Physiological Studies on *Origanum majorana* L. 1-Effect of Gibberellic Acid and Kinetin on Growth and Oil Yield Production

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ABSTRACT

The present study was designed to study the effect of Gibberellic Acid (GA_3) with concentrations 50,100,150 and 200 $mgL^{-1}$and Kinetin with concentrations 10, 20, 50 and 100 mgL^{-1} and the control treatment on the vegetative growth and quantity of the essential oil of *Origanum majorana* L. Plants. The experiment was carried out during 2005 and 2006 growing seasons in the Flowers and Ornamental Plants Research Gardens, Department of Floriculture & Ornamental Horticulture and Garden Design, Faculty of Agriculture, Alexandria University Alex., Egypt. The results of this work can be summerized as; GA_3 at the rate of 100 and/or150 mgL^{-1} had the most effect on (plant height, number of branches/plant, number of leaves, total fresh weight/plant, oil percentage in leaves) and 100 mgL^{-1} (GA_3) gave the highest oil percentage in racemes in both seasons. Also kinetin at the rate of 50 and/or100 mgL^{-1} gave the largest number of branches , the highest number of leaves, the heaviest total fresh weight, the highest leaves area , the highest values of total chlorophyll content and the highest oil percentage in leaves and Kinetin at the rate of 10 mgL^{-1} gave the highest oil percentage in racemes.

Key words: Medicinal and aromatic plants, Sweet marjoram, Essential oils and Growth Regulators

INTRODUCTION

Medicinal and aromatic plants contain substances that can be used for therapeutic purposes, it is known in the modern and ancient civilizations for their healing properties. Sweet marjoram (*Majorana hortensis* Moench, *Origanum majorana* L.) comprises several aromatic and medicinal labiatae herbs belonging to different species. Marjoram is native to Cyprus and the Eastern Mediterranean area (letswaart, 1980). The oil has been employed to treat flatulence, colic and rheumatism. It has also been applied externally as a liniment for sprains and bruises. Marjoram oil was used to promote perspiration in those suffering from measles, and was regarded as a treatment for spasms. Promotes menstruation so should not be taken during pregnancy, good for period pains and leucorrhoea. Eases respiratory difficulties and clears the chest by helping to loosen mucus in colds and bronchitis. The oil and oleoresin are used as fragrance components in soaps, detergents, cosmetics and perfumes. Employed in

most major food categories, especially meats, seasonings and sauces, as well as soft drinks and alcoholic beverages.

In Egypt during the year 2004, the cultivated area of Sweet marjoram was 4870 feddans, total production was 9170 tons, the quantity of exports was 2960 tons and the value of exports was 20520 pounds, this data according to Ministry of Agriculture and Land Reclamation, the central administration of the agricultural economy, the records of the census (2004). *O. majorana L.*, 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 carried out to study the effect of Gibberellic Acid (GA_3) on the vegetative growth and oil contents of some aromatic and medicinal plants, i.e., Balbaa and Laila (2002) found that 100 ppm of GA_3 increased chemical constituents and essential oil yield of *lavandula officinalis L.* plants. El-Naggar and Sharaf (2002) on tubrose plants (*Polyanthus tubrosa* C.V."double") found that, foliar application of gibberellic acid (200 mg/l) gave the best results for the leaves total chlorophyll content. Dhang and Biswas (2004) mentioned that spraying *Kaempferia galangal* plants with GA_3 500 ppm resulted in the maximum number of leaves per plant and the heighest rhizome length, rhizome fresh weight and rhizome yield. Baydar and Erdal (2004) studied the effects of GA_3 on the essential oil content, of oregano (*Origanum onites*). The gibberellic acid had important effect on the essential oil content in the leaf, carvacrol and thymol. Reda, *et al.*, (2005) studied the response of essential oils content of thyme plants (*Thymus vulgaris* L.) to different bioregulators including gibberellic acid. Essential oil percent as well as total phenolic compounds were significantly affected by the foliar application of bioregulators used. Kołodziej *et al.*, (2006) studied the effects of foliar application of GA_3 (100 or 200 mg l^{-1}) on ginsenoside content of American ginseng (*Panax quinquefolium* L.) roots. They found that the plant roots had a higher content of total ginsenosides in comparison to the control. GA_3 showed a decreasing effect in air-dry weight of roots and aboveground parts. Netherlands (2007) studied the effect of foliar sprays of 100 μM aqueous solutions of gibberellic acid (GA_3) on *Nigella sativa* (L.).He found that GA_3 appreciably increased chlorophyll and total protein contents and net photosynthetic rate in the leaves, along with capsule number and seed yield/plant, at harvest. Several researches carried out to study the effect of kinetin on the vegetative growth and oil contents of some aromatic and medicinal plants, i.e., Mahmoud (1996) found that using Kinetin at 50 ppm to basil plant promoting oil content (0.270% compared with 0.213% in the control). Gamal El Din *et al.*, (1997) showed on coriander plants that treatment with kinetin 10, 25 and 50 mg/l increased significantly oil yield.

