as well as the Zn substance used greatly affects the final response of plants to Zn supply.

Since Zn level has been reported to be nearly up to 300 ppm in soils (Alloway, 2004), the present study was designed to test one (corresponds to 250 ppm) and two (corresponds to 500 and 1,000 ppm) ZnO concentrations, below and above this soil Zn limit, respectively. According to the results from Table 4, 1 µM ZnO caused the highest length of leaf, leaf area, and leaf dry weight of seedlings in water substrate. However, all tested concentrations were able to improve the mentioned indices when applied to the soil substrate. For perlite medium, leaf area and leaf DW were increased at either middle or highest concentrations during the course of the experiment.

Table 5 shows the number of leaves, flowers, and fruits for tobacco plants grown in various types of media after exposure to different Zn treatments. In each condition, there was no significant difference among various treatments in connection with the leaf numbers. Nevertheless, the total number of leaves was much higher in water-based compared to perlite-base and soil-base conditions. This might imply that, this parameter is less sensitive to ZnO treatment, at least within the conditions used in the present study.

Differences among the three types of media were significant based on the combined Analysis

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

Table 4. Effect of ZnO supply on Length of Leaf (LL, cm), Leaf Area (LA, cm^2) and Leaf Dry Weight (LDW, mg) of tobacco plants grown in media over a period of 21 days and soil-based medium over a period of 45 days with supplementation with the indicated level of ZnO.[A]

	Water-based			Perlite- based		
ZnO (µM)	Leaf length (cm)	Leaf area (cm^2)	Leaf dry weight (mg)	Leaf length (cm)	Leaf area (cm^2)	Leaf dry weight (mg)
Control	19.8±0.71^{ab}	142.9±9.75^{ab}	88.0±4.92^{a}	15.47±0.68^{a}	48.00±0.53^{c}	81.6±3.36^{a}
0.2	16.7±1.34^{c}	79.0±8.69^{c}	59.0±7.48^{b}	14.50±0.74^{a}	26.60±3.71^{d}	111.0±7.51^{b}
1	21.8±0.68^{a}	170.1±15.53^{a}	102.9±8.07^{a}	15.92±0.45^{a}	85.01±2.27^{a}	84.5±5.14^{a}
5	19.5±0.71^{b}	93.7±14.10^{bc}	39.5±4.75^{b}	16.55±0.54^{a}	77.75±1.77^{a}	77.4±5.06^{a}
25	19.8±0.49^{ab}	95.8±33.34^{bc}	55.1±10.90^{b}	16.45±0.71^{a}	61.41±4.20^{b}	85.7±5.63^{a}
ZnO (mg Kg^{-1})	Soil-based					
Control	6.0±0.33^{b}	11.32±1.26^{c}	38.96±0.32^{b}			
250	6.7±0.51^{ab}	14.48±2.19^{bc}	52.16±1.22^{a}			
500	8.07±0.31^{a}	20.45±1.56^{a}	54.90±2.67^{a}			
1000	7.3±0.94^{ab}	16.93±2.22^{ab}	51.34±1.64^{a}			
LSD (0.05)	1.228	9.197	4.627			

[a] Means±*SD* followed by the same letter (s), in each column (each substrate-media) are not significantly different among treatments, as measured by Tukey's test ($P \leq 0.05$).

Table 5. Effect of ZnO supply on leaf, flower, and fruit numbers of tobacco plants grown in water- and perlite-based media over a period of 21 days and soil-based medium over a period of 45 days supplemented with the indicated level of ZnO.[a]

	Water-based			Perlite-based		
ZnO (µM)	Leaf No	Flower No	Fruit No	Leaf No	Flower No	Fruit No
Control	14.6±1.07^{a}	22.3±1.45^{a}	non	7.8±0.19^{a}	5.7±1.00^{ab}	5.0±0.33^{a}
0.2	14.4±0.69^{a}	00.0±0.00^{d}	non	8.1±0.84^{a}	5.1±0.38^{ab}	3.1±0.51^{b}
1	16.1±1.02^{a}	22.3±0.88^{a}	non	7.7±0.33^{a}	6.1±0.39^{a}	5.9±0.20^{a}
5	13.7±1.21^{a}	15.4±1.39^{b}	non	7.7±0.67^{a}	4.2±0.51^{bc}	5.2±0.51^{a}
25	13.3±1.16^{a}	8.9±1.17^{c}	non	7.8±0.19^{a}	3.3±0.33^{c}	5.1±0.39^{a}
ZnO (mg Kg^{-1})	Soil-based					
Control	6.6±0.04^{a}	non^{b}	non^{b}			
250	6.7±0.69^{a}	1.1±0.38^{ab}	1.1±0.38^{a}			
500	6.9±0.84^{a}	1.2±0.58^{a}	non^{b}			
1000	7.3±0.33^{a}	1.1±0.51^{ab}	non^{b}			
LSD (0.05)	1.388	1.479	0.505			

[a] Means±*SD* followed by the same letter (s), in each column (each substrate-media) are not significantly different, as measured by Tukey's test ($P \leq 0.05$).

Of Variance (ANOVA) displayed in Table 2. Although there was some kind of differences among various treatments in each media, the recorded flower numbers showed a strong higher tendency in water-based than the other two media. While the lowest recorded data was observed at 0.2 µM in water based medium, the total number of flowers decreased when plants were exposed to the highest Zn level (25 µM) in perlite-based medium (Table 5). On the other hand, the soil grown plants comparatively exhibited the lowest total number of flowers (about 1.1) for all treatments examined (Table 5). Therefore, when compared with perlite and soil-

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

based media, the number of tobacco flowers was more sensitive to Zn treatments when plants were cultured in water-based medium.

