Physiological Studies on *Origanum majorana* L. 2-Chemical Composition of The Essential Oil as Affected by The Application of Gibberellic Acid and Kinetin

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ABSTRACT

The present research was were carried out in the Flowers and Ornamental Plants Research Gardens, Department of Floriculture , Ornamental Horticulture and garden Design, Faculty of Agriculture, Alexandria University throughout two successive seasons 2005 and 2006. *Origanum majorana* L. was chosen for this study because of the importance of its essential oil, their medicinal and aromatic uses. The experiment was carried out to determine the effect of plant growth regulators on the quality of the essential oils of *Origanum majorana* L. Nine treatments consisted of four different concentrations of GA_3 (50,100,150 and 200 mgL^{-1}), four different concentrations of Kinetin (10, 20, 50 and 100 mgL^{-1}) and the control treatment were applied. Regarding the effects of applying Gibberellic acid, it was found that , spraying plants with GA_3 at the rate of 200 mgL^{-1} gave the highest percentage of terpinene in marjoram leaves in the two seasons. Using GA_3 at the rate of 150 mgL^{-1} gave the highest percentage of terpinene in marjoram racemes in the first season and GA_3 at the rate of 100 mgL^{-1} in the second season. In both seasons, using GA_3 at 200 mgL^{-1} gave the highest linalool percentage of marjoram leaves and racemes. Applying GA_3 at the rate of 200 mgL^{-1} gave the highest percentage of caryophyllene of marjoram leaves in the two seasons, and GA_3 at the rate of 150 mgL^{-1} gave the highest caryophyllene percentage in the oil of plant racemes.With respect to the effects of kinetin, it was noticed that using kinetin at the rate of 100 mgL^{-1} gave the highest terpinene percentage in the oil of marjoram leaves for both seasons and the rate of 50 mgL-1 increased terpinene percentage of racemes oil for the two seasons. The maximum increment in linalool percentage in the oil of marjoram leaves was obtained by spraying plants with kinetin at the rate of 100 mgL^{-1} in both seasons. The highest percentage of linalool was found by using kinetin at the rate of 100 mgL^{-1} in the first season and 50 mgL^{-1} in the second season in marjoram racemes. Using Kinetin at the rate of 100 mgL^{-1} in the two seasons had the most effect on caryophyllene percentage of *Origanum majorana* L. oil in the two seasons.

Key words: Medicinal and aromatic plants, Sweet marjoram, Essential oils, terpenene, linalool, caryophyllene

INTRODUCTION

Medicinal and aromatic plants contain substances that can be used for therapeutic purposes, it is known to modern and ancient civilizations for

their healing properties. These compounds called "active ingredient" and the plant is considered as a source for these compounds. Sweet marjoram (*Majorana hortensis* Moench, *Origanum majorana* L.) comprises several aromatic and medicinal labiatae herbs belonging to different species. Marjoram is annual, biennial or perennial plant depending on the habitant. It reaches a height of 15-50cm with erect branching stem. It is native to Cyprus and the Eastern Mediterranean area (Ietswaart, 1980).

Marjoram oil was used to promote respiration in those suffering from measles, and was regarded as a treatment for spasms. The oil and oleoresin are used as fragrance components in soaps, detergents, cosmetics and perfumes. The main compounds of the volatile fraction of marjoram are the monoterpene alcohols terpenene, linalool, caryophyllene, alpha-terpineol, terpinen-4-ol, carvacrol, sabinene, sabinol, thujanol, citral, linalyl acetate, camphor, estragol, eugenol, myrcene, phellandrene, pinene, *trans*-sabinene hydrate, *cis*-sabinene hydrate and the acetate of *cis*-sabinene hydrate (Novak *et al.,* (2000). *O. majorana*, used as aromatic plant in Europe, mainly comes from cultivation in Egypt, Germany, Poland, etc. It contains no *p*-cymyl compounds (Novak *et al.,* (2002). Several researches were carried out to study the main constituents of the essential oils of flowers, leaves and stems of *Origanum* species. .i.e., Arnold *et al.*, (1993) ; Baser *et al.,* (1993) ; Kokkini, *et al.,* (1997) ; Pino *et al.*, (1997) ; Novak *et al.,* (2000) ; Mockute *et al.,* (2001) ; Gotsiou *et al.,* (2002) ; Novak, *et al.,* (2002), Ruberto *et al.,* (2002) ; Dudai *et al.,* (2003) ; Schulz *et al.,* (2004) ; Tămaş *et al.,* (2004) and Jelali *et al.,* (2006).

