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Effect of Putrescine and Zinc on Vegetative Growth, Photosynthetic Pigments, Lipid Peroxidation and Essential Oil Content of Geranium (*Pelargonium graveolens* L.)

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Abstract: A pot experiments were carried out during 2007 and 2008 seasons at the greenhouse of National Research Centre, Dokki, Giza, Egypt. The aim of this work is to study the effect of foliar spray of putrescine (0, 10, 20, 40 mg/l) and Zinc (0, 50, 100, 200 mg/l) on vegetative growth and some chemical constituents of *Pelargonium graveolens* L. plants. All criteria of vegetative growth expressed as plant height, fresh and dry weight of plants were significantly affected by application of the two factor used in this study. Foliar application of putrescine and Zinc as $ZnSO_4$ promoted all the above mentioned criteria, especially at 20 mg/l putrescine and 200 mg/l Zinc sulphate. Chemical constituents i.e. chlorophyll (a), chlorophyll (b) and protein content were increased at all concentrations of the two factors especially at 40 mg/l putrescine and 200 mg/l Zinc. Treatment 200 mg/l zinc followed by that of 40 mg/l putrescine were more effective for decreasing lipid per-oxidation than other treatments. The contents of DNA and RNA, oil percent and yield were significantly increased at 20 mg/l putrescine and 200 mg/l Zinc especially at cutting (I). Major components as citronellal, geraniol and Linalool were improved and increased in the two cuttings at 20 mg/l putrescine and 200 mg/l Zinc sulphate.

Key words: Putrescine · Zinc · Lipid per-oxidation · Essential oil of geranium

INTRODUCTION

Pelargonium graveolens L. from Geraniaceae Family with common name geranium. The major production of geranium took place in China, Egypt and Morocco. Their leaves and green branches and fresh flowers contained essential oil mainly consisted from citronellal, geraniol and Linalool, as well as their aldehydes and esters. Their essential oils possess antibacterial, antifungal, antiviral and antioxidant properties [1], aromatherapy [2] and fragrance industries [3].

Putroscine belongs to polyamines which are considered as a group of plant growth regulators [4, 5]. They are characterized by low molecular mass and are found in all living organisms. Polyamines are part of the overall metabolism of nitrogenous compounds and influence the transcriptional and translational stages of protein synthesis [6], stabilize membranes [7] and also the regulation of gene expression for key enzymes of their biosynthesis and degradation [8].

Among micronutrients, zinc which is closely involved in the metabolism of RNA and ribosomal content in plant cell which lead to stimulation of carbohydrates, protein and the DNA metabolism [9]. Zinc is also, required for the synthesis of tryptophan, a precursor of IAA known to act as a growth promoting substance [10]. Zinc plays a significant role in many vital metabolic processes [11, 12] for instance; Zn is a cofactor for several enzymes such as anhydrates dehydrogenases, oxidase and peroxidase [13]. Zinc protects chloroplasts [12].

The aim of this work was to improve vegetative growth, essential oil percent and yield of geranium by foliar application of putrescine and zinc. To increase the main components of geranium oil such as citronellol, geraniol and linalool. Photosynthetic pigments, total protein, nucleic acid and lipid peroxidation were determined to correlate them with growth and essential oil pattern under putrescine and zinc application.

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MATERIALS AND METHODS

This work was conducted at the greenhouse of National Research Centre, Dokki, Giza, Egypt. Uniform cuttings of *Pelargonium graveolens* L. were planted in clay pots, 30 cm diameter during two successive seasons (2007 and 2008). Each pot was supplied with four gram calcium super phosphate (15.5 % P_2O_5) and one gram potassium sulphate (48 % K_2O) mixed with the soil before transplanting. Ammonium nitrate (33.5 % N) was applied twice started 30 days after transplanting. Fertilization was repeated after every cutting. The experimental design was complete randomized blocks with three replicates.

Treatments and Sampling: Seedlings of geranium plants were transplanted at 1st, 2007 and 3rd February, 2008. The treatments were three levels 50, 100 and 200 mg/l of zinc as zinc sulphate and three levels of putrescine, 10, 20, 40 mg/l in addition to the control (sprayed with distilled water). Zinc and putrescine solutions for treatments were freshly prepared before spraying. Treatments were applied 1st spray to plants after two months from transplanting and repeated ten days after 1st spray. The 1st and 2nd cuttings were drawn at 24 June and 15 November 2007 and 2008, respectively.