Surprisingly, plants in water-based medium failed to develop fruits 21 or even 45 days after exposure to ZnO, while those cultured in perlite or soil media exhibited few number of fruits, respectively, at the same time frames (Table 5). In sharp contrast, all flowers in water-based medium fully developed to fruits later, i.e. about 80 days after ZnO treatment. Available literature showed that plants usually enforced to complete their life cycles early if they experienced, or were exposed to, unfavorable conditions (Gupta and Sandalio, 2012). Thus, the recorded data here might be a part of such physiological adaptation.

Overall, the results indicated that the shoot and root FWs, length, dry weight and number of leaves, flowers, and fruits were comparatively lowest in the soil-grown plants, while those cultured in water-based system exhibited the highest (Tables 3 to 5). These results together suggest that nutrient and metal availability might be more restricted in soil-based system compared to hydroponics (Vázquez and Carpena-Ruiz, 2005). Apart from the restriction of nutrient/metals, the aeration of the root system might also partially affect the plant performance in the utilized culturing system. For instance, Lee *et al*. (2014) have shown that the root zone aeration level remarkable affected the growth and fruit yield of cucumber plants grown in pots filled with perlite. As one might expect, perlite- and soil-based substrates might not be highly convenient to ensure a proper aeration system for root respiration during the course of the experiment. Hence, poor gas exchange with the surrounding environment might greatly affect the reliability of the results, especially for soil-grown plants. Although the oxygen diffusion coefficient in the gaseous fraction of the inter soil particles is 11,300 times higher compared to water (Schulze *et al.*, 2005), this problem could be drastically reduced via continuous bubbling of water-based system. If the level of oxygen decreases in root zone, a concomitant reduction in the root growth will develop, thereby, leading to a great reduction in water beside nutrient acquisition (Janick, 1979; Gliński and Stepniewski, 1985; Vepraskas, 1994).

Photosynthetic Pigments

Although Zn is an essential micronutrient for plant growth and metabolism, the observed harmful responses of high Zn concentrations could be found closely connected with the generation of Reactive Oxygen Species (ROS), in addition to the displacement of some other metals from the active sites in proteins (Moura *et al.*, 2012). In this regard, excessive Zn level might suppress the uptake of iron and, in most cases examined, it was common to find symptoms of severe iron deficiency induced by Zn toxicity (Tewari *et al.*, 2008). Nevertheless, some researchers believe that the chlorosis induced by Zn toxicity is not truly a result of low leaf iron level, and most probably a result of the interaction of both micronutrients at the cellular active sites (Rosen *et al.*, 1977). To avoid any artifacts in the present study, Fe-EDDHA was adopted as a highly stable chelating substance, thereby, providing a more convenient iron source for easy access by the examined plants.

Here, various photosynthetic pigments were also quantified in response to different Zn treatments for plants grown at different substrates (Table 6). With exception of 1 µM, Chl *a*, *b* and total Chl greatly decreased in water- and perlite-grown plants, while Car content showed a reversible pattern to Chl and increased in water-grown plants. The unequivocal functions of Car in scavenging or quenching the singlet oxygen and protecting the chloroplast from lipid peroxidation and oxidative damages are well documented (Candan and Tarhan, 2003). The observed difference between treatments in various photosynthetic pigments is in agreement with the published results on the effects of Zn, for example, in pumpkin (Lalelou and Fateh, 2014), shepherd's-purse (Kozhevnikova *et al.*, 2014), and chickpea (Sharma *et al.*, 2010).

In this sequence of events, we do not dismiss the possibility that the photosynthetic pigments reduction under this experimental conditions could be due to the destruction of photosynthetic apparatus, interaction with ROS, impair of Chl biosynthesis coupled with higher increase in chlorophyllase activity (González *et al.*, 2012; Michael and Krishnaswamy, 2014). Chl content has been also classified as a reliable indicator in connection with the subject of

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

Table 6. Effect of ZnO supply on photosynthetic pigment contents (mg gr^{-1} FW) of tobacco plants grown in water- and perlite-based media over a period of 21 days and soil-based medium over a period of 45 days, supplemented with the indicated level of ZnO.

ZnO (µM)	Water-based				Perlite-based			
	Chl [a] (mg g^{-1} FW)	Chl [b] (mg g^{-1} FW)	Total Chl (mg g^{-1} FW)	Car (mg g^{-1} FW)	Chl (mg g^{-1} FW)	Chl (mg g^{-1} FW)	Total Chl (mg g^{-1} FW)	Car (mg g^{-1} FW)
Control	2.5±0.11^{a}	1.5±0.04^{b}	4.0±0.13^{a}	0.6±0.03^{c}	1.6±0.04^{a} ^{b}	0.6±0.15^{b}	2.2±0.05^{b}	0.4±0.62^{a}
0.2	1.7±0.16^{bc}	0.5±0.02^{d}	2.2±0.15^{c}	0.8±0.05^{b}	1.3±0.06^{c}	0.5±0.13^{b}	1.8±0.07^{c}	0.4±0.04^{a}
1	2.5±0.22^{a}	1.7±0.10^{a}	4.3±0.30^{a}	0. 5±0.02^{c}	1.7±0.05^{a}	0.7±0.16^{a}	2.3±0.07^{a}	0.4±0.05 0^{a}
5	2.0±0.02^{b}	1.2±0.13^{c}	3.1±0.15^{b}	1.2±0.07^{a}	1.5±0.03^{b}	0.5±0.18^{c}	2.0±0.04^{c}	0.4±0.06^{a}
25	1.5±0.10^{d}	0.6±0.05^{d}	2.1±0.05^{c}	1.1±0.04^{a}	1.2±0.05^{c}	0.4±0.14^{d}	1.6±0.7^{d}	0.5±0.02^{a}
ZnO (mg Kg^{-1})	Soil-based							
Control	1.6±0.06^{b}	0.6±0.06^{b}	2.3±0.12^{b}	0.3±0.01^{c}				
250	2.1±0.20^{a}	1.2±0.19^{a}	3.4±0.038^{a}	0.4±0.03^{ab}				
500	2.2±0.18^{a}	1.4±0.08^{a}	3.6±0.19^{a}	0.5±0.02^{a}				
1000	1.5±0.12^{b}	0.7±0.12^{b}	2.2±0.23^{b}	0.4±0.04^{bc}				
LSD (0.05	0.208	0.142	0.307	0.076				