Balbaa and Laila (2002) found that kinetin at 40 mg/L significantly increased total carbohydrates and volatile oil yield of *lavandula officinalis* L. plants. Youssef *et al.*, (2004) demonstrated that the foliar application of kinetin (40 ppm) on sage (*Salvia officinalis*) significantly increased the essential oil components. Fawzy (2005) studied the effect of kinetin on vegetative growth of *Mentha piperita* plant. The results showed that, kinetin at 30 mgL^{-1} gave the highest number of branches, total fresh weight per plant, chlorophyll content and leaf area. Kołodziej *et al.*, (2006) investigated the effects of foliar application of kinetin on ginsenoside content of American ginseng (*P. quinuefolium*) roots at a concentration of 100 or 200 mg l^{-1}. Kinetin significantly affected morphological features and accumulation of total ginsenosides in ginseng roots. An increase of roots size was observed using higher doses (200 mg L^{-1}) of kinetin. Netherlands (2007) studied the effect of foliar sprays of 100 μM aqueous solutions of kinetin (KIN) on *Nigella sativa* (L.). kinetin appreciably increased chlorophyll and total protein contents and net photosynthetic rate in the leaves.

The objectives of the present study were to investigate the effect of the application of four concentration of Gibberellic acid (GA_3) and Kinetin on the vegetative growth parameters and quantity of the essential oil of *Origanum majorana* L. Plants.

MATERIALS AND METHODS

This study was carried out 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 vegetative growth and quantity 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 experimental work was started on March 15th 2005 and 2006. Rooted cuttings (10-15cm in height) were transplanted in clay pots 30cm on April 15th 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 prepartion 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 high above the pot soil in the early morning (Shedeed *et al.*, 1990).

The parameters used to measure the effect of growth regulator on *Origanum majorana* L. growth at each harvest in the two seasons were vegetative growth (plant height (cm), plant diameter(cm), and fresh weight of leaves, racemes, branches and whole plant(g), number of leaves, number of branches, leaves area (cm^2) and total chlorophyll) and oil yield in leaves and racemes (%).

Total Chlorophyll on the fresh leaves at the harvesting time was measured according to the method described by Yadava (1986) using Minolta SPAD Chlorophyll Meter model-502.

The media used for pots were sandy loam soil and the chemical analysis of soil as following table:

Table 1: Chemical analysis of the media used in the pots of 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 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 until chemical analysis.

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).

RESULTS AND DISCUSSION

1- Effect of GA_3 and Kinetin on *Origanum majorana* L. growth at each cut in two seasons:

The observations that given in photo 1 and 2 showed the effects of foliar application of the different concentrations from GA_3 and Kinetin on the vegetative growth of *Origanum majorana* L compared with the control (sprayed with distilled water).

