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Growth and Chemical Constituents of *Cupressus sempervirens* L. Plant as Influenced by Kinetin and Iron Treatments at Nubaria

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Abstract: Pot experiment was carried out in two seasons (2005 and 2006) at Research and Production Station, Nubaria, National Research Centre, Dokki, Cairo, Egypt to study the effect of foliar application of kinetin (0, 20, 40 ppm) and iron (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 plants were significantly affected by application of the two factors which were used in this study. Foliar application of kinetin 40 ppm to cupressus plant significantly promoted plant height, stem diameter, number of branchlets, root length, fresh and dry weights of shoots and roots, as well as chemical constituents except total soluble phenols compared with control plants. Iron application increased stem diameter, number of branchlets, root length, fresh and dry weights of roots due to 40 and 20 ppm Fe, whereas Fe 20 ppm decreased plant height, fresh and dry weight of shoots as well as pigments content and total indoles than control plants in both seasons. 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 kinetin 40 ppm + Fe 40 ppm, it increased significantly all vegetative growth parameters, (chl a and b), total soluble phenols and essential oil percent, whereas decreased total indoles than control plants. Plants treated with kinetin 40 ppm + Fe 20 ppm significantly increased plant height, stem diameter, number of branchlets, root length, fresh and dry weight of shoots and roots as well as essential oil content, whereas decreased chl a, chl b, total indoles, total soluble phenols compared with control plants.

Key words: *Cupressus sempervirens* L. · kinetin (Ki) · iron (Fe)

INTRODUCTION

Cupressus sempervirens L., Family Cupressaceae, the Mediterranian cupresses is a species of cupressus native to the Mediterranean region in north Libya, south Greece, Turkey, Western Syria, Lebanon and western Jordan. It has been widely cultivated as an ornamental timber tree for its source of woods, shade and forage in different kinds of soils. Medicinal and windy resistance trees at California, south Africa, southern Australia and Italy, Chittendon [1], this species is known to have antiseptic, aromatherapy, astringent, balsamic and anti-inflammatory activities in the cones and young branches, Bean [2]. Polunin and Huxley [3] stated that the leaves contain about 2% essential oil, whereas the wood contains about 2.5%, it is used in perfume and soap making. 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 moths and impervious to wood warm. *Cupressus sempervirens* L. is a medium-sized evergreen tree, its length reached 35 m., with a conic crown, with level branches, it is very long lived to over 1000 years old. The foliage grows in dense sprays, dark green in colour, the leaves are scale-like 2-5 mm in length and produced on rounded shoots.

Cytokinins are important plant hormones that regulate various processes of plant growth and development, cytokinins appeared to play an important role in the regulation of cell division, differentiation and organogenesis in developing plants, enhancement of leaf expansion, nutrient mobilization and delayed senescence [4-6]. Shudo [7] reported that chemical structure of cytokinin-active substances has determined two groups of adnine cytokinins and urea cytokinins with similar physiological effects, it has pronounced effect on cotyledon growth and expansion and other processes.

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Karanov *et al.* [8] reported that application of kinetin on *Mentha spicata* and *Salvia officinalis* resulted an increases in total fresh weight including leaf weight and stem, kinetin affects plant growth. Runkova [9] revealed that it stimulated plant height, number of lateral branches on the main stem and number of leaves of some ornamental plants. The influences of cytokinins on the biosynthesis and accumulation of fixed oil and fatty acids were studied by many investigators, Vasudevan *et al.* [10] reported that spraying three sunflower cultivars with cytokinin produced the highest seed oil content.

The 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] Iron is among the essential micronutrients needed for better plant growth and high quality, Hussein *et al.* [12] on *Hibiscus* sp., who reported that Fe at 25 ppm stimulated growth characters. Misra and Srivastava [13] on *Mentha arvensis* L. reported that plant grown under iron deficiency had smaller leaves with smaller mesophyll cells compared with plants given sufficient iron, Liethy [14] on *Nigella sativa* added that application of iron increased plant height, number of leaves, fresh and dry weight/plant through its effect on enhancement of cell division and/or cell enlargement. The same author stated that application of iron increased chlorophyll contents and total carbohydrate.