Measurement of Growth Parameters: The plant herbage was harvested, by cutting above 10 cm over the soil surface and plant growth criteria in terms of plant height (cm), fresh and dry weights (g/plant) were determined.

Biochemical Criteria:

- Photosynthetic pigments (chlorophyll a, b) in fresh leaves using the method recommended by Metzner *et al.* [14].
- Total protein was determined using the method of Bradford [15].
- Lipid peroxidation, Lalondialdehyde (1,3-propandial) was measured using colorimetric method according to Heath and Parker [16].
- Total nucleic acids (RNA, DNA) were measured using the method by Sperin [17].
- Essential oil percent: 100 g samples of the fresh herbage of each treatment were separately subjected to hydro-distillation to determine the percentage of essential oil according to Egyptian Pharmacopoeia [18].
- Oil yield was calculated as ml per the total weight of fresh herbage per plant for different treatments.
- Identification of essential oil constituents by gas liquid chromatography (GLC) using Agilest Technologies 6890 Netuwork GC system model. The separation was carried out with 5 % phenyl-95% dimethyl polysiloxane capillary column ZB-S (FID). The column temperature was programmed from 70°C at the rate of 4°C/min. The detector was at 280°C and flow rate of the carrier gas (N_2) 30 ml/min. (H_2) was 30 ml/min and air was at 300 mn/min. The identification of essential oil constituents was achieved by matching their retention times (RT) with those of the authentic oil samples injected in GLC under the same conditions.

Data obtained were subjected to standard analysis of variance procedure. The values of L.S.D. were calculated at 5 % level according to Snedecor and Cochran [19].

RESULTS AND DISCUSSION

Effect of Putrescine and Zinc on Vegetative Growth Parameters: Data represented in Table 1 indicated that foliar application of all putrescine concentrations significantly promoted plant height, fresh and dry weights per plant of geranium plants in both cuttings. The increase in growth values was higher at treatment of 20 mg/l putrescine. The pronounced increases of vegetative growth were obtained when plants treated with Zn as zinc sulphate at 200 mg/l. In addition, significant increases in dry matter percentage of geranium occurred especially at 20 mg/l putrescine and 200 mg/l zinc in the two cuttings. In this respect, Abd El-Wahed and Gamal El-Din [20] mentioned that spermdine (belong to polyamines) stimulated vegetative growth characters as plant height and plant fresh and dry weights of chamomile plants. Also, putrescine enhanced the growth (fresh and dry weight) of *Mentha pipeprita* [21] and wheat plant [22]. Polyamines could be act as activators of physiological processes in plant [23]. Paschalidis and Roubelakis [24] reported that polyamines, their precursors and their biosynthetic enzymes were correlated with cell division, expansion, differentiation and vascular development in tobacco plant.

In this concern Farahat *et al.* [25] found that application of Zn significantly increased all tested morphological parameters of *Cupressus sempervirens* L. plants. Significant grain yield of maize plants was obtained in response to zinc foliar application [26].

Table 1: Effect of foliar spraying with putrescine or zinc on vegetative growth of Pelargonium graveolens L. (Average of 2007 and 2008 seasons)

	Plant height (cm)		Fresh wt. (g/plant)		Dry wt. (g/plant)	
Treatment mg/l	Cutting I	Cutting II	Cutting I	Cutting II	Cutting I	Cutting II
Putrescine						
10	41.33c	45.33 c	106.50 cd	94.53 bc	30.38 cd	21.43 cd
20	47.67 a	52.67 a	135.10 a	97.20 a	38.18 a	27.85 a
40	42.67 bc	46.00 c	113.20 bc	96.37 ab	30.66 bc	24.14 b
Zinc						
50	36.67 d	45.60 cd	97.88 d	79.28 d	26.45 cd	18.21 ef
100	41.33 c	47.00 bc	108.30 c	84.97 cd	29.22 cd	20.11de
200	45.64 ab	51.33 ab	121.20 b	92.67 bc	34.40 ab	22.70 bc
Control	29.00 e	40.00 d	86.92 e	74.17 d	25.93 d	16.83 f
L.S.D. 0.05	3.42	5.01	8.67	11.14	4.52	2.36