1-1 Effect of GA_3 and Kinetin on *Origanum majorana* L. height (cm):

Table 2 and figure 1 showed that, the GA_3 treatments had a significant effects compared with the control treatment. It was noticed that the GA_3 at 150 mgL^{-1} for the two cuts in the first season and the first cut for the second season had a significant effect on *Origanum majorana* L. height with the mean of (26.66, 25.96, 36.36 cm) respectively. Also, the GA_3 at 100 mgL^{-1} in the second cutfor the second season showed a high effect on *Origanum majorana* L. height with the mean of (31.66 cm). In general, there was a reduction in plant height with increasing GA_3 concentration than 150 mgL^{-1}.

The stimulatory effect of GA_3 on plant growth of marjoram plants might be due to an increase in internodes elongation, which in turn was often a consequence of increased cell wall extension. This might be explained on basis of striking increased in cell membrane permeability according to Wood and Paleg (1972), Brain and Lowe (1959) and Alvin

(1960). Also, these increase in plant height may be due to the effect of Gibberellic acid on stimulating the cell division and elongation of new cells growth formed on marjoram plants (Wasfy, 1995). This data coincided with El-Antably *et al.*, (1975) on *Origanum majorana*, El-Sharkawy (1981) on *Origanum majorana*, Farooqi *et al.*, (1998) on *Artimisia arzura* and Balbaa and Laila (2002) on *Lavendula officinalis*.

The result in the two cuts from the two seasons showed in table 1 and figure 3 indicated that all kinetin concentrations had no significant effect between each other and the control treatment. These results were in disagreement with those of Awad *et al.*, (1985) on basil plant and Marzou *et al.*, (1994) on *Ocimum basilicum*, Balbaa and Laila (2002) on *Lavendula officinalis*.

1-2 Effect of GA_3 and Kinetin on the diameter (cm) of *Origanum majorana* L.:

In general, the data illustrated in table 3, showed that the differences between all levels of GA_3 treatments in both seasons are not significant on *Origanum majorana* plant diameter compared with the control treatment. Data in table 3 and figure 2 clarified that all the different concentrations of kinetin had significant effect as compared with the control treatment. Whereas the level of kinetin 100 mgL^{-1} (in the two cuts of the two seasons) had the most effect on *Origanum majorana*_plant diameter with the mean of 26.56, 20.90, 22.83, 25.38 respectively.

The effect of kinetin can probably related to the action of cytokinin on the differentiation of areal parts since, the stimulation of shoot growth as a result of kinetin treatments has been occurred in poinsettia (Milbocker 1972) and (Yang and Clore 1973) on *Asparagus officinalis*.

1-3 Effect of GA_3 and Kinetin on the number of branches/plant of *Origanum majorana* L.:

The results in table 4 and figure 3 showed that, the GA_3 treatments gave significant effects compared with the control treatment. Whereas GA_3 at 150 mgL^{-1} for the two cuts in the first season and the second season gave the highest number of branches. There is no significant effect between $GA_3$100 and 150mgL^{-1}. These results were in agreement with El-Sharkawy (1981) on *Origanum majorana*, Brose and Dhumal (2001) on *solanum Khasianum* and Balbaa and Laila (2002) on *Lavendula officinalis*. Increment in branches number per plant may be due to enhancing the growth of more lateral buds leading to more branches. The effect of kinetin can probably related to the action of cytokinine on the differentiation of areal parts since, the stimulation of shoot growth as a result of kinetin treatments has been occurred in poinsettia Milbocker (1972) and (Yang and Clore 1973) on *Asparagus officinalis*. Cytokinins appeared to play an

important role in the regulation of cell division, differentiation and organogenesis in developing plant (Skoog and Armstrong, 1970 and Hall 1973).

These results are in agreement with Ahmed (1982), Al-Badawy *et al.*, (1984) on chamomile plant, Shedeed *et al.*, (1990) on basil plant, Hafez (1990) on marjoram plant and Balbaa (2002) on *Tagetes minata* plant.