Azza and El-Mesriy [15] on *Foeniculum vulgare* showed that iron application gave the highest values of N, P, K, Zn, Mn and Fe percentage in treated plants compared with control plants.

The aim of the present work is enhancing the vegetative growth of *Cupressus sempervirens* L. by foliar application with kinetin and iron, beside their effects on chemical constituents and essential oil content at Nubaria region, Egypt.

MATERIALS AND METHODS

The present investigation was carried out at 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 kinetin and iron on growth and chemical composition of *Cupressus sempervirens* L.

One year old seedlings of Cupressus were obtained from nursery of Forestery Department/Horticultural Research Institute. In the Agricultural Research Centre, the seedlings were planted on the first week of January during the two successive seasons 2005 and 2006 in plastic pots 30 cm. in diameter, filled with 10 kg of peatmoss and sandy soil (1:1 by v/v), one plant/pot, the average heights of seedlings were 15-20 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^{-1} in four doses after 4, 8, 16 and 20 weeks from transplanting.

Plants were sprayed twice with freshly prepared solutions of kinetin (0, 20, 40 ppm), Fe application used was ferrous sulphate as foliar spray at (0, 20, 40 ppm), interactions 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 kinetin and Fe was carried out two times of 30 days intervals, starting at the first week of February at both seasons. The experiments were sit in a Completely Randomized Design (CRD) with three replicates, two factors kinetin (0, 20, 40 ppm.) and iron (0, 20, 40 ppm.) concentrations and their interactions, all other horticulture practices were done as needed.

At the first week of January 2006 and 2007, the following data were recorded: plant height (cm), stem diameter (mm), number of branchlets, root length (cm), fresh and dry weights (g) of plant organs. Total soluble sugars were determined in the methanolic extract by using the phenol-sulphoric method according to Dubois *et al.* [16], photosynthetic pigments: including chlorophyll (a and b) as well as carotenoid contents were determined in fresh branchlets sample as mg mg^{-1} fresh weight, according to the procedure achieved by Saric *et al.* [17]. The total indoles were determined in the methanolic extract, using P. dimethyl amino benzaldhyde test "Erlic's reagent" according to Larsen *et al.* [18] and modified by Salim *et al.* [19]. Total soluble phenols were determined colourimetrically by using Folin Ciocaltea reagent AOAC [20]. Nitrogen content was determined according to the method described by Cottenine *et al.* [21]. Iron was determined by Atomic Absorption described by Chapman and Pratt [22]. A minimum of three representative 100 g samples of fresh shoots, weighed and 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.* [23].

The data were statistically analyzed test according to Steel and Torrie [24] and the treatments means were compared using LSD test.

RESULTS AND DISCUSSION

Effects of foliar application of kinetin and iron on growth: The results in Table 1 show that all growth characters increased by foliar application of kinetin at 40 and 20 ppm, the highest values of plant height, stem diameter, number of branchlets and root length were obtained at 40 ppm kinetin, whereas 20 ppm kinetin decreased number of branchlets. Application of kinetin at 40 ppm increased fresh and dry weights of shoots and roots by 7.94 and 6.41% respectively than the corresponding values of the control plants. Whereas 20 ppm kinetin decreased fresh weight of shoots by 1.38% and increased significantly fresh weight of roots by 18.42%. these results are in accordance with those obtained by Al-badawy *et al.* [25] on *Matricaria chamomilla* plants. Runkova [9] on some ornamental plants and Youssef *et al.* [26] they found that spraying *Matthiola incana* plants with kinetin at the rate of 20 or 40 ppm led to significant increases in all growth parameters. In this work, the increments in all studied parameters may be attributed to the effect of the used treatments on cell division and/or cell elongation [4, 14].