Table 2: Effect of foliar spraying with putrescine or zinc on photosynthetic pigments of Pelargonium graveolens L. (Average of 2007 and 2008 seasons)

	Chlorophyll (a)		Chlorophyll (b)	
Treatment mg/l	Cutting I	Cutting II	Cutting I	Cutting II
Putrescine				
10	1.10 ab	1.04	0.83 bcd	0.67 c
20	1.03 b	1.01	0.80 d	0.57 d
40	1.14 ab	1.12	0.86 b	0.77 b
Zinc				
50	0.91 c	0.94	0.86 b	0.79 ab
100	1.03 b	1.03	0.85 bc	0.82 ab
200	1.19 a	1.14	0.98 a	0.85 a
Control	0.76 d	0.71	0.45 e	0.43 e
L.S.D. 0.05	0.11	N.S	0.05	0.08

Effect of Putrescine and Zinc on Photosynthetic Pigments: Data given in Table 2 indicated that foliar application of putrescine and zinc sulphate significantly increased chl. (a), chl. (b) in foliage of geranium at the two cuttings. Putrescine effects increased the previous parameters as concentration increased to 40 mg/l putrescine. These increases might be attributed to putrescine function as anti-senescence activity. Polyamines have been found to affect protein synthesis and nitrogenous compounds metabolism [27]. In this connection, Li-Zhijun *et al.* [28] suggested that polyamines play a role in thermal acclimatization of photosynthesis in cucumber. In addition, Huiguo *et al.* [29] found that exogenous application of polyamines protects PSII against water stress at both transcriptional and translational levels and allow PSII to retain a higher activity level during stress, in wheat seedlings resulting in the increase in chlorophyll contents. El-Kausni *et al.* [30] reported that using putrescine resulted in the highest contents of photosynthetic pigments as compared with their control of *Bougainvillea glabra* L.

Concerning the effect of Zn on photosynthetic pigments the highest concentration of Zn i.e. 200 mg/l resulted in the highest increases in chl. a and b at the two cuttings. Regarding the beneficial effect of Zn on photosynthetic pigments, it may be due to its role in increasing the rate of photochemical reduction [31]. It was shown that the application of exogenous zinc to the leaves of tomato plants caused accumulation of chlorophyll content in leaves at both low and high concentrations [32]. Zn could also increase the biosynthesis of chlorophyll and carotenoids ultimately proving beneficial for the photosynthetic machinery of the plant system [12]. In this respect, the favorable effect of Zn on photosynthetic pigments was mentioned for many investigators, Farahat *et al.* [25] on *Cupresseus sempervirens* and Massoud *et al.* [33] on pea plants.

Effect of Putrescine and Zinc on Nucleic Acid (RNA and DNA): Data presented in Table 3 indicated that foliar application of putrescine caused the highest value of nucleic acid at all concentrations in the two cuttings

Table 3: Effect of foliar spraying with putrescine or zinc on RNA of DNA, protein and lipid per oxidation of the herb of Pelargonium graveolens L. (Average of 2007 and 2008 seasons)

	RNA		DNA		Protein		Lipid per oxidation	
Treatment mg/l	Cutting I	Cutting II	Cutting I	Cutting II	Cutting I	Cutting II	Cutting I	Cutting II
Putrescine								
10	32.14 d	27.73 d	30.96 e	25.64 d	2.21	2.65 bc	3.30 a	3.4
20	41.03 a	35.45 a	39.28 a	33.27 a	2.47	2.80 b	2.50 b	2.6
40	38.69 b	33.48 b	36.21 c	31.35 b	3.58	2.90 b	1.95 c	1.65
Zinc								
50	29.33 e	26.61 e	27.60 f	24.30 e	2.17	2.29 c	2.30 b	3.5
100	36.47 c	31.20 c	34.31 d	28.88 c	2.5	2.89 b	2.46 b	2.3
200	39.66 b	34.08 b	37.34 b	31.44 b	3.65	3.99 a	1.43 d	1.5
Control	28.10 f	25.52 f	26.93 f	23.14 f	1.98	1.85 d	2.50 b	3.8
L.S.D. 0.05	1.2	0.92	1.08	0.86	N.S	0.44	0.33	N.S

