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Response of Vegetative Growth and Some Chemical Constituents of *Cupressus sempervirens* L. to Foliar Application of Ascorbic Acid and Zinc at Nubaria

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Abstract: A pot experiment was conducted during 2005 and 2006 seasons at National Research Centre, Dokki, Cairo, Egypt, Research and Production Station, Nubaria. The aim of this work is to study the effect of foliar spray of ascorbic acid (0, 20, 40 ppm) and Zinc (0, 20, 40 ppm) and their interaction on vegetative growth and some chemical constituents of *Cupressus sempervirens* L plant. Most criteria of vegetative growth expressed as plant height, stem diameter, number of branchlets, root length, Fresh and dry weight of the plant organs were significantly affected by application of the two factors which were used in this study. Foliar application of ascorbic acid 40 ppm and Zinc 40 ppm separately promoted all the aforementioned characters in this study, as well as chemical constituents content. i.e. chl (a), chl (b), carotenoids, total soluble sugars, total indoles and total soluble phenols except Zinc 20 ppm compared with control Plants, whereas Zn at 20 and 40 ppm application gave insignificant effect on total soluble phenols. Essential oil content was significantly increased by the application of the two factors which were used in this study. The highest recorded data were obtained in plants treated with Asc. 40 ppm + Zn 40 ppm and Asc. 40 ppm + Zn 20 ppm, they increased significantly all growth parameters, except dry weight of shoots and fresh weight of roots compared with control plants. Chl (a) and chl (b) were significantly increased by the two factors collectively under study. Whereas decreased carotenoids content. Total soluble sugars, total indoles, total soluble phenols and oil percent content were significantly increased by the application of ascorbic acid and Zinc application collectively compared with control plants. These applications of ascorbic acid and Zinc may be recommended for increased vegetative growth of cupressus plant for its source of woods for different uses and the increment of oil percent for its medicinal uses.

Key words: *Cupressus sempervirens* L. · ascorbic acid (Asc.) · Zinc (Zn)

INTRODUCTION

Cupressus sempervirens L. Family Cupressaceae, Mediterranean Cupresses, has been widely cultivated as an ornamental tree for millennia away from its native range, mainly throughout the central and western Mediterranean region, in north Libya, south Greece, Turkey, western Syria, Lebanon and western Jordan and in other areas with similar hot, dry summer and mild, rainy winter, including California, south Africa and southern Australia and Italy. It can also be grown successfully in areas with cooler, moister summers, such as British Island, New Zealand and Pacific Northwest (costal Oregon, Washington and British Columbia), Chittendon [1]. It has been widely cultivated as an ornamental timber tree for its source of woods, windy resistance trees and medicinal, this species is known to have antipyretic, antirheumatic, antiseptic, aromatherapy, balsamic and astringent, it is used in the treatment of whooping cough, it influences flu and sore throats. Applied externally as a lotion or as a diluted essential oil (using an oil such as almond). A foot bath of the cones is used to clean the feet and counter excessive sweating, Chevallier [2]. Uphof [3] and Chopra *et al.* [4] reported that the leaves contain about 2% essential oil, whereas the wood contains about 2.5%, it is used in perfume and soap making, they added that wood is very hard and durable and it has scented wood, it is useful for building uses, cabinet making and wardrobes owing to retains its fragrance, repels mothes and imprevious to wood warm. *Cupressus sempervirens* L. is a medium sized

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evergreen tree, its length reached 35 m. with a conic crown, the foliage grows in dense sprays, dark green in colour, the leaves are scale-like, 2-5 mm long and produced on rounded (not flattened) shoots, Uphof [3].

Recently, a great attention has been focused on the possibility of using natural and safe substances in order to improve plant growth. In this respect, ascorbic acid is synthesized in the higher plants and affects plant growth and development, it is a product of D-glucose metabolism which affects some nutritional cycles activity in higher plants and play an important role in the electron transport system [5]. Ascorbic acid (vitamin c) is known as a growth-regulating factor which influences many biological processes.Price [6] reported that ascorbic acid increased nucleic acid content, Robinson [7] reported that ascorbic acid acts as a co-enzyme in the enzymatic reactions by which carbohydrates, proteins are metabolized and involved in photosynthesis and respiration. Blokhina *et al.* [8] stated that ascorbic acid is the most abundant antioxidant which protects plant cells, ascorbic acid is currently considered to be a regulator on plant growth and development owing to their effects on cell division and differentiation and added that ascorbic acid is involved in a wide range of important functions as antioxidant defense, photoprotection and regulation of photosynthesis and growth. Tarraf *et al.* [9] on lemon grass found that application of 50 mg L^{-1} ascorbic acid had positive effects on growth parameters and increased carbohydrates and total Nitrogen percent. Talaat [10] on sweet pepper detected that foliar application of ascorbic acid increased the content of macronutrients (N, P and K).