From the same table, data show that the highest increases in plant height, stem diameter, number of branchlets and root length were found in plants treated by Fe 40 and 20 ppm compared with control plants, whereas 20 ppm Fe decreased plant height, the increments of the aforementioned characters may be due to the positive effect of Fe on enhancement of cell division and/or cell enlargement, deficiency of iron inhibits cell division [12, 14] on *Hibiscus* sp. and *Nigella sativa*, respectively. Iron spraying could be explained in the light of its role in the oxidation or reduction in respiration and photosynthesis and hence, for that a marked effect on photosynthetic efficiency. Data presented in Table 1 show that application of Fe 40 ppm caused an increase in fresh weight of shoots and roots by 3.29 and 42.63%, respectively compared with control plant. The role of Fe enhancing the synthesis of chlorophyll and protein for optimum growth as well as increasing the enzyme systems activity, Fe enhancing the metabolism of carbohydrates and protein as well as the enzyme systems, consequently the vegetative growth, the fresh and dry weights of leaves. These results are on line with those obtained by Abd El-Salam and Inas [27].

Table 1: Effect of foliar application of kinetin and iron on the growth of *Cupressus sempervirens* L. plants (Means of the two seasons 2006 and 2007)

Treatments	Plant height (cm)	Stem diameter (mm)	Number of branchlets	Root length (cm)	Fresh weight of shoots (g)	Dry weight of shoots (g)	Fresh weight of roots (g)	Dry weight of roots (g)
Effect of kinetin								
Control	64.57	6.46	57.44	36.66	52.6	18.68	13.24	6.06
Ki 20 ppm	67.54	6.71	54.22	37.07	51.87	17.73	15.68	6.89
Ki 40 ppm	76.18	7.2	65.11	38.79	56.78	19.81	14.09	7.08
LSD at 5%	2.95	0.56	5.78	1.6	2.84	1.34	1.38	0.68
Effect of iron								
Control	70.28	6.23	55.78	36.65	53.43	18.71	11.54	6.19
Fe 20 ppm	67.09	7.1	63.22	37.3	52.62	17.9	15.01	6.48
Fe 40 ppm	70.92	7.03	57.78	38.57	55.19	19.61	16.46	7.36
LSD at 5%	2.95	0.56	5.78	NS	NS	1.34	1.38	0.68
Effect of interaction								
Control	53.67	5.63	50.33	33.5	46.53	16.03	8.73	3.63
Ki 20 ppm	79.73	6.73	56.33	36.13	58.03	20.5	12.47	6.83
Ki 40 ppm	79.37	6.33	60.67	40.33	55.73	19.6	13.43	8.1
Fe 20 ppm	70.57	6.43	59.67	37.8	54.9	18.13	14.2	6.47
Fe 40 ppm	69.47	7.3	62.33	38.67	56.37	21.87	16.8	8.07
Ki 20 + Fe 20 ppm	59.97	7.33	53.67	38.37	47.8	16.2	16.67	6.67
Ki 20 + Fe 40 ppm	62.93	6.07	52.67	36.73	49.77	16.5	17.9	7.17
Ki 40 + Fe 20 ppm	70.73	7.53	76.33	35.73	55.17	19.37	14.17	6.3
Ki 40 + Fe 40 ppm	78.43	7.73	58.33	40.3	59.43	20.47	14.67	6.83
LSD at 5%	5.12	0.97	10	2.78	4.92	2.32	2.39	1.17

Kinetin: Ki, Iron: Fe

Table 2: Effect of foliar application of kinetin and iron on chemical constituents of *Cupressus sempervirens* L. plants (Means of the two seasons 2006 and 2007)