[a] Means±*SD* followed by the same letter (s), in each column (each substrate-media) are not significantly different, as measured by Tukey's test ($P \leq 0.05$).

environmental quality, pollution, and toxicity of heavy metals in higher plants (De Gomes *et al*., 2014). Thus, if toxic levels of ZnO are applied to plant, a remarkable reduction in Chl content might result in response to oxidative stress. On the other hand, nontoxic Zn amounts are involved in the activity of enzymes including those for Chl biosynthesis (Beale, 1999). Some alleviating effects of sufficient Zn on Chl content has also been observed in plants under oxidative stress (Saeidnejad and Kafi, 2013).

In this study, all examined photosynthetic pigments were markedly increased with increasing Zn supply in soil-based medium, and reached the maximum at a Zn level of 500 mg kg^{-1}. Overall, 1µM ZnO for water and perlite grown plants and 250 and 500 mg kg^{-1} levels for soil-based ones caused the highest Chl content in tobacco grown in different substrates, which suggest them as useful levels for improving Chl content in future studies.

Anthocyanin Content

Unlike the plants grown in the other two media, the anthocyanin contents increased remarkably with increasing Zn supply in water-based medium, and reached the maximum at a Zn level of 1 µM followed by a steep reduction (Table 7). In perlite-based medium, increasing Zn treatment resulted in inconsistent response with 0.2 and 25 µM, showed the highest pigment contents (Table 7). The anthocyanin content significantly increased only when plants grown in the soil medium received the higher levels of Zn corresponding to 500 and 1,000 mg kg^{-1} (Table 7). Higher content of anthocyanin in response to Zn supply could be explained in terms of reciprocal aspects. In this regard, some published reports have implicated anthocyanins in several diverse functions including, but not limited to, cell protection through ROS scavenging, breakdown of peroxidation chain, hydrogen donation, quenching of singlet oxygen and peroxidase enzyme (Asad *et al*., 2015). Accordingly, one would expect a remarkable accumulation of anthocyanin if plants are exposed to metal stressful conditions. On the other hand, some other studies have suggested that the cellular level of

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

Table 7. Effect of ZnO supply on anthocyanin (µM gr^{-1} FW), FRAP (mM gr^{-1} FW) and Zn (ppm gr^{-1} DW) contents of tobacco plants grown in water- and perlite-based media over a period of 21 days and soil-based medium over a period of 45 days, supplemented with the indicated level of ZnO.[A]

	Water-based		Perlite- based	
ZnO (µM)	Anthocyanin (µM g^{-1} FW)	FRAP (mM g^{-1} FW)	Anthocyanin (µM g^{-1} FW)	FRAP (mM g^{-1} FW)
Control	0.10±0.004^{c}	0.15±0.003^{bc}	0.08±0.006^{bc}	0.35±0.026^{ab}
0.2	0.13±0.004^{ab}	0.11±0.018^{c}	0.10±0.005^{a}	0.36±0.005^{a}
1	0.14±0.006^{a}	0.25±0.023^{a}	0.07±0.007^{c}	0.24±0.018^{d}
5	0.12±0.011^{bc}	0.16±0.017^{b}	0.09±0.004^{ab}	0.30±0.013^{bc}
25	0.07±0.006^{d}	0.12±0.012^{bc}	0.10±0.007^{a}	0.29±0.025^{cd}
ZnO (mg Kg^{-1})	Soil-based			
Control	0.16±0.007^{b}	0.49±0.016^{b}		
250	0.18±0.004^{ab}	0.60±0.023^{b}		
500	0.21±0.005^{a}	1.23±0.091^{a}		
1000	0.19±0.0164^{a}	1.08±0.08^{a}		
LSD (0.05)	0.012	0.064		

[a] Means±*SD* followed by the same letter (s), in each column (each substrate-media) are not significantly different, as measured by Tukey's test ($P \leq 0.05$).

anthocyanin might be closely connected to the regulation of other nutrients uptake or a result of their improved and relatively higher content as a result of Zn supply (Asad *et al.*, 2015).

FRAP Assay

The response of FRAP to different levels of ZnO under various tested growing media are shown in Table 7. With the exception of 1 µM treatment, the levels of FRAP in water-based plants were found unaffected by the increase in Zn supplementation to the nutrient solution. For perlite-grown plants, application of most of the treatments induced a significant reduction in FRAP measurements. In accordance with the response of anthocyanin content, FRAP significantly increased when plants grown in the soil medium received higher levels of Zn corresponding to 500 and 1,000 mg kg^{-1}.