There are many beneficial effects of micronutrients on plants and their involvement in the other processes, carbohydrate and Nitrogen metabolism, as well as the resistance of plant to diseases and adverse environmental conditions. Micronutrients are also essential for organization and rapid alternation of nutrition compound within plant owing to their great importance in contribution to direct the enzymes way in metabolism [11].

Zinc plays an essential role in plant physiology where it activates some of enzymes such as dehydrogenases, proteinases, peptidases and phosphohydrolases, Rains [12], Moore and Patrick [13] and Romheld and Marschner [14] reported that the basic Zn functions in plant are related to metabolism of carbohydrates, proteins auxins, RNA and ribosome functions. Zn stimulates the resistance of plants to dry and hot weather and also to bacterial and fungal disease [15]. This element is also essential to multiple processes, critical to development and differentiation of cells [16]. El-Mansi *et al.* [17] reported that Zn application increased all growth parameters of pea plants.

Therefore, the present investigation aims to study the effect of ascorbic acid and Zinc application on growth, chemical constituents and essential oil content of *Cupressus sempervirens* L. plants.

MATERIALS AND METHODS

The present investigation was carried out in National Research Centre (Research and Production Station, Nubaria) during two successive seasons of 2005 and 2006. It intended to find out the individual and combined effects of foliar application of ascorbic acid and Zinc on growth and chemical composition of *Cupressus sempervirens* L.

One year old seedling of cupressus were obtained from the nursery of Forestry Department, Horticultural Research Institute, Agricultural Research centre, the seedling were planted at the first week of March during the two successive seasons 2005 and 2006, in plastic plots 30 cm in diameter, filled with 10 kg of peatmoss and sandy soil (1:1 by v/v), one plant per pot, the average height of the seedlings was 18-22 cm.. The available commercially fertilizer used through this experimental work was Kristalon (NPK 19:19:19) produced by Phayzen company, Holland. The fertilizer rates 5.0 g/pot in four doses after 4, 8, 16 and 20 weeks from transplanting.

Plants were sprayed twice with freshly prepared solutions of ascorbic acid (0, 20, 40 ppm) Zn application was used in the form of Zinc sulphate $Zn\ SO_4.\ 7H_2O$ as foliar spray at (0, 20, 40 ppm), interaction treatments of the different concentrations of the two factors had been also carried out, in addition to the untreated plants (control) which were sprayed with tap water. Foliar application of ascorbic acid and Zn was carried out two times of 30 days intervals, starting at the first week of April at both seasons. The experiments were sit in a Completely Randomized Design (CRD) with three replicates, two factors; ascorbic acid (0, 20, 40) and Zn (0, 20, 40) concentrations and their interactions. Other agricultural Processes were performed according to normal practice.

At the first week of March 2006 and 2007, the following data were recorded: plant height (cm), stem diameter (mm), number of branchlets, root length (cm), fresh and dry weights (gm) of plant organs. Total soluble sugars were determined in the methanolic extract by using the phenol-sulphoric method according to Dubois *et al.* [18], photosynthetic pigments including Chlorophyll (a and b) as well as carotenoids content were determined in fresh branchlets as mg/gm fresh weight, according to the procedure achieved by Saric *et al.* [19]. The total indoles were determined in the methanolic extract, using p-dimethyl aminobenzaldhyde test "Erlic's reagent" according to Larsen *et al.* [20] and modified by Salim *et al.* [21]. Total soluble phenols were determined colourimetrically by using Folin Ciocaltea reagent A.O.A.C [22], Nitrogen content was determined according to the method described by Cottenine *et al.* [23]. Zn was measured using the atomic absorption spectrophotometer model Perkin Elmer 3300 Chapman and Pratt [24]. A minimum of three representative 100 gm samples of fresh shoots, weighed, homogenized and finally extracted with N- hexane solvent which was added to cover the sample…etc.

The extracted essential oil was kept in freezer to be ready to calculate oil percent according to Badawy *et al.* [25].

The data were statistically analyzed using L.S.D test according to Steel and Torrie [26].