Sartoratto *et al.,* (2004) recorded the chemical composition of *Origanum vulgare* and *O. applii* oils in Table 1. Thymol was the main constituent of *O. applii* (Domin) Borus (64.5%) and *O. vulgare* L. (38.0%). The oils were analyzed by GC and GC-MS techniques in order to determine the major compounds. Baranauskienė *et al.,* (2005) studied the composition of volatile constituents of sweet marjoram (*Origanum majorana* L.) grown in Lithuania. Terpinen-4-ol, α-terpineol, γ-terpinene, α-terpinene, *cis*-sabinene hydrate and *p*-cymene were the major constituents in sweet marjoram. Jelali *et al.,* (2006) studied the composition of the essential oil of *Origanum majorana* fresh leaves. Essential oil extraction was done by hydrodistillation and its analysis was investigated by means of capillary gas chromatography (CGC). The results showed that, *Origanum majorana* leaf essential oil (EO) yield was 0,003 %. It was constituted by volatile compounds which belong to different chemical classes, the most represented class was monoterpenic alcohols one it accounted for 60,7 % of the whole EO and these compounds were followed by monoterpenes, sesquiterpenes and ethers which accounted respectively for 36 %, 4 % and 1,3 %. The most prominent compounds of the essential oil of *Origanum majorana* were terpinen-4-ol, γ-terpinene, α-terpinene and α-terpineol

whose respective rates were 47,4 %, 16,4 %, 7,9 % and 6,4 % and the other compounds accounted for 21,9 %.

The aim of this work was to study the effect of Gibberellic acid (GA_3) and kinetin on the oil yield quantity of *Majorana hortensis*.

Table 1. Identification the oil compounds from *O. vulgare* and *O. applii*.

Analyte	RI[a]	*Origanum vulgare*	*Origanum applii*
1-octen-3-ol	978	0.68[b]	-
p-cimene	1024	1.50	-
trans-β-ocimene	1035	0.39	-
γ-terpinene	1057	1.99	-
α-terpinolene	1083	0.31	-
Linalool	1093	-	4.92
cis-*p*-menth-2-en-1-ol	1122	1.47	-
trans-*p*-menth-2-en-1-ol	1140	0.86	-
Borneol	1169	2.52	-
Terpin-4-ol	1176	33.3	3.08
Cimen-8-ol	1182	-	0.64
α-terpineol	1188	4.25	-
trans-piperitol	1208	0.16	-
Thymol methyl ether	1231	0.24	1.47
Carvacrol methyl ether	1241	1.33	5.91
Thymol	1294	38.0	64.5
Geranyl acetate	1379	0.33	-
β-bourbonene	1385	-	1.35
trans-caryophillene	1422	2.66	-
β-gurjunene	1430	-	0.36
allo-aromadendrene	1461	-	0.28
Germacrene D	1482	1.47	4.79
β-bisabolene	1508	1.05	1.98
γ-cadinene	1516	-	0.89
Espatulenol	1580	1.44	1.62
Caryophillene oxide	1586	1.07	-
epi-α-muurolol	1643	-	0.72
α-cadinol	1656	0.29	1.53
SUM OF IDENTIFIED PEAKS		**95.3**	**94.1**

(a) **RI** = retention index; **(b)** Results expressed as % Area.

MATERIALS AND METHODS

The study was carried out the in Flowers and Ornamental Plants Research Gardens, Department of Floriculture & Ornamental Horticulture and Garden Design, Faculty of Agriculture, Alexandria University, throughout two successive seasons 2005 and 2006.

The experimental work was carried to study the effect of two plant growth regulators (GA3 and kinetin) on the quality of the essential oils of *Origanum majorana* L. Nine treatments consisted of four concentrations of GA_3 as a solution (50,100,150 and 200 mgL^{-1}), four concentrations of Kinetin as a solution (10, 20, 50 and 100 mgL^{-1}) and the untreated plants (control) were used. The experiment was designed as Randomized Complete Blocks Design (RCBD) using nine treatments with three blocks. Each treatment in the replicate contains "5" pots which were used as a plot (Snedecor and Cochran, 1967).The experimental work was started on March 15 th 2005 and 2006. Rooted cuttings (10-15cm in height) were transplanted in clay pots 30cm on April 15 th 2005 and 2006. One month after transplanting plants were sprayed (until run off point) with (50,100,150 and 200 mgL^{-1}) GA_3 and with (10, 20, 50 and 100 mgL^{-1}) kinetin. The plants were sprayed with the same concentrations of GA_3 and Kinetin 2 weeks after the first cut of each season. The control plants were sprayed with distilled water at the same time.The plants were fertilized with calcium super phosphate "16% P_2O_5" (5gm per plant) during soil preparing or planting. Ammonium nitrate "33.5% NH_4" (5gm per plant) and potassium sulphate"48% K_2O" (5gm per plant) were added after 45 days from transplanting the plants and after 21 days from the first cut (Sleem, 1973).