As the result of ZnO application, the increase in anthocyanin and FRAP levels could help plants to overcome unfavorable conditions (e.g., ROS production) for better response and adaptation. In addition to the role of anthocyanin in ROS scavenging and cell protection (Asad *et al.*, 2015), FRAP levels in stressed plants are reliable indicators of the tissue antioxidant power and widely used as a good indicator of plant potential antioxidant capacity (Cervilla *et al*., 2007; Szôllôsi *et al*., 2011). Therefore, compared to other concentrations, application of 1 µM ZnO and 250/500 mg kg^{-1} caused a relatively higher increment in the content of Chl, anthocyanin and FRAP. Hence, future studies might consider using these Zn regimes for better growth of plants.

Flavonoid Content

An overriding question regarding our approach is the response of flavonoids content in the examined plants. In this study, application of 0.2 and 25 µM ZnO to water grown plants resulted in a significant higher absorbance of extracts (presumably flavonoids) at 270, 300 and 330 nm (Table 8). With the exception of 5 µM ZnO, no other Zn treatment showed a significant lower absorbance of extracts (at 330 nm) for water grown plants. In perlite-based medium, 0.2 µM ZnO significantly reduced the flavonoid contents at 270 and 300 nm, while Zn levels above 0.2 µM remarkably increased this parameter at both wavelengths excluding 25 µM at 300 nm. For

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

Table 8. Effects of ZnO supply on Flavonoid (Fla) content of tobacco plants grown in water- and perlite-based media over a period of 21 days and soil-based medium over a period of 45 days supplemented with the indicated level of ZnO. The Fla content was measured at 270, 300 and 330 nm wavelengths.[a]

ZnO (µM)	Water-based			Perlite- based		
	Fla 270	Fla 300	Fla 330	Fla 270	Fla 300	Fla 330
Control	0.39±0.035^{c}	0.32±0.022^{b}	0.31±0.011^{c}	0.36±0.013^{b}	0.22±0.016^{b}	0.24±0.027^{ab}
0.2	0.58±0.025^{a}	0.49±0.025^{a}	0.49±0.031^{a}	0.29±0.004^{c}	0.17±0.002^{c}	0.21±0.009^{b}
1	0.35±0.035^{c}	0.27±0.027^{b}	0.27±0.019^{cd}	0.44±0.017^{a}	0.25±0.013^{a}	0.25±0.016^{ab}
5	0.34±0.012^{c}	0.28±0.037^{b}	0.23±0.019^{d}	0.46±0.015^{a}	0.25±0.006^{a}	0.27±0.007^{a}
25	0.50±0.30^{b}	0.44±0.031^{a}	0.39±0.011^{b}	0.46±0.025^{a}	0.24±0.010^{ab}	0.26±0.016^{a}
ZnO (mg Kg^{-1})	Soil-based					
Control	0.29±0.026^{c}	0.32±0.018^{b}	0.39±0.420^{b}			
250	0.36±0.026^{b}	0.41±0.021^{b}	0.44±0.028^{b}			
500	0.61±0.018^{a}	0.75±0.100^{a}	0.75±0.010^{a}			
1000	0.66±0.038^{a}	0.64±0.039^{a}	0.64±0.063^{b}			
LSD (0.05)	0.043	0.063	0.068			

[a] Means±*SD* followed by the same letter (s), in each column (each substrate-media) are not significantly different, as measured by Tukey's test ($P \leq 0.05$).

soil-based plants, application of 500 mg kg^{-1} Zn treatment significantly increased the flavonoid content at the three wavelengths used to detect the absorbance. At 270 nm, 250 mg kg^{-1} Zn treatment reflected the higher significance effect, while, at 270 and 300 nm, the highest Zn treatment was able to reach this edge. Overall, Zn treatments significantly increased the flavonoid content in the plants grown under conditions of the three different media. Mostly, it occurred in extreme Zn levels (0.2 and 25 µM in water-based and 500 and 1,000 mg kg^{-1} in soil-based media), probably, as an adaptation response. In addition to flavonoids content, some increases in FW of organs, leaf length, area and DW, number of flowers, flavonoid content, anthocyanin and FRAP levels were observed mostly at 500 or even 1,000 mg kg^{-1} on soil-based medium (similar to 1 µM at water-based condition) compared to the control, thus suggesting a better condition.

Although 1,000 mg kg^{-1} caused less positive effects than 500 mg kg^{-1}, it showed relatively better results over the control. Pending on the test plant and conditions adopted, the substrate conditions for the examined substrate might vary greatly from one study to another. For instance, Alia and coauthors (2015) reported some toxic effects and reduction in growth indices of *Spinacia oleracea* at 250, 500, and 700 ppm of Cd, Pb and/or Zn in soil, which are not comparable to the results of the present study. Apparently, the main reason for this discrepancy might be related to the test plant used, pH, and organic matter of soils in both studies (Alia *et al*., 2015). Although higher compared to the control, the relative similar Zn contents in plants grown in pots at all ZnO concentrations (Table 3) might give a sense that no more Zn entered the plants at higher concentrations.

To summarize, ZnO is insoluble in water but remains soluble in most acids. The level of ZnO solubility is highly depending on the pH of the medium (Wesolowski *et al*., 1998). While the pH can be easily adjusting (~ 5.6) in water and perlite-based mediums, it remains relatively hard to correct in the soil medium (pH= 7.8). Apart from the pH, other reports have shown that organic acids (e.g., oxalic acid, malic acid, citric acid, and succinic acid) could be exudated from the plant roots, thus would affect the pH, around the roots (Wu *et al*., 2014). On the other hand, microbial soil inoculation might also affect the plant growth, either positively by providing more availability of metal contents and their uptake, or negatively due to the competition (Chauhan and Rai, 2009; Barber, 1973). In this regard, perlite is known to have special properties, which make it a candidate adsorbent for the removal of toxic heavy metals from aqueous solutions. Thus, it

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

can successfully be used as sorbent for the removal of some inorganic and organic pollutants (Sarı *et al*., 2012). All these, in addition to a better handling of gases exchange into water-based condition, could make some different and inconsistent results from ZnO treatment on plants grown in the three media in question.

