

PHYSIOLOGICAL EFFECT OF SOME GROWTH REGULATORS AND MICRONUTRIENTS ON THE GROWTH AND CHEMICAL CONSTITUENTS OF (*Lavandula Officinalis*, L.) PLANT

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ABSTRACT

A pot experiment was conducted in the screen of the National Research Centre to study the effect of foliar application of kinetin, gibberellic acid and micronutrients on the growth and chemical constituents of *Lavandula officinalis* L. plant. The obtained results indicated that plant height, number of branches, fresh and dry weights of herb were significantly promoted as a result of foliar application of kinetin, gibberellic acid and microelements or their combinations influenced the vegetative growth of Lavender plants especially when plants sprayed with Ki (40 mg/L) and GA₃ (100 mg/L) and with these combinations of microelements (Fe, Mn and Zn) 0.15 %. These effects increased chemical constituents and essential oil yield per plant. The most promising results were obtained from plants treated with kinetin (40 mg/L) combined with microelements and meanwhile, the best oil qualities, i.e., having low content of 1,8-cineole and camphor and high content of linalool and linalyl acetate were obtained from the same treatments.

INTRODUCTION

Nowdays, considerable attention has been paid to cultivate, the aromatic and medicinal plants in Egypt which considered in this time as one of the most important sources of income and also the main sources of natural compounds instead of the synthetic products. Most of the essential oils of aromatic plants are rich in aromatic, flavouring chemicals and colored materials which are used in cosmetic, perfume, pharmaceutical purposes and food industries (Duke, 1985).

Lavender (*Lavandula officinalis* L.) is one of the most important aromatic plants grown in Egypt. Oil extracted from Lavender plants is one of the most important volatile oils. Which is widely used for the scenting of soaps. It is used in many cosmetics and technical preparations, both salts room sprays and disinfectants (Guenther, 1973).

Plant growth regulators are chemicals which originally regulate growth and development processes by altering the endogenous hormones level. They are responsible for the most metabolic processes of cell division, elongation and differentiation and they could be expected to influence the biosynthesis of essential oils in aromatic plants.

Cytokinins, being a group of growth regulating substances, which play an important roles in the regulation of cell division, differentiation of the cultures, and organogenesis in developing plants (Skoog and Armstrong, 1970 and Hall, 1973) and also influence on the biosynthesis and accumulation of monoterpene components of essential oils in the aromatic plants Shedeed et

al., 1990 reported that kinetin treatments increased the oil percentage and yield of *Mentha piperita* L. plants.

Gibberellic acid (GA₃) is the most famous growth regulator. It has many effects on plant growth phenomena such as germination, vegetative growth and flowering and the well known promotive effect of GA₃ on stem elongation. The response of aromatic plants to GA₃ application was reported by many investigators, El-Khateeb (1989) found that GA₃ at all rates (50-200 ppm) markedly increased plant height, fresh and dry weights of *Rosmarinus officinalis* L.

Sharma *et al.* (1988) on *Mentha citrate* and Shedeed *et al.* (1990) on basil stated that treating these plants with GA₃ increased plant growth and their yield.

Micronutrients are considered as one of the main important practices that can increase the productivity and quality of plants (Lindsay, 1972). Foliar application is widely used to provide micronutrients, or to compensate the deficiency of a specific micronutrients. Their deficiencies cause great disorders in the physiological and metabolic processes of the plants, Kanwer and Dhingra (1962). Fe, Mn and Zn were reported to stimulate the growth of various plants due to their enhancement effect on most metabolic processes Price *et al.*, 1972.

Little work concerning the effect of growth regulators combined with micronutrients on the aromatic plants, so, the present work, was carried out to study the effect of foliar application from different concentrations of kinetin (0, 20, 40 mg/L.) gibberellic acid (0, 50, 100 mg/L) and in combination with micronutrients (Fe, Mn and Zn) 0.15 % on the vegetative growth and essential oil production as well as some metabolic constituents of lavender plants.

MATERIALS AND METHODS

Pot experiment had been conducted at the experimental Farm of National Research Centre, Dokki, Cairo during two successive seasons of (1998/1999-1999/2000). Two uniform cuttings of Lavender were planted in each pot (30 cm diameter) on 11th and 14th November for the two successive seasons respectively.