The first cut was carried out during flowering period at 50% to full blooming stage (this period would be the last week of July 2005 and 2006) and the second cut was carried out in the end of September 2005 and 2006 (after 60 days from the first cut). The plants were cut at 7cm above the pot soil in the early morning (Shedeed *et al.*, 1990).

The medium used for pots was a sandy loam soil and the chemical analysis of soil is presented following table:

Table 2: Chemical analysis of the media used in the pots of the first and second seasons experiments.

Variable	Value first season	Value second season
pH	7.57	7.51
E.C	3.31 ds/m	2.95 ds/m
Chloride	18 meq/L	16meq/L
Bicarbonate	1.4 meq/L	1.4 meq/L
Calcium	11.8 meq/L	9 meq/L
Magnesium	23.4 meq/L	13 meq/L
Sodium	17.87 meq/L	12.09 meq/L
Potassium	4.1 meq/L	30.17 meq/L
Phosphorus	41.51 ppm	39.5 ppm
Soil Texture	Sandy Loam	Sandy Loam

Oil percentage determination:

The essential oil was extracted by water distillation method according to Novak, *et al.*, (2002), where the leaves and racemes (50gm of each) were placed in the two liters capacity flask. Sufficient distilled water was added to correspond 3/4 the final volume of plant material. A proper essential oil trap (Clevenger's apparatus) and condenser were attached to the flask. The distillation was continued from forty-five minutes to an hour until no further increase in the oil volume was observed. The amount of oil obtained from five plants (plot) was measured and the oil percentage was calculated according to (Charles and Simon 1990). The volatile oil of each part of the plant (leaves and racemes) was kept dry in sealed brown bottles at 4°C to until chemical analysis.

Analysis of *Origanum majorana* L.Oil:

Samples were taken from the oil obtained for each treatment in the first cut of each season to analyze by using Gas Liquid Chromatography (GLC) to determine their main constituents at National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt.The volatile oil was dissolved in *n*-hexane 99.99% and 3 μl was injected in the gas liquid chromatography (GLC) (HP-5: Hewlett Packard series II) that was equipped with a splitless injector at 250°C, and a flame ionization detector (FID) held at 280°C using hydrogen as a carrier gas (flow rate 1.5 ml/min). Samples were separated on a stainless steel capillary column (30m long, and 0.32mm internal diameter) of 0.25μm film thickness using 5% diphenyl

and 95% dimethylpolysiloxane as a stationary phase with polarity non-polar. The temperature of the column was programmed from 50°C to 150°C at a heating rate of 5°C /minute.Identification of the chemical constituents of the essential oil were made using authentic reference samples (External chemical standers) by comparing the peak area of constituents with that appear in the chromatogram of standard essential oil. Concentration of such compound was calculated according to Heftam (1967) and Gunther and Joseph (1978). Sources of the principal components of Marjoram oil, which used as reference for determined in essential oil of Marjoram by GLC, were Sipa Gigi NY, USA for Caryopheline, Linalool and Terpenine.

The experiment was repeated in the second year (2006) at the same site in the Experimental Station, Department of Floriculture & Ornamental horticulture and Garden, Design,Faculty of Agriculture, Alexandria University. The same steps and the technique of the experiment were exactly followed in the second year (2006) to compare the results of the two successive experiments.

RESULTS AND DISCUSSION

Chemical composition of volatile oil of *Origanum majorana* L.:

The following data showed the effect of Kinetin and GA_3 on different oil components of *Origanum majorana* L. analyzed by GC through the two growing seasons 2005 and 2006. Qualitative essential oil analyzed by GC presented in figures (3, 4, 5 and 6) showed the effect of GA_3 and kinetin on the major oil constituents (terpinene, linalool and caryophyllene).