especially at 20 mg/l in cutting (I), while application of zinc sulphate significantly increased RNA and DNA contents especially at 200 mg/l in the same cutting. Similar promoting effects of putrescine and zinc were reported by other investigators who suggested that, DNA and RNA were significantly higher in polyamines-treated plants as reported by Kakkar and Nagar [34] on *Camellia sinensis*, Sood and Nagar, [35] on rose and Bekheta and El-Bassiouny [36] on wheat. Zinc plays critical roles in the defense system of cell against reactive oxygen species (ROS) and thus represented an excellent protective agent against the oxidation of several vital cell components such as membrane lipid and proteins, chlorophyll, SH-containing enzymes and nucleic acid [37].

Effect of Putrescine and Zinc on Protein Content and Lipid Peroxidation: Data given in Table 3 revealed that protein content (mg/g f.w.) was significantly increased as a result of foliar application of all concentrations of puterscine especially at 40 mg/l, the increments were 3.58, 2.90 mg/g f.w. compared with control (1.98, 1.85 mg/g f.w.) in the two cutting, respectively. In, addition application of Zn gave the highest increment in protein content of geranium plant especially at 200 mg/l Zn in the two cutting. Similar results were true by many investigators, Evans and Malmberg [5] and Kumar *et al.*[38] reported that polyamines regulated cellular ionic environments maintenance of membrane in terrify, prevented chlorophyll loss and stimulation of synthesis of proteins, nucleic acid and protective alkaloids. Hussein *et al.*[39] reported that putrescine application improved plant growth, photosynthetic pigments content and increasing protein content of pea plants. Abd El-Wahed and Gamal El-Din [20] reported that nitrogenous compounds (total N and crude protein) of chamomile leaves significantly increased with putrescine application at 50 mg/l.

Concerning the effect of Zn on protein content, Babhulkar ***et al.*** [40] working on safflower and Abdel Aziz and Balbaa [41] working on *Salvia farinaceous* reported that application of Zn at 200 ppm significantly increased free amino acids, nitrogen and protein content. Sawan *et al.*[42] reported that Zn application slightly increased the seed protein content compared with the untreated control of cotton seed and stated that Zn was directly involved in both gene expression and protein synthesis.

Data given in Table 3 showed that lipid peroxidation decreased at all treatments especially at 40 mg/l of putrescine application while, these decreases were pronounced at all concentrations of zinc sulphate especially at 200 mg/l Zn in the herb at the two cuttings. These significant decreases in lipid peroxidation were in favour for vegetative growth (Table 1) and essential oil content (Table 4). This could be attributed to the protection of polysaturated fatty acids of cell membrane from oxidative damage [43]. In addition, polyamines acted as antioxidants and they counteracted oxidative damage in plants, which as a consequence reduced free radicals and alleviate lipid per-oxidation [44]. Borrell *et al.* [45] suggested that inhibition of lipid per-oxidation might be one of the mechanisms responsible for the anti-senescence action of the polyamines in the leaves. Verma and Mishra [46] reported that exogenous putrescine affected the activities of several antioxidant enzymes when added to *Brassica juncea* seedlings, which occurred concomitantly with a reduction of H_2O_2 and lipid per-oxidation, implying that the positive effects of exogenous polyamines might be related to its antioxidant properties. Polyamines were also potent reactive oxygen species (ROS) scavengers and inhibitors of lipid per-oxidation on chamomile and sweet marjoram seedlings [47]. Thus, it could be concluded that putrescine application to geranium plants increased chlorophyll (a) and (b), protein,

Table 4: Effect of foliar spraying with putrescine or zinc on essential oil percent and yield (plant-1) (Average of 2007 and 2008 seasons).