All plants were irrigated as needed and each pot was fertilized twice with 1 gm ammonium nitrate (33.5 %). 2 gm calcium superphosphate (15.5 % P₂O₅) and 1 gm potassium sulphate (48 % K₂O) . Fertilization was applied after 2 months from planting.

Plants were foliarly sprayed with kinetin (0, 20, 40 mg/L) gibberellic acid (0, 25, 50 mg/L) and interaction treatments with the different concentrations, had been also carried out with microelements (0.15 %) mixture (Fe, Mn and Zn). The plants were cut two times the first cut of herb was on 8th and 11th June and the second cut was collected at 12th and 17th Nov. in the two successive seasons, respectively.

The experimental design used was complete randomized with three replicates each replicate represented by three pots. The volume of the spraying solution was maintained Just to cover completely the plant foliage till drip.

The plant herbage was cut above 10 cm from above the soil surface and plant growth characters in terms of plant height, number of branches and fresh and dry weight of the herb were recorded.

Total carbohydrates in the dry herb were determined according to Dubois *et al.*, (1956). Also, total nitrogen was determined using the modified micro-kjeldahl method according to A.O.A.C. (1980). While essential oil percentage in the fresh herb of each treatment was determined by hydro-distillation according to the Egyptian Pharmacopoeia (1984).

Qualitative and quantitative determination of the different main constituents of lavender oils obtained from the first cut from each treatment had been carried out in parallel with authentic samples of different oil components by GLC, technique. The qualitative identification of the main oil fractions was carried out by comparing the relative retention time of different peaks with those of the pure authentic samples. The quantitative determination was achieved by the peak area percentage, which was measured for each fraction : to study the changes in the constituents of lavender oil as a result of the effect of different treatments applied .

For this purpose gas liquid chromatographic apparatus (VARIAN-3700) equipped with FID. Hp4270 Integrator was used for the separation of lavender oil fractions of the samples. The analysis conditions were as follows : The chromatography was fitted with (2 m.x 1/8) columns peaked with Diatomic G.Hb. (100-120) mesh and coated with 10 % DEGS. 12Ft-SS. The columns were operated using a temperature program a linear increase with rate of 4°C/min. from 70°C to 190°C) : with nitrogen at 30 ml. min. as a carrier gas the flow rates for hydrogen and air were 30 and 3000 ml/min. respectively.

Detector temperature was 280°C. Chart speed was 0.5 cm/min. range : 32 : sample size was about 2 ml. sensitivity of the apparatus was 18 X 32. The standard material was injected with the samples of Lavender oil under the same conditions.

Data obtained (mean of the two growing seasons), were subjected to standard analysis of variance procedure. The values of L.S.D. were calculated at 5 % level according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Effect on plant growth, carbohydrate and nitrogen content :

I. Effect of kinetin :

Kinetin at (20 and 40 mg/L) significantly increased plant height, number of branches and fresh and dry weights of herb in both cuts as compared to the untreated plants (Table 1) Foliar application of kinetin at 40 mg/L gave the highest significant increase in plant growth. The biochemical parameters Table (2) have been studied indicated the same trend obtained with vegetative growth, i.e. kindin at 40 mg/L significantly increased total carbohydrates, nitrogen and volatile oil percent as well as oil yield of herb.

Cytokinins appeared to play an important roles in the regulation of cell division, differentiation and organogenesis in developing plants (Skoog and Armstrong, 1970 and Hall, 1973).

Table (1) : Effect of kinetin, gibberellic acid and micronutrients on the growth of lavender (*Lavandula officianlis* L.) plants. (mean of two seasons)