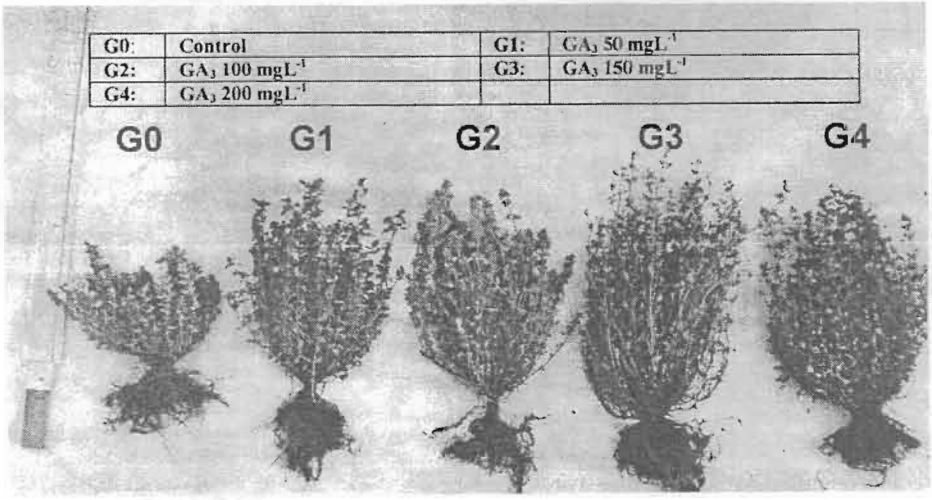


Photo 1. Effect of different concentrations of GA_3 on the growth of *O. majorana* L. compared with control.

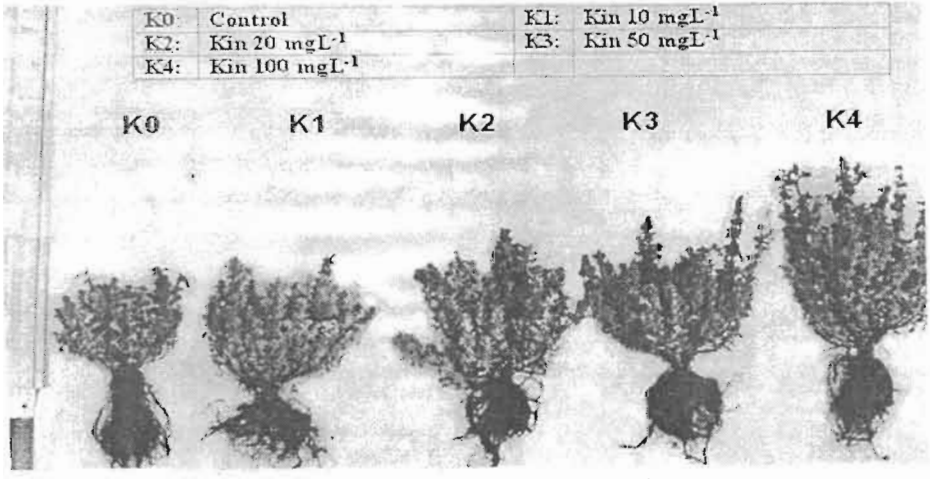


Photo 2. Effect of different concentrations of Kinetin on the growth of *O. majorana* L. compared with control.

Table 2: Effect of GA_3 and Kinetin on *O.majorana* L. height (cm.).

Treatments (mgL^{-1})	First Season		Second Season	
	First Cut	Second Cut	First Cut	Second Cut
Control	16.13 ^{d}	15.13^{de}	23.25^{d}	22.85^{ns}
GA_3 50	22.00^{bc}	20.30^{bc}	32.35^{bc}	27.66^{ns}
GA_3 100	22.80^{abc}	24.63^{ab}	34.63^{ab}	31.66^{ns}
GA_3 150	26.66 ^{a}	25.96 ^{a}	36.36^{a}	29.66^{ns}
GA_3 200	24.20^{ab}	19.43^{cde}	30.26^{c}	25.63^{ns}
Kin. 10	16.60 ^{d}	15.06 ^{e}	24.20^{d}	25.53^{ns}
Kin. 20	16.80 ^{d}	16.80^{cde}	24.43^{d}	26.16^{ns}
Kin. 50	18.96^{cd}	18.80^{cde}	25.43^{d}	27.00^{ns}
Kin. 100	19.60^{cd}	19.86^{cd}	23.23^{d}	26.10^{ns}

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

Table 3: Effect of GA_3 and Kinetin on the plant diameter (cm) of *O. majorana* L..