RESULTS AND DISCUSSION

Effect of foliar application of ascorbic acid and Zinc on growth: Data presented in Table 1 indicated that all growth characters increased by foliar application of ascorbic acid at 20 and 40 ppm, the highest values of plant height, stem diameter, number of branchlets and root length were obtained at 40 ppm ascorbic acid. Application of ascorbic acid 40 ppm increased fresh weight of shoots and roots by 25.52 and 16.62% respectively than the control plants. These results are on line with those obtained by Tarraf *et al.* [9] on Lemon grass. Smirnoff [27] and Tarraf *et al.* [9] mentioned that ascorbate has been implicated in the regulation of cell division. In this connection, the author pointed out cell wall ascorbate and cell wall localized ascorbate oxidase has been implicated in content of growth, high ascorbate oxidase is associated with rapidly expanding cells. Shaddad *et al.* [28] assumed that the effect of ascorbic acid on plant growth may be due to substantial role of ascorbic acid in many metabolic and physiological processes.

Results in Table 1 show that Zinc 40 ppm application significantly increased all tested morphological parameters compared with those obtained by low level and untreated control plant. Data on the response of all growth characters as well as fresh weight of shoots and roots of the plant to Zinc 40 ppm indicate that there were significant increases in the aforementioned characters. Fresh weights of shoots and roots were exceeded by 12.51 and 8.03% respectively than the control plant. Similar results were obtained by El-Mansi *et al.* [17], whereas root length decreased by 2.53% due to Zinc 20 ppm.

Zinc plays an important role in plant physiology, where it activates some enzymes, Rains [12], Moore & Partrich [13] and Romheld and Marschner [14] reported that Zn functions are related to metabolism of carbohydrates and proteins, which are essential for multiple processes, critical to development and differentiation of cells [16]. Concerning the effect of interaction between ascorbic acid and Zinc application, it was noticed that ascorbic acid 40 ppm combined with Zinc 40 ppm followed by ascorbic 40 ppm+Zinc 20 ppm, Asc. 20 ppm+Zinc 40 ppm and Asc. 20 ppm +Zinc 20 ppm significantly increased all growth characters, i.e. plant height, stem diameter, number of branchlets, root length as well as fresh weight of shoots which exceeded by 45.86, 36.48, 24.36 and 20.19% respectively than the corresponding values of the control plants, whereas data show insignificant effect of the aforementioned treatments on fresh weight of roots.

Effect of ascorbic acid and zinc on chemical constituents

Pigment content: According to Table 2 it was found that the leaves content of three photosynthetic pigments Chlorophyll (a and b) and carotenoids were gradually increased as the concentrations of ascorbic acid and Zinc were increased to 40 ppm. The highest mean levels of Chlorophyll contents were obtained at the highest dose (40 ppm) of used treatments, in both seasons. These results are in agreement with those reported by Smirnoff [27] on the function and metabolism of ascorbic acid. Bokhina *et al.* [8] stated that ascorbic acid has a wide range of important functions as antioxidant defense, photoprotection and regulation of photosynthesis and growth while the Chlorophyll contents of cupressus leaves obtained in response to Zn were in agreement with those showed by Massoud *et al.* [11]. Regarding the beneficial

Table 1: Effect of foliar application of ascorbic acid and Zinc on the growth of *Cupressus sempervirens* L. plants (average of the two seasons 2006 and 2007)

Treatments	Plant height (cm)	Stem diameter (mm)	Number of branchlets	Root length (cm)	Fresh weight of shoots (gm)	Dry weight of shoots (gm)	Fresh weight of roots (gm)	Dry weight of roots (gm)
Effect of ascorbic acid								
Control	58.40	7.13	52.07	34.81	60.30	22.96	25.39	12.36
Asc. 20 ppm	67.77	8.26	55.78	38.80	67.76	25.77	27.42	13.42
Asc. 40 ppm	67.19	9.14	61.19	42.39	75.69	28.98	29.61	15.84
LSD at 5%	1.64	0.36	2.00	1.62	2.09	1.58	1.11	1.08
Effect of Zinc								
Control	58.88	7.96	51.49	37.83	63.61	23.47	26.12	12.28
Zn 20 ppm	69.56	7.99	56.87	36.87	68.57	25.84	28.08	14.13
Zn 40 ppm	73.92	8.59	60.68	41.30	71.57	28.39	28.22	15.21
LSD at 5%	1.64	0.36	2.00	1.62	2.09	1.58	1.11	1.08
Effect of interaction								
Control	49.93	6.40	47.63	32.33	56.10	31.50	29.20	10.60
Asc. 20 ppm	61.10	8.17	52.77	41.20	66.07	26.17	27.43	12.17
Asc. 40 ppm	65.60	9.30	54.07	39.97	68.67	25.90	28.33	14.07
Zn 20 ppm	60.07	6.27	53.10	31.77	61.70	29.53	31.30	11.40
Zn 40 ppm	65.20	8.73	55.47	40.33	63.10	27.27	28.63	15.07
Asc. 20+Zn 20ppm	69.37	8.77	54.03	36.80	67.43	26.40	26.83	13.60
Asc. 20+Zn 40ppm	72.83	7.83	60.53	38.40	69.77	21.83	25.50	14.50
Asc. 40+Zn 20 ppm	79.23	8.93	63.47	42.03	76.57	23.87	26.20	17.40
Asc. 40+Zn 40 ppm	83.73	9.20	66.03	45.17	81.83	20.63	23.83	16.07
LSD at 5%	2.83	0.62	3.47	2.81	3.62	N.S.	N.S.	1.87