Treatment mg/L.	Plant height (cm)		No. of branches		Fresh wt. of herb (g)		Dry wt. of herb (g.)	
	First cut	Second cut	First cut	Second cut	First cut	Second cut	First cut	Second cut
Control	30.23	26.12	6.12	5.33	63.71	41.14	14.52	10.82
Ki 20 mg/L.	37.56	28.14	11.33	7.14	72.14	51.31	25.30	19.12
Ki 40 mg/L.	42.12	33.21	13.22	8.12	81.54	60.33	27.31	21.25
GA ₃ 50 mg/L.	35.56	32.11	10.33	9.12	77.19	57.12	23.21	17.22
GA ₃ 100 mg/L.	38.13	30.51	11.65	10.12	89.22	67.51	26.12	21.13
Microelements (M)	31.14	27.14	7.15	6.11	65.31	39.33	15.57	11.38
Ki 20 mg/L. + M	39.14	35.13	13.22	11.51	74.33	58.42	26.22	22.13
Ki 40 mg/L. + M	47.16	40.13	16.17	13.22	84.32	62.31	28.14	23.14
GA ₃ 50 mg/L. + M	39.22	32.12	14.21	11.33	79.28	58.17	24.53	19.80
GA ₃ 100 mg/L.+M	40.13	34.33	15.13	11.21	91.12	64.17	26.14	21.12
L.S.D. at 5 % level	0.78	0.62	0.72	0.51	4.83	4.22	1.03	1.22

Application of kinetin at concentrations of 1-4 ppm on *Mentha piperita* and *Salvia officinalis* resulted in a 30-60 % increase in total fresh weight compared to control, 40 % increase in leaf weight and 10 % in the number of branches (El-Ketawi and Croteau, 1987).

The essential oil yield of cytokinin treated plants is also increased up to two folds on fresh weight relative to controls, with only a minor influence on oil composition in most cases (El-Keltawi and Croteau, 1987). They added that the increase in oil yield in *Mentha piperita* under the influence of cytokinin is a result of increase monoterpene biosynthesis.

II. Effect of gibberellic acid :

Data presented in Table (1) show that GA₃ treatments significantly promoted plant height, number of branches, fresh and dry weight of herb in both cuts. GA₃ at 100 mg/L resulted, in the tallest plants in most cases. These findings are in agreement with those obtained by El-Keltawi (1981) on spearmint plants, Harridy (1981) on *Calamintha officinalis* and Dessouky (1986) on *Vinca* sp.

The stimulatory effect of gibberellic acid (GA₃) on plant growth of lavender plants might be due to an increase in internode elongation which in turn was oftenly a consequence of increased cell wall extension. This might be explained on basis of striking increase in cell membrane permeability according to Wood and Paleg (1972), Vliotose and Most (1973) and Zee (1976). It may also be due to the stimulatory effect of GA₃ on auxin action as previously described by Kuraishi and Muir (1963).

Data presented in Table (2) show that foliar application of GA₃ to lavender plants; significantly increased total carbohydrates and total nitrogen % especially at 100 mg/L. Similar results were obtained by Abou-Leila *et al.* (1994) who reported that foliar spray of GA₃ at 75 mg/L increased carbohydrate content of basil plants. On the other hand, GA₃ increased the essential oil % and oil yield this could be attributed to GA₃ effect on metabolism and enzyme

levels responsible for mono or sesquiterpene biosynthesis as reported by Lawrence (1978). Furthermore, it was found that the enzymes responsible for the biosynthesis of higher terpenes arise in plastid as reported by Amelunxen and Arbeiter (1967) and these enzymes could cause an increase in the essential oil.

III. Effect of micronutrients :

It was evidence from the present data Table (1) that micronutrients treatments (Fe + Mn + Zn) slightly favoured the vegetative growth of *Lavandula officinalices* plants. This slight effect of micronutrients foliar application on plant height, number of branches and fresh and dry weight reported by several investigators, Abou-Zeid (1980) on *Ocimum* El-Sherbeny (1990) on *Trigonella foenumgraceum* L. and El-Sherbeny *et al.* (1988) on *Ocimum basilicum*. They reported that spraying the above mentioned plants with microelements had a slight effect on vegetative growth. This favorable effect on growth of lavender plants might be due to the enhancement of most of the metabolic processes as a result of micronutrients foliar application these treatments may extent their effect on biosynthesis of some metabolites required for the formation and elongation of internodes concluded by Agui *et al.* (1985) on *Phaseolus vulgaris*.

