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Effects of $ZnSO_4$ Foliar Application on Vegetative Growth and Phenolic and Essential Oil Content of Geranium (*Pelargonium odoratissimum* L.)

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Abstract

Pelargonium odoratissimum is a perennial evergreen used as a fragrant ornamental plant. Leaves, green branches and fresh flowers contain essential oils, that have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. In order to study the effect of foliar spray of $ZnSO_4$ (0, 1000, 2000, 3000 and 4000 mg L^{-1}) on vegetative growth and some physiological characteristics of *Pelargonium odoratissimum* L. plants, a pot experiment was carried out during 2015 at the Research Greenhouse of Azarbaijan Shahid Madani University, Iran. Vegetative growth (plant height, fresh and dry weight) was significantly affected by the application of $ZnSO_4$. Foliar application of $ZnSO_4$ promoted plant height especially at 2000 mg L^{-1}. Chlorophyll a, b, total chlorophyll and carotenoid contents were affected with $ZnSO_4$ treatment as well. The content of essential oil was not significantly influenced with $ZnSO_4$ spray. $ZnSO_4$ application had significant effect on total phenolic and anthocyanins (at 1000 and 2000 mg L^{-1}) and total flavonoids (at 2000 mg L^{-1}) content.

Keywords: Essential oil, Growth, *Pelargonium odoratissimum* L., Phenolic compound, $ZnSO_4$.

INTRODUCTION

Pelargonium odoratissimum (from Geraniaceae) is a perennial evergreen used as a fragrant ornamental plant, commonly grows in tropics, Mediterranean and subtropical climates. Leaves, green branches and fresh flowers contain essential oils mainly consisted of citronellal, geraniol and linalool, as well as their aldehydes and esters. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties, and is commonly used in aromatherapy and fragrance industries (Ayad *et al*., 2010).

Zinc is closely involved in RNA metabolism and ribosomal content in plant cell which lead to the stimulation of carbohydrate, protein and DNA metabolism (Fox and Guerimot, 1998). Among micronutrient; Zn is also required for the synthesis of tryptophan, a precursor of IAA known to act as a growth promoting substance (Amberger, 1982). Zinc plays a significant role in many vital metabolic processes (Amberger, 1982) for instance; zinc is a cofactor for several enzymes such as anhydrates dehydrogenases, oxidases and peroxidases (Nasiri and Najafi, 2015). Zn deficiency retards the activity of carbon metabolism enzymes such as carbonic anhydrase (Ohki, 1976 and 1978), ribulose 1,5-bisphosphate carboxylase/oxygenase and fructose-1,6-bisphosphate (Marschner, 1986). Zinc also protects chloroplasts. Zn-deficiency reduces plant growth and inhibits photosynthesis in many plants (Misra and Srivastava, 2010). Zn is involved in antioxidants pools which scavenge free radicals (Chakmak and Engles, 1999). Essential oil biosynthesis in geranium is strongly influenced by Zn-acquisition and the influences caused by Zn on nutrition and growth. The aim of this work was to study the effect of Zn foliar application on yield, vegetative growth, essential oil and phenolic content of *Pelargonium odoratissimum* L.

MATERIALS AND METHODS

This work was conducted at the research greenhouse of Azarbaijan Shahid Madani University of Tabriz, Iran. Homogenous plants of *Pelargonium* were planted in plastic pots (5 liters), each pot containing a 1:1:1 mixture of soil, leaf manure and animal manure. The treatments were five levels (0, 1000, 2000, 3000 and 4000 mg L^{-1}) of $ZnSO_4$. Zinc solution for the treatments was freshly prepared before spraying. The first treatment was applied after 1 month from transplanting and was repeated 15 days after 1st spray. Forty five days after the last spray, the plants were harvested. The plant herbage was harvested by cutting from above 10 cm over the soil surface and plant growth criteria including plant height (cm) and fresh and dry weight (g/plant) were determined. The experimental design was complete randomized block with three replications.