	Essential oil percent		Essential oil yield (ml/plant)	
Treatment mg/l	Cutting I	Cutting II	Cutting I	Cutting II
Putrescine				
10	0.26 cd	0.28 cd	0.28 bc	0.26 bc
20	0.32 b	0.35 a	0.43 a	0.38 a
40	0.25 d	0.25 d	0.28 bc	0.24 cd
Zinc				
50	0.25 d	0.30 bc	0.25 c	0.24 cd
100	0.30 bc	0.26 cd	0.32 b	0.22 cd
200	0.40 a	0.33 ab	0.48 a	0.31 b
Control	0.30 bc	0.28 cd	0.26 c	0.20 d
L.S.D. 0.05	0.04	0.05	0.05	0.06

Table 5: Effect of foliar spraying with putrescine or zinc on the major constituents of essential oil of Pelargonium graveolens L. (Average of 2007 and 2008 seasons)

	Treatments					
	Putrescine (20 mg/l)		Zn (200 mg/l)		Control	
Oil constituents	Cutting I	Cutting II	Cutting I	Cutting II	Cutting I	Cutting II
α-Pinene	1.55	0.958	1.558	0.952	1.107	0.707
β-Pinene	6.586	6.018	6.698	6.622	6.209	6.001
1,8-Cineal	0.373	0.374	0.195	0.183	0.16	0.119
Linallol	9.981	13.296	10.243	15.823	9.871	11.019
Citronellol	35.45	29.773	35.863	30.328	33.935	26.847
Geraniol	10.026	9.959	9.432	9.967	8.255	8.968
Carvon	2.533	2.016	2.429	3.165	8.231	3.507
Engenol	2.547	2.32	2.236	2.479	1.1	2.193

RNA and DNA reflected by higher vegetative growth and essential oil content and yield. Cakmak [37] and Rathinavel *et al.* [48] speculated that Zn deficiency stress might inhibit the activation of a number of antioxidant enzymes, resulting in extensive oxidative damage to membrane lipids.

Effect of Putrescine and Zinc on Oil Percent and Yield: Data given in Table 4 showed that foliar application of putrescine at 20 mg/l was the only level significantly increased essential oil percent at the two cuttings. While, concentration at 10, 40 mg/l of putrescine significantly decreased essential oil percent in the two cuttings. While, foliar application of zinc sulphate caused significant increases in oil percent, especially at 200 mg/l in cutting (I), while the increases in oil percent at 200 mg/l and 50 mg/l zinc sulphate in cutting (II) were less than cutting (I).

It could be revealed that putrescine and zinc sulphate treatments caused significant increment of oil yield (Table 4) of geranium plants at the two cuttings, especially at 20 mg/l putrescine and 200 mg/l Zn. Similar results were reported by Gamal El-Din [49] for fenugreek plant. Farahat *et al.* [25] reported the highest oil percent in *Cupressns simper virens* L. plants treated with zinc 40 ppm, while Abd El-Wahab [50] reported that the application of Zn at 50 or 100 ppm significantly increased the oil percent and yield of *Trachyspermum ammi* L. (ajowan) plants.

Effect of Purescine and Zinc on Essential Oil Constituents: Data represented in Table 5 indicated that the essential oil of treated and control plant mainly consisted of citronellol as major constituent (26.84 - 35.86 %), followed by geranial (8.25- 10.02 %) and linalool (7.52- 15.82 %) at the two cuttings. Gas liquid chromatography showed that the minor constituents of the oil were carvon, á-â-pinene eugenol and 1, 8-cineol at all treatments. Citronellol content was pronouncedly increased in geranium plants of treatment 20 mg/l putrescine or at 200 mg/l zinc sulphate at cutting (I) as well as cutting (II).

Geranial content in the oil was slightly increased at all treatments, especially at 20 mg/l putrescine in the two cuttings, followed by 200 mg/l zinc application. Linalool was markedly increased in the oil produced from the herb of treatment 200 mg/l zinc at cutting II as well as cutting I, followed by 20 mg/l putrescine. These results are in agreement with those obtained by Abd El-Wahed and Gamal El-Din [20], who found that spermedine application on chamomile plant, stimulated some terpenic constituents of essential oil and the major components of chamomile essential oil were higher at 25 mg/l putrescine. In addition, Abd El-Wahab [50] reported that the main component of essential oil of *Trachyspermum ammi* L. (ajowan) plants was increased in response to application of zinc at 50 or 100 ppm.

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