Conclusions

As an essential element, both deficient and excessive amounts of Zn are very important subjects for the international research community. Although Zn deficiency is more common in crops and human bodies, the toxicity of this element has been widely increased due to the industrial applications (e.g. ZnO fertilizers) in the environment. Among the different kinds of Zn, $ZnCl_2$, $ZnSO_4$ as well as ZnO NPs are the most studied; however, several researchers have recently focused on the differences between ZnO NPs and its bulk forms. According to the literature, the nutrient uptake and consequent influences on plant indices could be closely related to the rhizospheric characteristics. Apart from the soil, numerous tobacco producers extensively use soilless systems in order to produce transplants for tobacco industry. In the present study, the effect of bulk form of ZnO on three substrate media (water-based, perlite-based and soil based) was evaluated by using tobacco as a model crop plant. Water- and perlite-based plants were relatively more sensitive to Zn supply than soil-based plants. However, perlite substrate caused some inconsistent results, possibly due its modicum uptake properties. Soil substrate showed improvement in plant growth indices in response to ZnO concentrations including the higher levels (500 and 1,000 mg kg^{-1}). This response could be partly due to the chemical properties of the soil e.g. pH and organic matter parameters. Generally, water-grown plants showed a more consistent response to ZnO application compared to the other two media. This response might be related to a better gas exchange, accurate pH adjustment, and effective nutrient uptake system. Moreover, the variation between treatments looks more specific, thus reflecting a better experimental control and handling over soil and perlite. The findings also revealed that tobacco had a better performance in water-based systems, with 1 µM ZnO reflecting the best performance compared to the other levels. Under the conditions of the present study, the potential capacity of root spread increases according to the following sequence: soil-grown <perlite-grown <water-grown plants. Collectively, these results suggest the water-based medium as a plausible alternate for future investigations of Zn trials.

REFERENCES

1. Abdollahi, M., Eshghi, S., Tafazzoli, E. and Moosavi, N. 2011. Effects of Paclobutrazol, Boric Acid and Zinc Sulfate on Vegetative and Reproductive Growth of Strawberry cv. Selva. *J. Agr. Sci. Tech.*, **14:** 357-363.
2. Alia, N., Sardar, K., Said, M., Salma, K., Sadia, A., Sadaf, S., Toqeer, A. and Miklas, S. 2015. Toxicity and Bioaccumulation of Heavy Metals in Spinach (*Spinacia oleracea*) Grown in a Controlled Environment. *Int. J. Environ. Res. Public Health*., **12(7):** 7400-7416.
3. Alloway, B. J. 2004. *Zinc in Soils and Crop Nutrition*. International Zinc Association Brussels, IZA publications, Belgium.
4. Alloway, B. J. 2013. Heavy Metals and Metalloids as Micronutrients for Plants and Animals. *In "Heavy Metals in Soils*". Springer, The Netherlands, PP 195-209.
5. Asad, S. A., Muhammad, S., Farooq, M., Afzal, A., Broadley, M., Young, S. and West, H. 2015. Anthocyanin Production in the Hyperaccumulator Plant *Noccaea caerulescens* in Response to Herbivory and Zinc Stress. *Acta Physiol. Plant*., **37**: 1-9.
6. Bellasio, C., Olejníčková, J., Tesař, R., Šebela, D. and Nedbal, L., 2012. Computer Reconstruction of Plant Growth and Chlorophyll Fluorescence Emission in Three Spatial Dimensions. *Sensors*, **12(1):** 1052-1071.
7. Bandyopadhyay, S., Plascencia-Villa, G., Mukherjee, A., Rico, C. M., José-Yacamán, M., Peralta-Videa, J. R. and Gardea-Torresdey, J. L. 2015. Comparative Phytotoxicity of ZnO NPs, Bulk ZnO, and Ionic Zinc onto the Alfalfa Plants Symbiotically Associated with *Sinorhizobium meliloti* in Soil. *Sci. Total Environ.*, **515**–516:60–69.
8. Barber, D. A. 1973. Effects of Micro-Organisms on the Absorption of Inorganic