MEASERMENTS

Total chlorophyll and carotenoids content

Chlorophyll and carotenoids content were calculated according to Prochazkova *et al*. (2001).

Determination of total phenolic content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method. Briefly, 1ml of diluted sample solutions was shaken for 5 min with 0.2ml of Folin-Ciocalteu reagent. Then, 1ml of 2% Na_2CO_3 was added and the mixture was shaken once again for 5 min. Finally, after dilution of solution with distilled water, the absorbance at 760 nm was evaluated using a spectrophotometer (T+80). Gallic acid was used as an internal standard for the calibration curve. The phenolic content was expressed as mg of gallic acid equivalent per gram of dry sample (mg GAE/g) using the linear equation based on a calibration curve (Kim *et al.*, 2006).

Determination of total flavonoids content

The flavonoids content in the extracts was determined spectrophotometrically according to Quettier-Deleu *et al.* (2000) using a method based on the formation of a complex flavonoid-aluminium, having the maximum absorption at 415 nm. The flavonoids content was expressed in mg of rutin equivalent per gram of dry plant extract (mg rutin/g).

Determination of total anthocyanin content

Total anthocyanin content was determined spectro-photometrically according to Wenger (1979).

Essential oil extraction

The oil extraction was done from 30g dry plant material by water distillation during 5 hrs using a Clevenger-type apparatus and the oils were dried with anhydrous sodium sulphate.

Data analysis

The data were analysed by MSTATC and SAS (Ver. 9.2) softwares. Mean comparisons were done by LSD at 1 and 5 percent probability levels.

RESULTS AND DISCUSSION

Effect of zinc on vegetative growth parameters

The results indicated that foliar application of all the concentrations of $ZnSO_4$ significantly promoted plant height and fresh and dry weight in geranium plant (Table 1, 2). The treatments increased leaf and stem fresh weight at 2000 and 4000 mg L^{-1}. The highest amount for root dry weight and plant height was observed in 2000, 4000 and 2000 mg L^{-1} $ZnSO_4$ respectively (Table 2). Ayad *et al*. (2010) reported that the application of zinc (200 mg l^{-1}) and putrescine 20 mg L^{-1} significantly increased fresh and dry weight in geranium. A research done by Cakmak (2008) in wheat and also the results of another study by Salehi Sardoei *et al.* (2014) on petunia plants revealed that Zn foliar application drastically increased the biomass of the plants. Micro-nutrients foliar application moreover, influences the soil-based micronutrients availability and absorption and in-line promotes the plant growth, development and yield components. This in main-part is due to the fundamental role of the trace elements in enzymes activation and dynamics, proteins biosynthesis, photosynthesis enhancement and the intrinsic effect they have on cell division and differentiation.

Table 1. ANOVA for the effects of Zn foliar application on some morphological traits of *Pelargonium odoratissimum*

S.o.V	df	Stem fresh weight	Stem dry weight	Root fresh weight	Root dry weight	Plant lenght	Leaf fresh weight	Leaf dry weight
Replication	2	11.26^{ns}	0.357^{ns}	14.64^{ns}	0.04^{ns}	2.46^{ns}	4.98^{ns}	0.55^{ns}
Treatment	4	281.4**	4.62**	106.16^{ns}	6.78**	22.06**	1992.09**	8.41**
Error	8	34.85	0.632	37.29	0.078	2.21	8.55	0.92
Non additive	7	32.84^{ns}	1.121^{ns}	17.98^{ns}	0.016^{ns}	0.006*	0.37^{ns}	1.52^{ns}
Residual	1	35.14	0.562	40.05	0.087	2.52	9.72	0.86
C.V (%)		9.21	7.51	28.91	6.57	4.02	3.70	8.01

ns,* and ** show non-significant and significant at P<0.05 and P<0.01, respectively

Table 2. Means comparison for the effects of Zn foliar application on some morphological traits of *Pelargonium odoratissimum*