Ascorbic acid: Asc., Zinc : Zn

Table 2: Effect of foliar application of ascorbic acid and Zinc on chemical constituents of *Cupressus sempervirens* L. plants (average of the two seasons 2006 and 2007)

	Chlorophylls as mg/gm F. W.				
Treatments	Chl (a)	Chl (b)	Total chl (a + b)	Carotenoids mg/gm	Total soluble sugars mg/gm
Effect of ascorbic acid					
Control	0.26	0.20	0.46	0.16	0.45
Asc. 20 ppm	0.35	0.24	0.59	0.19	0.65
Asc. 40 ppm	0.38	0.26	0.64	0.20	0.46
LSD at 5%	0.01	0.02	0.02	0.03	0.02
Effect of Zinc					
Control	0.31	0.20	0.52	0.12	0.40
Zn 20 ppm	0.33	0.24	0.56	0.21	0.63
Zn 40 ppm	0.36	0.26	0.62	0.23	0.79
LSD at 5%	0.01	0.02	0.02	0.03	0.02
Effect of interaction					
Control	0.22	0.19	0.41	0.27	0.17
Asc. 20 ppm	0.31	0.21	0.52	0.22	0.42
Asc. 40 ppm	0.39	0.21	0.60	0.11	0.61
Zn 20 ppm	0.27	0.18	0.45	0.23	0.51
Zn 40 ppm	0.30	0.22	0.52	0.22	0.68
Asc. 20+Zn 20ppm	0.35	0.27	0.62	0.19	0.74
Asc. 20+Zn 40ppm	0.38	0.25	0.63	0.17	0.80
Asc. 40+Zn 20 ppm	0.35	0.27	0.62	0.13	0.79
Asc. 40+Zn 40 ppm	0.40	0.30	0.70	0.12	0.98
LSD at 5%	0.02	0.03	0.04	N.S.	0.03

Ascorbic acid: Asc. Zinc : Zn Fresh Weight: F. W.

effect of Zinc on photosynthetic pigments, it may be due to its role in increasing the rates of photochemical reduction [29], chloroplast structure, photosynthetic electron transfer as well as photosynthesis [14]. In this respect, El-Kabbany [30] indicated that Zn increased Chlorophyll content of wheat leaves. Concerning the effect of interaction between ascorbic acid and Zinc application, all the interaction of used treatments increased significantly photosynthetic pigments Chlorophyll (a and b) and carotenoids in the leaves of *Cupressus sempervirens* L. plant.

Table 3: Effect of foliar application of ascorbic acid and Zinc on chemical constituents of *Cupressus sempervirens* L. plants (average of the two seasons 2006 and 2007)

Treatments	Total indoles mg/g F. W.	Total soluble phenols mg/g FW	Mineral ions as Total N %	Mineral ions as Total Zn as ppm	Essential oil %
Effect of Ascorbic acid					
Control	2.25	3.75	0.34	58	2.58
Asc. 20 ppm	3.31	4.23	0.46	72	2.67
Asc. 40 ppm	3.13	4.31	0.71	128	3.04
LSD at 5%	0.6	0.09	-	-	0.05
Effect of Zinc					
Control	2.95	4.06	0.34	58	2.57
Zn 20 ppm	2.34	4.13	0.71	82	2.77
Zn 40 ppm	3.40	4.11	0.74	98	2.96
LSD at 5%	0.06	NS	-	-	0.05
Effect of interaction					
Control	2.15	3.46	0.34	58	2.18
Asc. 20 ppm	3.55	5.25	0.46	72	2.49
Asc. 40 ppm	3.16	3.46	0.71	128	3.03
Zn 20 ppm	1.89	3.35	0.71	82	2.57
Zn 40 ppm	2.70	4.45	0.74	98	2.98
Asc. 20+Zn 20ppm	2.62	4.17	0.52	72	2.83
Asc. 20+Zn 40ppm	3.77	3.27	0.63	92	2.69
Asc. 40+Zn 20 ppm	2.61	4.87	0.79	60	2.90
Asc. 40+Zn 40 ppm	3.73	4.61	0.81	72	3.20
LSD at 5%	0.11	0.15	-----	-----	0.09