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

Nutrients by Plants. *Pest Manage. Sci.*, **4**(3): 367-373.
9. Barceló, J. U. A. N. and Poschenrieder, C. 1990. Plant Water Relations as Affected by Heavy Metal Stress: A Review. *J. Plant Nutr.*, **13(1):** 1-37.
10. Beale, S.I. 1999. Enzyme of Chlorophyll Biosynthesis. *Photosynthesis Res.*, **60**:43-73.
11. Beutler, A. N., Silva, V. N., Deak, E. A., Burg, G. M., Schmidt, M. R. and Toebe, M. 2014. Zinc Doses, Sources and Application Times: Seed Physiological Potential and Flooded Rice Yield. *Aus. J. Crop Sci.*, **8(11):**1517-1525.
12. Broadley, M. R., White, P. J., Hammond, J. P., Zelko, I. and Lux, A. 2007. Zinc in Plants. *New Phytol.*, **173:** 677-702.
13. Candan, N. and Tarhan, L. 2003. Changes in Chlorophyll-Carotenoid Contents, Antioxidant Enzyme Activities and Lipid Peroxidation Levels in Zn-Stressed *Mentha pulegium*. *Turk. J. Chem.*, **27:** 21-30.
14. Cervilla, L. M., Blasco, B., Ríos, J. J., Romero, L. and Ruiz, J. M. 2007. Oxidative Stress and Antioxidants in Tomato (*Solanum lycopersicum*) Plants Subjected to Boron Toxicity. *Ann. Bot-London*, **100:** 747-756.
15. Chauhan J. S. and Rai. J. P. N. 2009. Phytoextraction of Soil Cadmium and Zinc by Microbes-Inoculated Indian Mustard (*Brassica juncea*). *J. Plant Interact*., **4(4):** 279-287.
16. Farhad, S., Ahmad, M., Akbar Anjum, M., Hussain, S. 2014. The Effect of Micronutrients (B, Zn and Fe) Foliar Application on the Growth, Flowering and Corm Production of Gladiolus (*Gladiolus grandiflorus* L.) in Calcareous Soils. *J. Agr. Sci. Tech*., **16**: 1671-1682.
17. Feng, Y. L., Lei, Y. B., Wang, R. F., Callaway, R. M., Valiente-Banuet, A., Li, Y. P. and Zheng, Y. L. 2009. Evolutionary Tradeoffs for Nitrogen Allocation to Photosynthesis *versus* Cell Walls in an Iinvasive Plant. *Proc. Natl. Acad. Sci.*, **106(6):** 1853-1856.
18. García-Gómez, C., Obrador, A., González, D., Babín, M. and Fernández, M. D. 2017. Comparative Effect of ZnO NPs, ZnO bulk and $ZnSO_4$ in the Antioxidant Defenses of Two Plant Species Growing in Two Agricultural Soils under Greenhouse Conditions. *Sci. Total Environ*., **1**: 589: 11-24.
19. Ganapathi, T. R., Suprasanna, P., Rao, P. S. and Bapat, V. 2004. Tobacco (*Nicotiana tabacum* L.): A Model System for Tissue Culture Interventions and Genetic Engineering. *Int. J. Biotechnol*., **3**: 171-184.
20. Ghehsareh, A. M. and Kalbasi, M. 2012. Effect of Addition of Organic and Inorganic Combinations to Soil on Growing Property of Greenhouse Cucumber. *Afr. J. Biotechnol.*, **11**: 9102-9107.
21. Ghehsareh, A. M., Hematian, M. and Kalbasi, M. 2012. Comparison of Date-Palm Wastes and Perlite as Culture Substrates on Growing Indices in Greenhouse Cucumber. *Int. J. Recycl. Org. Waste Agric.*, **1**: 1-4.
22. Gliński, J. and Stępniewski, W. 1985. *Soil Aeration and Its Role for Plants.* CRC Press, Boca Raton.
23. González, Á., Chumillas, V. and del Carmen Lobo, M. 2012. Effect of Zn, Cd and Cr on Growth, Water Status and Chlorophyll Content of Barley Plants (*H. vulgare* L.). *Agric. Sci.*, **3**: 572-581.
24. De Gomes, S. M., De Lima, V. A., De Souza, A. P., Do Nascimento, J. J. V. R. and Do Nascimento, E. S. 2014. Chloroplast Pigments as Indicators of Lead Stress. *Eng. Agrí. Jaboticabal*., **34**(5): 877-884.
25. Gupta, D. K. and Sandalio, L. M. 2012. *Metal Toxicity in Plants: Perception, Signaling and Remediation.* Springer-Verlag, Berlin Heidelberg.
26. Haslett, B., Reid, R. and Rengel, Z. 2001. Zinc Mobility in Wheat: Uptake and Distribution of Zinc Applied to Leaves or Roots. *Ann. Bot.*, **87:** 379-386.
27. Houshmandfar, A. and Moraghebi, F. 2011. Effect of Mixed Cadmium, cCopper, Nickel and Zinc on Seed Germination and Seedling Growth of Safflower. *Afr. J. Agric. Res.*, **6:** 1182-1187.
28. Hwang, S. S., Park, J. S. and Namkoong, W. 2007. Ultrasonic-assisted Extraction to Release Heavy Metals from Contaminated Soil. *J Industrial Engine Chem-Seoul*, **13**: 650-656.
29. Ingram, D. L. 2014. Understanding Soilless Media Test Results and Their Implications on Nursery and Greenhouse Crop Management. Horticulture, University of Kentucky College of Agriculture, Food and Environment. Agriculture and Natural Resources Publications. 161
30. Janick, J. 1979. *Horticultural Reviews.* AVI, Publishing Co. Westport, Cunnecticut, E. E. UU.
31. Kalra, Y. 1997. Handbook of Reference Methods for Plant Analysis. CRC Press.
32. Kołodziejczak-Radzimska, A., and Jesionowski, T. 2014. Zinc oxide: From