Zn concentration (mg L^{-1})	Stem fresh weight (g)	Stem dry weight (g)	Root dry weight (g)	Plant height (cm)	Leaf fresh weight (g)	Leaf dry weight (g)
Control	61.33 ^{ab}	8.86 ^{b}	2.90 ^{c}	34.67 ^{c}	40.70 ^{c}	11.67 ^{b}
1000	51.33 ^{b}	10.07 ^{ab}	4.46 ^{b}	37.67 ^{b}	74.67 ^{b}	15.32 ^{a}
2000	70.67 ^{a}	11.97 ^{a}	5.43 ^{a}	41.00 ^{a}	100.1 ^{a}	14.87 ^{a}
3000	60.67 ^{ab}	10.43 ^{ab}	2.53 ^{c}	34.33 ^{c}	74.17 ^{b}	9.33 ^{c}
4000	76.33 ^{a}	11.59 ^{a}	5.93 ^{a}	37.67 ^{b}	105.5 ^{a}	15.01 ^{a}
LSD 1%	16.17	2.17	0.76	4.53	8.013	2.19
LSD5%	11.12	1.49	0.52	3.06	5.50	1.50

Similar letters in the columns are non-significant based on LSD test

Effect of zinc on photosynthetic pigments content

Data given in Table 3-4 indicated that foliar application of zinc sulphate meaningfully increased chl (a) at 1000 mg L^{-1}, chl (b) at 3000 mg L^{-1} and total chlorophyll content at 3000 and 4000 mgL^{-1} as well as carotenoid content at 1000 mg L^{-1} in foliage of geranium (Table 4). Regarding, the beneficial effects of Zn on photosynthetic pigments content, it may be due to its role in increasing the rate of photochemical reduction (Ayad *et al*., 2010). It was shown that the application of exogenous zinc to the leaves of tomato plant increased the accumulation of chlorophyll molecules (Kaya and Higgs, 2002). Overall, Zinc is involved in the biosynthesis of chlorophyll and carotenoids and ultimately stimulates the photosynthetic machinery of the plant (Kaya and Higgs, 2002). In this respects, the favorable effects of Zn on photosynthetic pigment content was in line with many previous reports such as; Song *et al*. (2015) in *Vitis vinifera* and Fahad *et al.* (2014) in

Table 3. ANOVA for the effects of Zn foliar application on some physiological characteristics of *Pelargonium odoratissimum*

S.o.V.	df	Total phenolic content	Flavonoids content	Anthocyanin content	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotinoid content	Essential oil content
Replication	2	30.32^{ns}	1.79^{ns}	3.47^{ns}	0.026*	0.489^{ns}	4.050^{ns}	0.036*	0.673**
Treatment	4	474.56**	42.9**	11.74*	0.022*	13.93**	12.67**	0.92**	0.138^{ns}
Error	8	26.93	0.92	2.58	0.005	0.141	1.01	0.006	0.061
Non additive	1	7.16^{ns}	0.15^{ns}	3.24^{ns}	0.001^{ns}	0.001^{ns}	2.97^{ns}	0.034**	0.474^{ns}
Residual	7	29.74	1.03	2.48	0.006	0.161	0.74	0.003	0.004
C.V (%)		5.65	23.23	6.41	3.33	5.55	9.42	5.31	50.53

ns,* and ** show non-significant and significant at P<0.05 and P<0.01, respectively

Table 4. Means comparison for the effects of Zn foliar application on some metabolites of *Pelargonium odoratissimum*

Zn concentration (mgL^{-1})	Total phenolics content (mg GA g^{-1}DW)	Flavonoid content (mg Rutin g^{-1}DW)	Anthocyanin (µg^{-1} FW)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Carotenoid content (mg g^{-1} FW)
Control	71.96^{c}	1.86^{bc}	27.4^{a}	2.05^{b}	4.07^{d}	8.18^{c}	2.06^{a}
1000	97.46^{ab}	2.1^{b}	26.7^{a}	2.23^{a}	5.18^{c}	9.58^{c}	2.16^{a}
2000	105.10^{a}	10.62^{a}	24.7^{ab}	2.01^{b}	7.09^{b}	10.18^{bc}	1.10^{c}
3000	96.31^{ab}	4.39^{ab}	23.48^{b}	2.14^{ab}	9.42^{a}	13.10^{a}	1.21^{b}
4000	88.05^{b}	1.73^{c}	22.91^{b}	2.10	8.03^{b}	12.52^{ab}	1.01^{c}
LSD 1%	14.22	2.63	4.40	0.19	1.02	2.76	0.15
LSD5%	9.77	1.81	3.02	0.13	0.70	1.90	0.10