On the other hand data presented in Table (2) indicated that microelements foliar application significantly increased the oil % and oil yield, total carbohydrate and N. These results are in agreement with finding obtained by Abou-Zeid (1980) on *Ocimum basiliam* El-Halwagy (1981) on pelargonium and El-Sherbeny *et al.* (1988) on *Ocimum basilicum* they found that microelements led to more accumulation of volatile oily in

Table (2) : Effect of kinetin, gibberellic acid and micronutrients on the chemical constituents of lavender (*Lavandula officianlis* L.) plants. (mean of two seasons)

Treatment mg/L.	Oil %		Oil yield (ml/plant)		Total carbohydrate %		Total nitrogen %	
	First cut	Second cut	First cut	Second cut	First cut	Second cut	First cut	Second cut
Control	0.151	0.112	0.096	0.046	10.26	8.14	2.24	1.19
Ki 20 mg/L.	0.234	0.194	0.169	0.019	17.69	15.33	4.70	3.14
Ki 40 mg/L.	0.267	0.216	0.218	0.130	19.83	17.12	5.86	4.22
GA ₃ 50 mg/L.	0.212	0.178	0.164	0.105	14.22	12.14	3.80	2.12
GA ₃ 100 mg/L.	0.234	0.181	0.208	0.122	15.46	13.22	4.12	3.88
Microelements (M)	0.165	0.124	0.108	0.048	12.31	10.14	3.71	2.25
Ki 20 mg/L. + M	0.246	0.203	0.183	0.118	18.33	16.03	5.12	3.89
Ki 40 mg/L. + M	0.277	0.212	0.233	0.132	20.12	18.17	6.22	4.91
GA ₃ 50 mg/L. + M	0.233	0.183	0.184	0.106	15.24	13.65	4.71	3.24
GA ₃ 100 mg/L.+ M	0.251	0.192	0.228	0.123	16.33	14.21	5.33	4.19
L.S.D. at 5 % level	0.016	0.014	0.008	0.013	2.33	1.14	0.64	0.41

Ocimum and *Pelargonium* plants this was concluded also by Crane and Steward (1962) on *Mentha piprita* and El-Habbal and Mostafa (1983) on peanut and sesame found microelements raise the photosynthetic efficiency and increase metabolic products of plant such as carbohydrate and protein.

VI. Combined effect of growth regulators and micronutrients :

The present results revealed that the combined treatment of GA₃ and micronutrients (Fe + Mn + Zn) showed higher positive effect on morphological characters of lavender plant than the single treatments such as plant height, number of branches and fresh and dry weight and the highly positive effect was obtained from the combined treatment of GA₃ (100 mg/L) with microelements. These results are in agreement with the findings obtained by Aly (1988) on *Hyoscyamus muticus* L. plants, Abou-Zeid (1978) on *Ocimum*, they found the combined treatment of GA₃ + fulli-fertile increased vegetative growth and essential oil content than the single treatments. On the other hand, combined treatments of kinetin and micronutrient gave higher value than the single treatment especially will Ki 40 mg/L which increased both vegetative growth, oil % and carbohydrate content. The present result are in agreement with those obtained by Abou-Zeid (1978, 79, 80) on *Ocimum* plant, claimed that the combination of growth regulators; with microelements have more pronounced influence on the biosynthesis of pigments which increase photosynthetic which cause a highly stimulatory effect upon metabolic products.

Effect on essential oil components :

The oil of lavender herb from different treatments in addition to that of the untreated control were subjected to fractionation using G.L.C. presented in Table (3). The identified

Table (3) : Gas-Liquid chromatographic analysis of essential oil components of *Lavandula officinalis* L. plants as affected by Kinetin, Gibberellic acid and micronutrients.