1-Percentage of terpinene in *Origanum majorana* L..

1-1 Percentage of terpinene in the leaves:

The results in table 3 and figure 5 showed that, the percentage of terpinene was increased by increasing GA_3 concentrations in both seasons, whereas the highest percentage was obtained by using GA_3 at the rate of 200 mgL^{-1}.

There was significant effect of kinetin concentrations on terpinene percentage in leaves. Kinetin at the rate of 100 mgL^{-1} gave the highest terpinene percentage in both seasons. Whereas, terpinene percentage was increased in the levels by increasing kinetin concentrations.

1-2 Percentage of terpinene in the racemes:

Data presented in table 3 and figure 6 showed that GA_3 at the rate of 150 mgL^{-1} increased terpinene percentage in the first season (29.47%) while GA_3 at the rate of 100 mgL^{-1} in the second season was (34.46%).

There was a significant effect of kinetin treatments on terpinene percentage compared with the control. The highest percentage of terpinene found by using kinetin at the rate of 50 mgL^{-1} in the two seasons with the mean of 26.85 - 29.29% respectively.

2- Percentage of linalool in *Origanum majorana* L.

2-1 Percentage of linalool in the leaves:

Data in table 4 and figure 7 showed that there was significant effect of GA_3 treatments on linalool percentage in leaves oil. Linalool percentage was increased by increasing GA_3 concentrations whereas; the highest percentage was obtained by GA_3 at the rate of 200 mgL^{-1} through out the two seasons.

There was a significant effect of kinetin treatments on linalool percentage in leaves oil. The highest percentage of linalool was produced by using kinetin at the rate of 100 mgL^{-1} in the two seasons with the mean of 7.65 - 7.19 % respectively.

Table 3: Effect of GA_3 and Kinetin on terpinene percentage of *Origanum majorana* L. oil.

Treatments (mgL^{-1})	Leaves		Racemes	
	First Season	Second Season	First Season	Second Season
Control	22.71^{i}	22.78^{g}	24.76^{g}	10.28^{i}
GA_3 50	25.86^{g}	25.36^{f}	25.27^{f}	26.19^{e}
GA_3 100	30.49^{d}	33.05^{e}	26.32^{e}	34.46^{a}
GA_3 150	35.40^{c}	37.55^{d}	29.47^{a}	34.36^{b}
GA_3 200	47.01^{a}	41.65^{a}	29.16^{b}	33.64^{c}
Kin. 10	23.82^{h}	20.91^{i}	24.19^{i}	16.77^{h}
Kin. 20	27.14^{f}	21.51^{h}	24.41^{h}	22.98^{g}
Kin. 50	29.32^{e}	39.30^{c}	26.85^{c}	29.29^{d}
Kin. 100	39.64^{b}	41.30^{b}	26.42^{d}	24.26^{f}

Means with the same letter in the same column are not significantly different ($P<0.05$).

Table 4: Effect of GA_3 and Kinetin on linalool percentage of *Origanum majorana* L. Oil.

Treatments (mgL^{-1})	Leaves		Racemes	
	First Season	Second Season	First Season	Second Season
Control	3.87^{i}	3.82^{h}	5.93^{g}	2.16^{i}
GA_3 50	6.88^{d}	4.14^{g}	6.73^{c}	4.51^{g}
GA_3 100	6.14^{e}	6.09^{e}	6.85^{b}	6.28^{c}
GA_3 150	7.14^{c}	7.12^{c}	6.66^{d}	7.20^{a}
GA_3 200	9.76^{a}	7.27^{a}	7.26^{a}	6.98^{b}
Kin. 10	5.08^{h}	3.78^{h}	5.73^{h}	4.30^{h}
Kin. 20	5.69^{g}	4.23^{f}	6.07^{f}	4.98^{f}
Kin. 50	5.86^{f}	7.05^{d}	6.45^{e}	5.96^{d}
Kin. 100	7.65^{b}	7.19^{b}	6.78^{c}	5.47^{e}

Means with the same letter in the same column are not significantly different (P<0.05).

2-2 Percentage of linalool in the racemes:

It was obvious in table 4 and figure 8 that linalool percentage of *Origanum majorana* L. increased by using GA_3 concentration up to 200 mgL^{-1} in the first season and up to 150 mgL^{-1} in the second season.There was a significant effect of kinetin treatments on linalool percentage compared with the control. The highest percentage was found by kinetin at the rate of 100 mgL^{-1} in the first season and by 50 mgL^{-1} in the second season.