Similar letters in the columns are non-significant based on LSD test

Gladiolus grandiflorus. Ayad *et al*. (2010) reported that the application of Zn at 200 mg L^{-1} induded the highest protein content of geranium. They also reported that Zn application retained the chlorophyll pool and declined the lipids peroxidation and simultaneously promoted the biosynthesis of proteins and nucleic acids.

Effect of zinc on total phenolics, flavonoids and anthocyanin content

Data presented in Table 3-4 indicates that Zn foliar application lead to the highest values for total flavonoids content at 2000 mg L^{-1} and for anthocyanins at 1000 and 2000 mg L^{-1}. Highest data for the total phenolic compounds was observed with 1000 and 2000 mgL^{-1} Zn foliar application (Table 4). Song *et al*. (2015) noted that the foliar spray of $ZnSO_4$ had promoting effects on photosynthesis rate and total phenolics, total flavonoids, flavanols and anthocyanins content in *Vitis vinifera*. It have been suggested that Zn, significantly influences the expression of phenolics biosynthesis pathway genes during berry development. The expression of the related genes in phenolics biosynthesis (PAL, STS229, CHS, CHI, FLS4, DFR, LDOX and MYBF1) in grape berry skin was all significantly influenced by the foliar spray. They also suggested that PAL expression was highly up-regulated at 61 and 97 days after full-bloom in the Zn treated plants (Song *et al.,* 2015). Song *et al*. (2015) also suggested that flavanols are the major metabolites of anthocyanin biosynthetic pathway. Therefore, the more transformation of flavanols into anthocyanidins may also cause the higher content of total anthocyanins in Zn-treated grapes. Zinc plays critical roles in the defense system of cells against reactive oxygen species and stimulates the production of several antioxidant enzymes and compounds which have crucial role in the prevention of membrane lipids peroxidation and even prevent the structural modification in the proteins and nucleic acids which are commonly prevailing under oxidative situations (Cakmak, 2000).

Effect of zinc on essential oil content

Result showed that foliar application of zinc had no significant effect on essential oil content in pelargonium (Table 3). Nasiri and Najafi (2015) reported that flower yield, essential oil content and essential oil yield were markedly increased by the soil and foliar applications of Fe + Zn in chamomile. In addition, Abd El-Wahab (2008) reported that essential oil of *Trachyspermum ammi* plants were increased in response to the application of Zinc at 50 or 100 ppm. It seems that micronutrients (zinc for example) greatly influence plants growth and development. Zinc is an essential element which plays roles either as metal component of various enzymes or as a functional, structural or regulatory cofactor and is thus linked with photosynthesis and carbohydrate metabolism (Farahat *et al.*, 2007). Overall, Zn is involved in carbon assimilation, saccharids accumulation, reactive oxygen radicals scavenging and finally carbon utilization in volatile oils biosynthesis and accumulation (Nahed and Balbaa, 2007).

CONCLUSION

Overly, the result obtained reveals that zinc application had highlighted effects on the growth and gross content of some metabolites except essential oil content. Considering; the optimized application of Zn containing solutions would be a preferable way to increase the growth potential and to manage the accumulation of preferred metabolites in the plants. However, the recommendation of the suggested idea to the extension sections need more deep studies to trace the effects of Zn application on the content of individual major constituents. Briefly, the foliar application of minerals has the advantage of high nutrients use efficiency and the application feasibility and, will be a good nutrient management tool for the promotion of the plant growth criteria in sustainable agricultural production systems especially on the times of water and nutrients scarcity.

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