Ascorbic acid: Asc., Zinc : Zn

Total soluble sugars content: According to Table 2 it was found that total soluble sugars content was significantly affected as a result of foliar application with different concentrations of ascorbic acid and Zinc application separately or collectively. Such increments might be attributed to the significant increase in photosynthetic pigments content which reflect on photosynthesis process and led to an increase in carbohydrates content of cupressus plant. These results are in accordance with those obtained by Tarraf *et al.* [9] on lemon grass and Romheld and Marschner [14]. As for the interaction between ascorbic acid and Zinc application, the higher values were obtained by Asc. 40 ppm + Zn 40 ppm, followed by Asc. 20 + Zn 40 ppm, Asc. 40 ppm + Zn 20 ppm and Asc. 20 ppm + Zinc 20ppm, in total soluble sugars content of cupressus plant as compared with control plants. Talaat [31] on lavender stated that foliar application of ascorbic acid significantly increased total carbohydrate and total soluble sugars.

Total indoles: According to the data illustrated in Table 3 the total indole levels which were determined in branchlets of cupressus plant were highly significantly affected by the application of ascorbic acid at 20 and 40 ppm, it increased by 47.1 and 39.1% respectively than control plants. Zinc 40 ppm application significantly increased indoles content by 15.25%, whereas Zn 20 ppm decreased indoles content than control plants. The highest values of total indoles were obtained from interaction treated plants with ascorbic acid and Zinc, which exceeded by 75.34, 73.48 and 21.39% due to ascorbic acid 20 ppm combined with Zn 40 ppm followed by ascorbic acid 40 ppm + Zn 40 ppm and ascorbic 40 ppm + Zn 20 ppm, respectively than control plants.

Total soluble phenols: The results in Table 3 emphasized that amounts of total soluble phenols were significantly increased by ascorbic acid application at 40 and 20 ppm, it exceeded by 14.93 and 12.8% respectively. These results are in accordance with those obtained by Reda and Gamal El-Din [32] on chamomile than control plants. Data on the response of total soluble than control plants after phenols content to the interaction of ascorbic acid and Zinc, it exceeded by 40.75, 33.23 and 20.52% due to ascorbic acid 40 ppm + Zn 20 ppm, ascorbic 40 ppm + Zn 40 ppm and ascorbic acid 20 ppm + Zn 20 ppm respectively than the control plants.

Mineral ions: It appears from the data in Table 3 that spraying cupressus plants with ascorbic acid and Zinc at all the used levels lead to an increase in total Nitrogen percent compared with control plants. These increments lead to higher proteins contents, which acted positively in cell division and cell elongation resulting in a higher growth production. These results are in agreement with those obtained by Tarnaf *et al.* [9] on lavander and Gamal El-Din and Reda [33], on wheat. At the same time, all the used treatments either separate or combined lead to significant increases in Nitrogen and Zinc content compared with control plants. The aforementioned treated plants lead to increased Zinc content in cupressus plant compared with control plants.

Oil content: Data presented in Table 3 show that oil percent in *Cupressus sempervirens* branchlets were significantly affected as a result of foliar application with different concentrations separately or collectively, the highest recorded oil percent in plants treated with ascorbic acid 40 ppm and Zinc 40 ppm, it significantly exceeded by 17.82 and 15.17% respectively than control plants. These results are in agreement with those obtained by Gamal El-Din [34] on sunflower plants, that ascorbic acid significantly increased plant growth and oil percentage of seeds. The highest values of oil percent in cupressus plant were obtained from ascorbic 40 ppm + Zn 40 ppm followed by Asc. 40 ppm + Zn 20 ppm, Asc. 20 ppm + Zn 20 ppm and Asc. 20 ppm + Zn 40 ppm, it increased significantly by 46.78, 33.02, 29.81 and 23.39% respectively than the corresponding values of the control plants.

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