3- Percentage of Caryophyllene in *Origanum majorana* L.

3-1 Percentage of Caryophyllene in the leaves:

Regarding to the results presented in table 5 and figure 9, it was cleared that the level of GA_3 concentrations had a significant effect on caryophyllene percentage of *M. hortensis* oil compared with the control treatment. The caryophyllene percentage was increased by increasing GA_3 concentration, whereas the highest percentage was obtained by using GA_3 at the rate of 200 mgL^{-1}.

In addition, the data showed that, the levels of kinetin were significantly affected caryophyllene percentage in the leaves compared with

the control. Kinetin at rate of 100 mgL^{-1} in the two seasons had the most effect on caryophyllene percentage of *Origanum majorana* L. oil with means 1.88 - 1.78%, respectively.

3-2 Percentage of Caryophyllene in the racemes:

Regarding to the results presented in table 5 and figure 10, it was cleared that GA_3 concentrations had a significant effect on caryophyllene percentage of *Origanum majorana* L. racemes oil compared with the control treatment. GA_3 at the rate of 150 mgL^{-1} gave the highest percentage in two seasons (2.20- 2.24%) respectively.

There was a significant increase in caryophyllene precentage by increasing kinetin concentrations. The highest percentage was found by using kinetin at the rate of 100 mgL^{-1} in the two seasons (1.36, 2.31%) respectively.

These increases in oil components may be due to the use of gibberrellin, which increase mevalonic acid, which is considered as a basic compound of biosynthesis of principal compounds of volatile oils (EL-Sayed 1993). In addition, this could be attributed to the effect of GA_3 on metabolism and enzyme levels responsible for mono sesqueiterpene 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 component.

According to El-keltawi and Croteau, (1987), cytokinins plays a role in increasing monoterpene biosynthesis. Gershenzon *et al.*, (2002) showed that increasing menthol percentage especially in peppermint oil might be due to enhancement of the rate of biosynthesis specially the first committed step in monoterpene biosynthesis.

Table 5 : Effect of GA_3 and Kinetin on caryophyllene percentage of *Origanum majorana* L oil.

Treatment (mgL^{-1})	Leaves		Racemes	
	First Season	Second Season	First Season	Second Season
Control	1.38^{g}	0.69^{g}	1.18^{de}	0.76^{f}
GA_3 50	1.37^{g}	0.86^{f}	1.36^{c}	1.52^{e}
GA_3 100	2.02^{c}	1.12^{d}	1.43^{bc}	1.83^{c}
GA_3 150	2.08^{b}	1.24^{c}	2.20^{a}	2.24^{b}
GA_3 200	2.45^{a}	1.71^{b}	1.47^{b}	1.46^{e}
Kin. 10	1.50^{f}	0.92^{e}	1.16^{e}	1.59^{d}
Kin. 20	1.76^{e}	1.15^{d}	1.24^{d}	1.83^{c}
Kin. 50	1.81^{e}	1.14^{d}	1.36^{c}	1.85^{c}
Kin. 100	1.88^{d}	1.78^{a}	1.36^{c}	2.31^{a}

Means with the same letter in the same column are not significantly different ($P<0.05$).

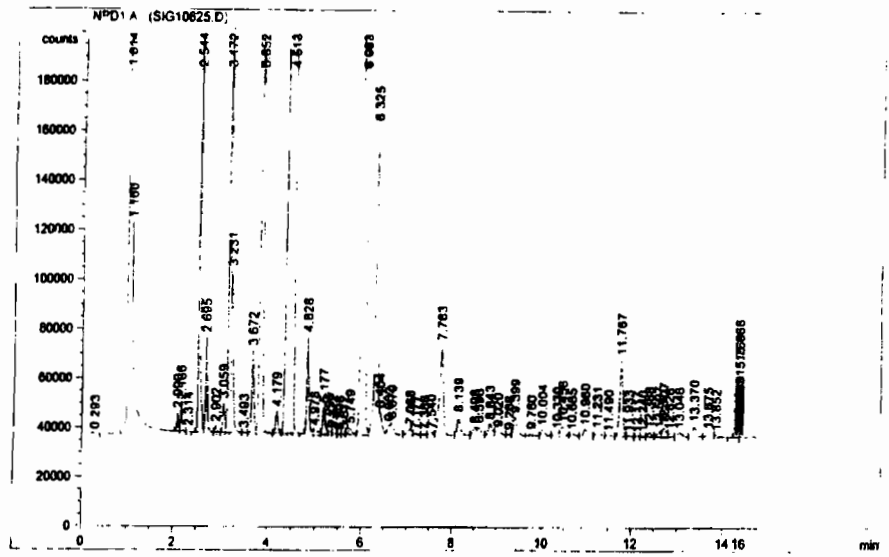

Figure 1: Effect of kinetin on oil component of marjoram leaves.