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

Synthesis to Application. *Material.*, **7**: 2833-2881.
33. Kozhevnikova, A. D., Erlikh, N. T., Zhukovskaya, N. V., Obroucheva, N. V., Ivanov, V. B., Belinskaya, A. A., Khutoryanskaya, M. Y. and Seregin, I. V. 2014. Nickel and Zinc Effects, Accumulation and Distribution in Ruderal Plants *Lepidium ruderale* and *Capsella bursa-pastoris*. *Acta Physiol. Plant*, **36:** 3291-3305.
34. Krizek, D. T., Britz, S. J. and Mirecki, R. M. 1998. Inhibitory Effects of Ambient Levels of Solar UV-A and UV-B radiation on Growth of cv. New Red Fire Lettuce. Physiol Plant., 103: 1-7.
35. Lalelou, F. S. and Fateh, M. 2014. Effects of Different Concentrations of Zinc on Chlorophyll, Starch, Soluble Sugars and Proline Content in *Cucurbita pepo*. *Int. J. Biosci.*, **4:** 6-12.
36. Lee, J., Lee, B., Kang, J., Bae, J., Ku, Y., Gorinstein, S. and Lee, J. 2014. Effect of Root Zone Aeration on the Growth and Bioactivity of Cucumber Plants Cultured in Perlite Substrate. *Biologia.*, **69:** 610-617.
37. Lichtenthaler, H. K. and Buschmann, C. 2001. Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. *Curr. Protocols Food Analyt. Chem*., F4. **3:** 821-828.
38. Marinou, E., Chrysargyris, A. and Tzortzakis, N. 2013. Use of Sawdust, Coco Soil and Pumice in Hydroponically Grown Strawberry. *Plant Soil Environ.*, **59:** 452–459.
39. Michael, P. I. and Krishnaswamy, M. 2014. Membrane Damage and Activity of Antioxidant Enzymes in Response to Zinc and High Irradiance Stress in Cowpea Plant. *Int. J. Curr. Res. Acad. Rev.*, **2:** 112-128.
40. Moura, D. J., Péres, V. F., Jacques, R. A. and Saffi, J. 2012. Heavy Metal Toxicity: Oxidative Stress Parameters and DNA Repair. In: "*Metal Toxicity in Plants: Perception, Signaling and Remediation*". Springer, PP. 187-205
41. Pandey, S. K. and Singh, H., 2011. A Simple, Cost-Effective Method for Leaf Area Estimation. *J. Bot.*, 1-6.
42. Roca-Pérez, L., Boluda, R. and Pérez-Bermúdez, P. 2004. Soil-Plant Relationships, Micronutrient Contents, and Cardenolide Production in Natural Populations of *Digitalis obscura*. *J. Plant Nutr. Soil Sci.*, **167:** 79-84.
43. Rosen, J. A., Pike, C. S. and Golden, M. L. 1977. Zinc, Iron, and Chlorophyll Metabolism in Zinc-Toxic Corn. *Plant Physiol.*, **59**: 1085-1087.
44. Saeidnejad, A. H. and Kafi, M. 2013. Alleviative Effects of Zinc on Physiological Properties and Antioxidants Activity of Maize Plants under Salinity Stress. *Int. J. Agr. Crop Sci*., **5(5):** 529-537.
45. Sagardoy, R., Morales, F., López-Millán, A. F., Abadía, A. and Abadía, J. 2009. Effects of Zinc Toxicity on Sugar Beet (*Beta vulgaris* L.) Plants Grown in Hydroponics. *Plant Biol.*, **11**: 339-350.
46. Sarı, A., Şahinoğlu, G., and Tüzen. M., 2012. Antimony (III) Adsorption from Aqueous Solution Using Raw Perlite and Mn-Modified Perlite: Equilibrium, Thermodynamic, and Kinetic Studies. *Ind. Eng. Chem. Res.*, **51(19):** 6877–6886.
47. Schaeffer, S., Koepke, T. and Dhingra, A. 2012. Tobacco: A Model Plant for Understanding the Mechanism of Abiotic Stress Tolerance. *Improve Crop Resist Abio Stress*, **1-2:** 1169-1201.
48. Schulze, E-D., Beck, E. and Mu¨ller-Hohenstein, K. 2005. Plant Ecology. Berlin / Heidelberg, Germany: Springer.
49. Sharma, S., Sharma, P., Datta, S. P. and Gupta, P. 2010. Morphological and Biochemical Response of *Cicer arietinum* L. var. pusa-256 towards an Excess of Zinc Concentration. *Life Sci.*, **7**: 95-98.
50. Siddiqi, M. Y., Glass, A. D., Ruth, T. J. and Fernando, M. 1989. Studies of the Regulation of Nitrate Influx by Barley Seedlings Using $13NO_3^-$. *Plant Physiol*., **90**: 806-813.
51. Sobrino-Plata, J., Ortega-Villasante, C., Flores-Cáceres, M. L., Escobar, C., Del Campo, F. F. and Hernández, L. E. 2009. Differential Alterations of Antioxidant Defenses as Bio Indicators of Mercury and Cadmium Toxicity in Alfalfa. *Chemosphere*, **77:** 946-954.
52. Szôllôsi, R., Kálmán, E., Medvegy, A., Petô, A. and Varga, S. L. 2011. Studies on Oxidative Stress Caused by Cu and Zn Excess in Germinating Seeds of Indian Mustard (*Brassica juncea* L.). *Acta Biol. Szeged*, **55**: 175-178.
53. Tewari, R. K., Kumar, P. and Sharma, P. N. 2008. Morphology and Physiology of Zinc-Stressed Mulberry Plants. *J. Plant Nutr. Soil Sci.*, **171**: 286-294.
54. Vázquez, S. and Carpena-Ruiz, R. 2005. Use of Perlite in Cadmium Plant Studies: An Approach to Polluted Soil Conditions. *J. Environ. Monit*., **7:** 1355-1358.