Treatments Compound (%)	Control	Ki (20) mg/L	Ki (40) mg/L	GA ₃ (50) mg/L	GA ₃ (100) mg/L	Micro. (M)	Ki (20) mg/L + M	Ki (40) mg/L + M	GA ₃ (50) mg/L + M	GA ₃ (100) mg/L + M
a-Pinene	1.102	1.539	1.423	1.355	0.713	1.109	0.003	2.137	0.999	1.008
β-Pinene	1.015	0.511	1.486	2.622	0.750	1.020	0.242	1.934	1.526	1.578
α-Terpinene	4.887	trch	0.615	3.848	7.315	4.890	0.649	0.759	4.591	3.637
α-Caryophyllene	1.118	0.615	1.092	1.843	4.066	1.120	0.529	1.613	0.986	1.124
Sesquiterpenes	8.456	1.092	2.596	7.538	5.881	8.470	1.713	0.935	1.877	3.661
β-Bisablene	2.893	2.5964	1.842	5.466	5.433	2.899	2.818	4.455	3.491	3.738
1,8-cineol	29.461	46.931	46.931	25.715	29.691	29.466	5.954	23.536	35.081	31.090
Linalool	17.462	19.426	19.426	12.891	17.041	17.465	24.522	29.271	15.762	20.528
Camphor	8.167	4.001	4.001	0.509	9.070	8.170	6.156	6.175	9.861	8.801
Linalyl acetate	1.340	4.605	4.605	1.130	1.178	1.350	6.834	7.355	2.801	0.915
Geraniol	3.816	3.223	3.223	4.060	4.261	3.820	0.437	1.455	1.156	3.106
Lavandulylacetate	3.554	1.252	1.252	3.60	1.089	3.560	1.765	1.365	2.814	3.001
Geranyl acetate	1.566	0.214	0.214	3.057	1.814	1.569	0.326	0.678	2.376	3.164
Total hydrocarbon	19.470	9.052	9.052	22.241	24.161	19.480	46.322	11.831	13.477	4.752
Total oxyganted hydrocarbon	65.332	79.653	79.653	54.771	64.154	65.339	86.361	69.837	70.461	70.604
Unknown	15.191	11.291	11.291	22.991	11.680	15.196	7.684	18.333	16.06	14.644

compounds ranged from (7.685 to 22.987 %) hydrocarbon and oxygenated terpenes were markedly identified which are grouped into three classes i.e. major, minor, and traces constituent. Accordingly it is clear from the obtained data that 1,8-cineol and linalool represent the major compounds which ranged from 23.536 to 46.931 and 12.891 to 29.271 respectively.

Hydrocarbon terpenes ranged from 5.953 to 24.163 while the oxygenated compound ranged for 54.271 to 86.361 %. It is also clear from data presented in Table (3) that plants treated with (Ki 20 + M) recorded the highest level of total hydrocarbons (46.322 %).

Meanwhile, plants treated with (GA₃ 50 mg/L.) recorded the lowest content of total oxygenated compounds (54.771 %).

These results are in agreement with those obtained by Piccaglia and Marotti (1993) who reported that the main components in *Lavandula angustifolia* p. are 1,8-cineol, linalool, camphor and linalyl acetate which define the oil quality generally foliar application of Ki 40 + M possessed the best quality of the essential oil because of its low content of 1,8-cineol and high content of linalool and linalyl acetate.

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لتأثير الفسيولوجى لبعض منظمات النمو والعناصر الصغرى على النمو والمحتوى
الكىماوى لنبات اللافندر
لىلى كمال بلىع
قسم النبات - المركز القومى للبحوث - الدقى - القاهرة

تمت زراعة تجرية اصص فى الصوبية الزراعية بالمركز القومى للبحوث خلال الموسمين الزراعيين (١٩٩٩/٩٨-١٩٩٩/٢٠٠٠). وذلك لدراسة تأثير الرش بالكينتين والجبرلين وكذا تأثير بعض العناصر المغذية الصغرى مثل الحديد، المنجنيز، والزنك وايضا دراسة المعاملة المشتركة بهذه الهرمونات والعناصر الصغرى على نمو نبات اللافندر وانتاجها من الزيت الطيار وكذا نمو النبات من الكربوهيدرات الكلية والتغير فى محتوى مكونات الزيت.

وقد اثبتت الدراسة ان رش النباتات بالمعاملة المنفردة سواء للهرمونات او العناصر الصغرى ادى الى تشجيع النمو الخضري وزيادة محتوى العشب من الزيت الطيار - كما ادت المعاملة المشتركة مع العناصر الصغرى الى افضل النتائج بالنسبة للنمو الخضري ومحتوى العشب من الكربوهيدرات الكلية والنتروجين الكلى والزيت الطيار ومحصول الزيت الطيار.

وكانت افضل النتائج حصلت عليها المعاملة المشتركة بين كينتين ٤٠ ملجم/لتر + العناصر الصغرى - كما ادت الى افضل النتائج بالنسبة لصفات الزيت حيث ادت الى زيادة محتوى الزيت من اللينالول وڤالات الليناليل.