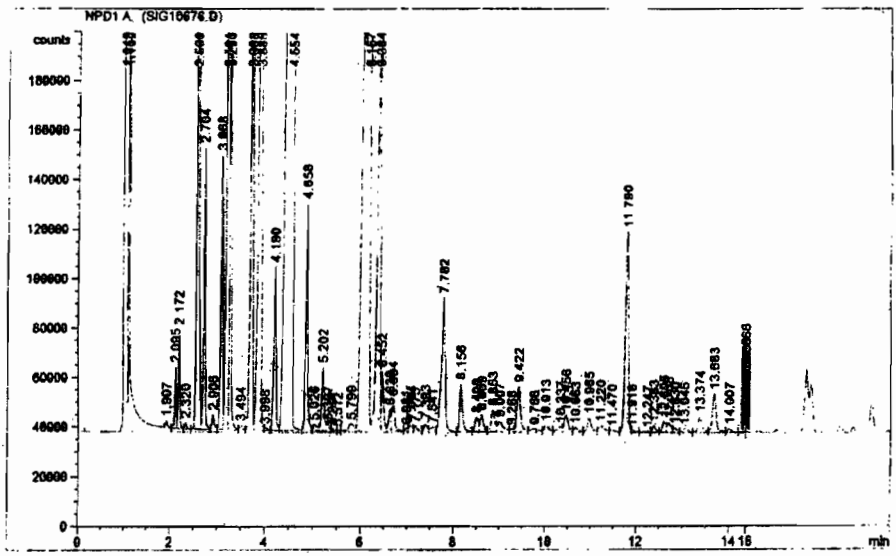

Figure 2: Effect of kinetin on oil component of marjoram racemes.

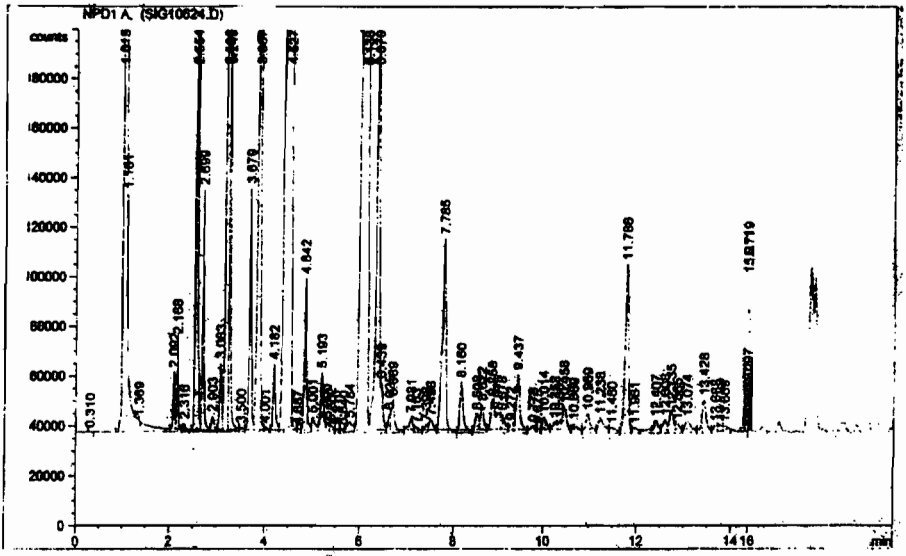

Figure 3: Effect of GA_3 on oil component of marjoram racemes.