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

55. Vepraskas, M. J. 1994. Plant Response Mechanisms to Soil Compaction. In: "*Plant-Environment Interactions*", (Ed.): Wilkinson, R. E. Marcel Dekker, Inc., USA, PP. 263–287.
56. Wang, P., Menzies, N. W., Lombi, E., McKenna, B. A., Johannessen, B., Glover, C. J., Kappen, P. and Kopittke, P. M. 2013. Fate of ZnO Nanoparticles in Soils and Cowpea (*Vigna unguiculata*). *Environ. Sci. Technol.*, **47(23):** 13822–13830.
57. Wang, X., Yang, X., Chen, S., Li, Q., Wang, W., Hou, C., Gao, X., Wang, Li. And Wang, S. 2016. Zinc Oxide Nanoparticles Affect Biomass Accumulation and Photosynthesis in *Arabidopsis*. *Front. Plant Sci.,* 12 January.
58. Wagner, G. J. 1979. Content and Vacuole/Extravacuole Distribution of Neutral Sugars, Free Amino Acids, and Anthocyanin in Protoplasts. *Plant Physiol*., **64**: 88-93.
59. Wagner, G., Korenkov, V., Judy, J. D. and Bertsch, P. M. 2016. Nanoparticles Composed of Zn and ZnO Inhibit *Peronospora tabacina* Spore Germination *In Vitro* and *P. tabacina* Infectivity on Tobacco Leaves. Nanomaterials., **6(50):** 1-10.
60. Weisany, W., Sohrabib, Y., Heidarib, G., Siosemardehb, A., Badakhshanb, H. 2014. Effects of Zinc Application on Growth, Absorption and Distribution of Mineral Nutrients under Salinity Stress in soybean (*Glycine max* L.). *J Plant Nutr*., **37**(14): 2255-2269.
61. Wesolowski, D. J., Ezeth, P., Palmer, D. A., 1998. ZnO Solubility and Zn21 Complexation by Chloride and Sulfate in Acidic Solutions to 290°C with *In-Situ* pH Measurement. *Geochimica et Cosmochimica Acta*., **62(6):** 971–984.
62. Wojdyło, A., Oszmiański, J. and Czemerys, R. 2007. Antioxidant Activity and Phenolic Compounds in 32 Selected Herbs. *Food Chem*., **105**: 940-949.
63. Wu, K., Yuan, S., Guanhua, X., Shi, W., Pan, B., Guan, H., Shen, B. and Shen, Q. 2014. Root Exudates from Two Tobacco Cultivars Affect Colonization of *Ralstonia solanacearum* and the Disease Index. *Eur J Plant Pathol*., **141:** 667-677.
64. Yoon, J., Cao, X., Zhou, Q. and Ma, L. Q. 2006. Accumulation of Pb, Cu, and Zn in Native Plants Growing on a Contaminated Florida Site. *Sci. Total Environ*., **368**: 456-464.

بررسی مقایسه‌ای تاثیر اکسید روی بر گیاه تنباکو (*Nicotinana Tabacum* L.) در محیط های رویشی متفاوت

م. مظاهری تیرانی، م. مددکار حق‌جو، س. سلیمان و ا. اسماعیلی

چکیده

اکسیدروی دارای کاربردهای گسترده صنعتی است. ازاینرو، تجمع مقدار عمده‌ای از آن در محیط زیست غیرقابل اجتناب است. هدف این مطالعه، بررسی و مقایسه تاثیر غلظت‌های مختلف اکسیدروی در محیط‌های کشت متفاوت (آبی، پرلیت و خاک) بر خصوصیات رشد گیاه مدل تنباکو بود. در محیط فاقد خاک (بویژه آبی)، غلظت‌های پایین‌تر و یا بالاتر از 1 میکرومولار، بر روی وزن‌تر، برخی از شاخص های برگی و تعداد گل‌ها و میوه‌ها تاثیر منفی نشان دادند. در محیط خاک، 250 و 500 میلی‌گرم بر کیلوگرم اکسیدروی، اغلب بیشترین مقدار شاخص‌های ذکر شده را موجب شدند. با اینحال کلیه شاخص‌ها در محیط آبی، بالاتر از دو محیط کشت دیگر بودند. مقدار فلاونوئیدها، آنتوسیانین وFRAP درگیاهان رشد یافته در خاک و پرلیت، تحت تاثیر غلظتهای بالای اکسید روی افزایش یافتند، اما مقدار عنصر روی گیاهچه، در کلیه غلظتها در خاک یکسان بود.رنگدانه‌های فتوسنتزی بر اثر

[DOR: 20.1001.1.16807073.2018.20.4.11.9]

غلظت 1000 میلی‌گرم برکیلوگرم،در محیط خاک کاهش یافتند. بطورکلی، حساسیت نسبت به تغییرات اندک غلظت اکسیدروی، در محیط آبی بسیار بیشتر از دو محیط دیگر بود، درحالیکه روی سبب بهبود برخی شاخص‌ها در محیط خاک گردید. محیطهای خاک و پرلیت، محدودیت‌های آزمایشی ویژه‌ای (نظیر جذب سطحی، pH غیرمطلوب، تبادلات گازی پایین، محدودیت در گستردگی ریشه‌ها و کمپلکس‌های غیرمحلول روی) را سبب می‌شوند، درحالیکه کنترل و رسیدگی به گیاه رشدیافته در محیط آبی، بهتر از سایر محیط‌ها بود. این نتایج، تاثیرات مختلف غلظت‌های اکسیدروی در محیط‌های کشت مختلف را نشان داده و نیز محیط آبی را بعنوان یک مورد قابل قبول برای انجام تحقیقات دقیق آتی در انجام آزمون‌های عنصر روی معرفی می‌نماید.

[DOR: 20.1001.1.16807073.2018.20.4.11.9]