	Chlorophylls as mg g^{-1} fresh weight				
Treatments	Chl (a)	Chl (a)	Total chl (a+b)	Carotenoids (mg g^{-1})	Total soluble sugars (mg g^{-1})
Effect of kinetin					
Control	1.6	5.42	7.02	0.15	0.37
Ki 20 ppm	1.54	4.7	6.24	0.14	0.56
Ki 40 ppm	1.61	5.72	7.33	0.14	0.8
LSD at 5%	0.054	NS	NS	NS	0.02
Effect of iron					
Control	1.63	5.64	7.27	0.15	0.35
Fe 20 ppm	1.46	4.48	5.95	0.14	0.36
Fe 40 ppm	1.65	5.85	7.5	0.14	0.78
LSD at 5%	0.054	NS	NS	NS	0.02
Effect of interaction					
Control	1.55	5.19	6.74	0.15	0.19
Ki 20 ppm	1.64	6.02	7.66	0.16	0.27
Ki 40 ppm	1.77	5.72	7.49	0.14	0.61
Fe 20 ppm	1.61	5.96	7.57	0.15	0.25
Fe 40 ppm	1.65	5.21	6.86	0.15	0.66
Ki 20 + Fe 20 ppm	1.42	4.06	5.48	0.14	0.69
Ki 20 + Fe 40 ppm	1.55	4.04	5.59	0.11	0.74
Ki 40 + Fe 20 ppm	1.33	3.43	4.76	0.13	0.88
Ki 40 + Fe 40 ppm	1.72	5.4	7.12	0.15	0.93
LSD at 5%	0.094	1.66	1.94	0.026	0.03

Kinetin: Ki, Iron: Fe

Hence only through interaction like those reported above kinetin can play an important role in the regulation of cell division, differentiation and enhancement of leaf expansion [5], in addition to iron is the among essential micronutrients needed for plant growth and high quality [12], the highest increases in plant height, stem diameter, number of branchlets, root length, fresh and dry weights of shoots were found in plants treated with 40 ppm kinetin combined with 40 ppm Fe, it significantly increased by 46.13, 37.30, 15.89, 20.29, 27.72 and 68.04% respectively than the corresponding values of the control plants, while 40 ppm kinetin + 20 ppm Fe increased significantly number of branchlets, fresh weight of shoots and roots by 51.65, 18.65 and 62.81% respectively than control plants.

Effect of kinetin and iron on chemical constituents

Pigment content: Data presented in Table 2 show that foliar application of kinetin 40 ppm significantly affected chl (a), chl (b) content of cupressus branchlets compared with control plant. These results were in accordance with those obtained by Youssef *et al.* [26] on Matthiola incana L. this phenomenon may be due to the enhancement of leave expansion, nutrient mobilization and chlorophyll content [4, 5], foliar application of Fe 40 ppm significantly increased chl (a) content, whereas it had insignificant effect of chl (b) content of cupressus branchlets as compared with control plant. These results are in line with those obtained by leithy [14] on *Nigella sativa* L. Considering the interaction, foliar application with kinetin 40 ppm combined with iron 40 ppm gave the highest value of chl (a) and chl (b) content of cupresses branchlets as compared with control plant.

Total soluble sugars: Data in Table 2 show that total soluble sugars content significantly increased in plants treated with the two concentrations of kinetin and iron compared with control plant. The positive effect of Fe on enhancing the total soluble sugar may be due to the importance in contribution to direct the enzymes way in metabolism [11]. As for the interaction between kinetin and iron application the higher values obtained by kinetin 40 ppm + Fe 40 ppm followed by kinetin 40 ppm + Fe 20 ppm, kinetin 20 ppm + Fe 40 ppm and kinetin 20 ppm + Fe 20 ppm, in total soluble sugars content of cupressus plant as compared with control plant.

Total indoles: According to the data illustrated in Table 3 the total indole levels were determined in branchlets of cupressus plant were highly significantly affected by the application of kinetin at 20 and 40 ppm, it

Table 3: Effect of foliar application of kinetin and iron on chemical constituents of *Cupressus sempervirens* L. plants (Means of the two seasons 2006 and 2007)