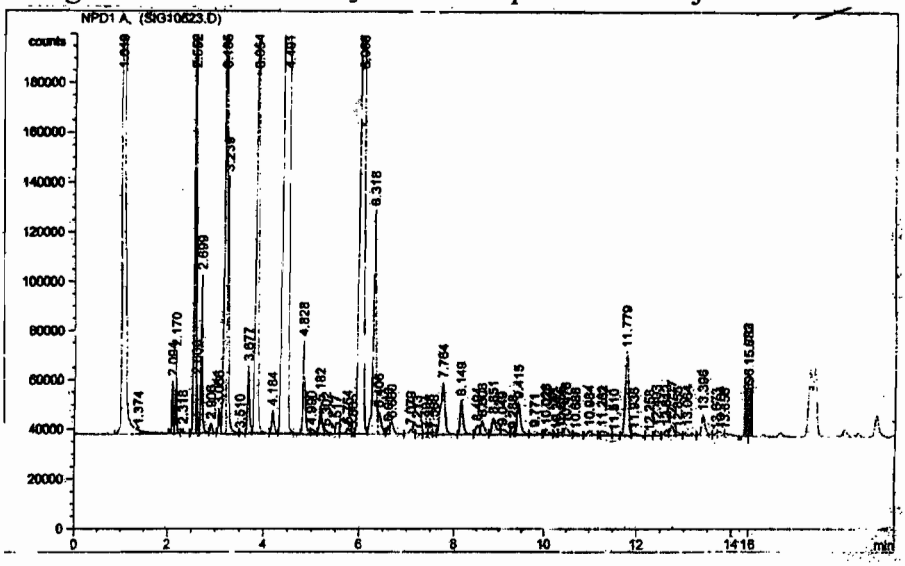

Figure 4: Effect of GA_3 on oil component of marjoram racemes.

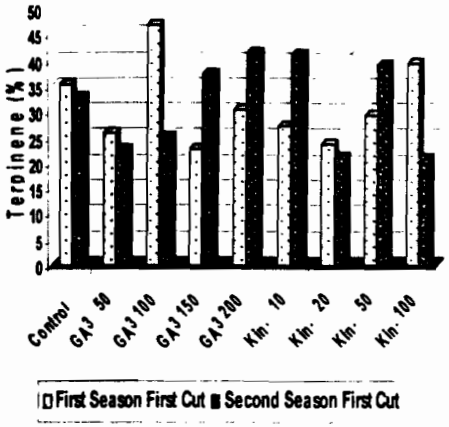

Figure 5. Effect of GA_3 and Kinetin treatments on terpinene percentage of *O. majorana* L. Leaves oil.

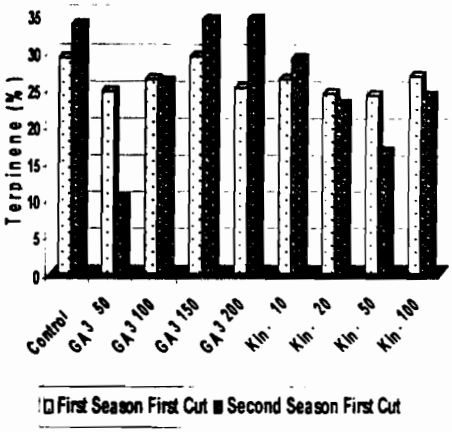

Figure 6. Effect of GA_3 and Kinetin treatments on terpinene percentage of *O. majorana* L. racemes.

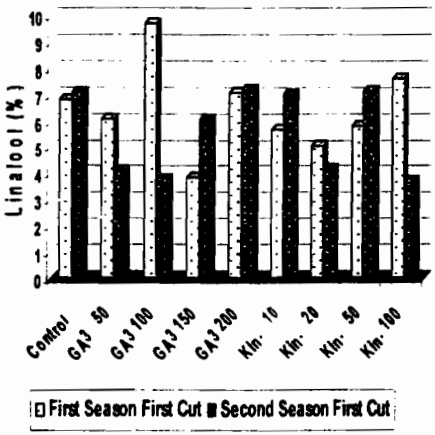

Figure 7. Effect of GA_3 and Kinetin treatments on linalool percentage of *O. majorana* L. leaves.

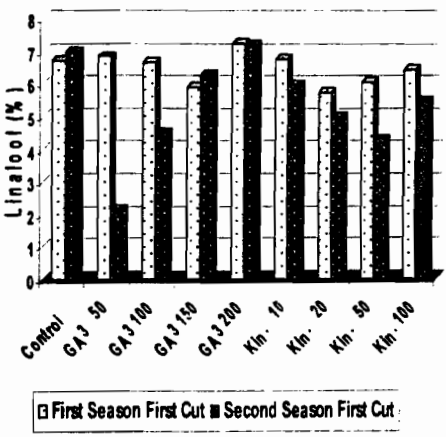

Figure 8. Effect of GA_3 and Kinetin treatments on linalool percentage of *O. majorana* L. racemes.

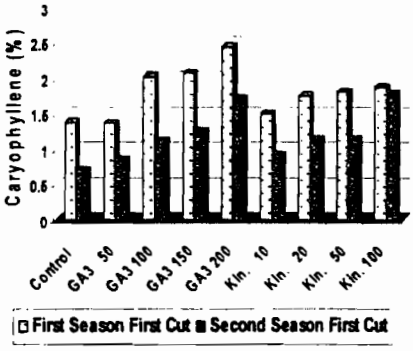

Figure 9. Effect of GA_3 and Kinetin treatments on caryophyllene (%) of *O. majorana* L. leaves.

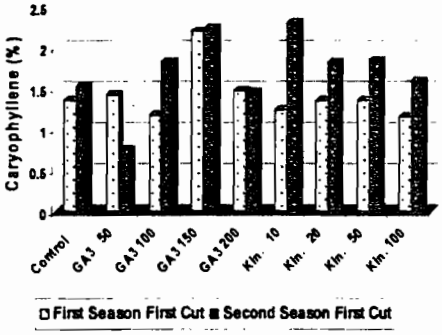

Figure 10. Effect of GA_3 and Kinetin treatments on caryophyllene (%) of *O. majorana* L. racemes.

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الملخص العربى

دراسات فسيولوجية على نباتات البرَدقوش

٢- التحليل الكيماوي للزيت الطيار في نباتات البردقوش المتأثر بالمعاملة بحامض الجبيريلليك والكينيتين

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قسم الزهور ونباتات الزينة وتنسيق الحدائق

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أجري هذا البحث في حدائق أبحاث الزهور ونباتات الزينة بقسم الزهور ونباتات الزينة بكلية الزراعة جامعة الإسكندرية ، ويهدف هذا البحث دراسة تأثير الرش بتركيزات مختلفة من منظمات النمو (حامض الجبريلليك بتركيزات ٥٠- ١٠٠ - ١٥٠- ٢٠٠ مجم/لتر و الكينيتين بتركيزات ١٠- ٢٠-٥٠ - ١٠٠ مجم/لتر) إضافة لمعاملة المقارنة (الكنترول) على محتوي أوراق وأزهار نبات البردقوش من الزيت العطري كما ونوعا. وقد أوضحت النتائج أن إضافة حامض الجبريلليك أوالكينيتين أدت إلى تحسن معنوي في مكونات الزيت حيث تحققت أعلى نسبة من التربينين في الأوراق باستخدام حامض الجبريلليك بتركيز ٢٠٠ مجم/لتر لكل من الموسمين بينما كانت أعلى نسبة حصل عليها في النورات عند الرش بتركيز ١٥٠ مجم/لتر في الموسم الأول و ١٠٠ مجم/لتر في الموسم الثاني, بينما كانت أعلى نسبة من الليناالول فى زيت الأوراق والنورات لنبات البردقوش حصل عليه باستخدام تركيز ٢٠٠ مجم/لتر فى كل من الموسمين,وقد تحققت أفضل النتائج من الكاريوفيلين فى الأوراق برش حامض الجبريلليك عند تركيز ٢٠٠ مجم/لتر لكل من الموسمين و ١٥٠مجم/لتر بالنسبة للنورات لكل من الموسمين.

كما أوضحت نتائج التحليل الكيماوي أن رش الكينيتين بتركيز١٠٠مجم/لتر أعطى أعلى نسبة من التربينين في الأوراق في كل من الموسمين بينما تحققت أعلى نسبة في النورات عند الرش بتركيز ٥٠ مجم/لتر لكل من الموسمين, وأن أعلى نسبة من الليناالول حصل عليها فى الأوراق باستخدام كينيتين ١٠٠ مجم/لتر فى كل من الموسمين , كذلك فان اعلى نسبة من الليناالول فى زيت النورات حصل عليها باستخدام كينيتين ١٠٠ مجم/لتر في الموسم الأول و ٥٠ مجم/لتر فى الموسم الثانى , بينما الرش بالكينيتين بتركيز١٠٠ مجم/لتر أعطى أعلى نسبة من الكاريوفيلين في زيت أوراق و نورات نبات البردقوش لكل من الموسمين.