Treatments	Total indoles mg g^{-1} FW	Total soluble phenols mg g^{-1} FW	Mineral ions as Total N (%)	Mineral ions as Fe as ppm	Essential oil (%)
Effect of kinetin					
Control	2.4	4.8	0.59	170	2.64
Ki 20 ppm	3.74	4.28	0.95	548	2.66
Ki 40 ppm	3.04	4.67	1.17	622	2.77
LSD at 5%	0.33	0.22	-	-	2.46
Effect of iron					
Control	3.94	4.34	0.59	170	2.65
Fe 20 ppm	2.79	4.64	0.83	204	2.61
Fe 40 ppm	2.45	4.76	1.14	246	2.63
LSD at 5%	0.33	0.22	-	-	NS
Effect of interaction					
Control	3.39	4.69	0.59	170	2.36
Ki 20 ppm	4.31	4.32	0.95	548	2.78
Ki 40 ppm	4.11	4.03	1.17	622	2.8
Fe 20 ppm	1.93	5.38	0.83	204	2.42
Fe 40 ppm	1.87	4.32	1.14	296	2.6
Ki 20 + Fe 20 ppm	3.36	4.33	0.71	392	2.63
Ki 20 + Fe 40 ppm	3.56	4.2	0.74	194	2.57
Ki 40 + Fe 20 ppm	3.07	4.2	1.02	290	2.77
Ki 40 + Fe 40 ppm	1.92	5.77	0.98	486	2.73
LSD at 5%	0.58	0.39	-	-	0.18

Kinetin: Ki, Iron: Fe

increased total indoles by 55.83 and 26.66%, respectively than control plants. The highest values of total indoles were obtained at interaction treated plants with kinetin and iron, which exceeded by 5.01% due to ki 20 ppm + Fe 40 ppm than control plants. Whereas Fe application at 40 and 20 ppm produced lower values which were less than control plants by 3.78 and 29.18%, respectively than control plants.

Total soluble phenols: The results in Table 3 emphasized that the amounts of total soluble phenols were highly significantly influenced by iron application at 40 and 20 ppm, it increased by 9.67 and 6.91%, respectively than control plant. Data on the response of total soluble phenols content to the interaction of kinetin and iron significantly increased by 23.02% due to ki 40 ppm + Fe 40 ppm, than that of control plants. This strongly leads to the conclusion that application of iron and the interaction of kinetin and iron had an inductive effect on increasing the concentration of total soluble phenols. These results are in agreement with those obtained by Essam [28] on *Acacia saligna* L.

Mineral content: Regarding the effect of kinetin and iron foliar application on nitrogen concentration of cupressus plant was gradually increased by increasing the concentrations of kinetin and iron to 40 ppm compared with control plant, these increments lead to quantitative and qualitative changes in proteins content which acted positively in cell division and cell elongation resulting in addition to vegetative growth. These results are in line with those obtained by Bekhata and Mahgoub [29]. Concerning the interaction, foliar application with kinetin 40 ppm + Fe 20 ppm followed by ki 40 ppm + Fe 40 ppm were lead to significant increase in this criterion. As regarding that spraying cupressus plants with kinetin and iron at all the used levels lead to increases in Fe content compared with control plants. These increments led to positive effects on growth parameters and increased total nitrogen and Fe percent. In relation to the effect of iron concentrations on the percentage of the previous mineral in the leaves were gradually increased by increasing Fe level, these increments due to enhancing effects of Fe on the absorption and/or translocation of those minerals. Such phenomenon may be due to its effect on enhancing the plant metabolism [30].

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 of individual kinetin and iron or

collectively with iron treatment. The highest recorded oil percentage in plants treated with kinetin 40 and 20 ppm, it significantly exceeded by 12.60 and 8.13%, respectively than control plants.

These results are in line with those obtained by Vasudevan *et al.* [10] who reported that spraying three sunflowers cultivars with cytokinin produced the highest seed oil content, whereas Fe 40 and 20 ppm it produced less oil content by 0.75 and 1.50% due to Fe 40 and Fe 20 ppm, respectively than control plants.

The interactions application of the two factors, the highest values of oil percentage of cupressus were obtained from ki 40 ppm combined with Fe 20 ppm, followed by ki 40 ppm + Fe 40 ppm, ki 20 ppm + Fe 20 ppm and ki 20 ppm + Fe 40 ppm, it increased significantly by 17.37, 15.6, 11.44 and 8.89%, respectively than the corresponding values of the control plants.

From the above mentioned results, it could be concluded that foliar application of kinetin 40 ppm combined with Fe at 40 or 20 ppm promoted growth parameters and possessed the best oil percentage in *Cupressus sempervirens* L. plant.

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