Treatments (mgL^{-1})	First Season		Second Season	
	First Cut	Second Cut	First Cut	Second Cut
Control	23.46^{bc}	17.06^{b}	18.93^{b}	18.26^{b}
GA_3 50	21.76 ^{c}	19.20^{ab}	18.43^{b}	18.21^{b}
GA_3 100	22.86^{bc}	19.86^{ab}	19.16^{b}	18.64^{b}
GA_3 150	25.16^{ab}	19.86^{ab}	18.40^{b}	18.76^{b}
GA_3 200	22.90^{bc}	19.23^{ab}	18.13^{b}	19.81^{b}
Kin. 10	23.20^{bc}	18.63^{ab}	22.60^{a}	23.49^{a}
Kin. 20	23.90^{bc}	19.13^{ab}	22.76^{a}	25.23^{a}
Kin. 50	24.73^{ab}	21.40^{a}	23.03^{a}	26.52^{a}
Kin. 100	26.56 ^{a}	20.90^{a}	22.83^{a}	25.38^{a}

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

Table4: Effect of GA_3 and Kinetin on the number of branches/plant of *Origanum majorana* L.

Treatments (mgL^{-1})	First Season		Second Season	
	First Cut	Second Cut	First Cut	Second Cut
Control	134.13^{bc}	130.13^{e}	114.00^{d}	134.40^{d}
GA_3 50	124.90^{c}	156.10^{de}	127.26^{cd}	165.77^{cd}
GA_3 100	136.17^{bc}	188.07^{abcd}	137.73^{bc}	202.17^{abc}
GA_3 150	155.83^{ab}	199.87^{abc}	152.90^{b}	216.77^{ab}
GA_3 200	128.40^{bc}	168.93^{bcd}	126.63^{cd}	168.87^{cd}
Kin. 10	136.93^{bc}	152.80^{de}	116.80^{d}	174.50^{cd}
Kin. 20	145.50^{bc}	164.33^{cde}	135.46^{bc}	184.60^{bc}
Kin. 50	182.57^{a}	205.23^{ab}	175.63^{a}	227.30^{a}
Kin. 100	185.97^{a}	214.17^{a}	153.03^{b}	198.57^{abc}

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

1-4 Effect of GA_3 and Kinetin on number of leaves/plant of *Origanum majorana* L.:

Data in table 5 and figure 4 showed that GA_3 100 mgL^{-1} had a statistically high effect on number of *Origanum majorana* L. leaves compared with the other concentrations and control treatment in the second cut of the first season and the two cuts of the second season with the means of 4255.6, 2201.80, and 4333.80; respectively. The stimulating effects of gibberellic acid may be due to activating apical meristems, enhancing the biosynthesis of the protein and carbohydrates. These together lead to enhancing the initiation of leaves primordial and consequently more leaves would be formed (EL-Naggar and Sharaf 2002). Shedeed *et al.*, (1990), found similar results on *Mentha piperita*, Brose and Dhumal (2001) on *Solanum khasianum*, Pablo (2001) on *Eryngium foetidum* and Vijayakumari (2002) on *Andrographis paniculata*.

From the results in table 7 and figure 4, it was cleared that the Kinetin treatments gave significant effect on number of *O. majorana* leaves per plant with increase Kinetin concentration up to 100 mgL^{-1} with the means of 3286.10, 6149.80, 2181.50, 3271.90; respectively. This

increasing was a result of enhancing the growth of more lateral buds leading to more branches and more leaves number. The effect of kinetin can probably related to the action of cytokinin on the differentiation of areal parts since, the stimulation of shoot growth as a result of kinetin treatments has been occurred in Poinsettia Milbocker (1972) and (Yang and Clore 1973) on *Asparagus officinalis*. Cytokinins appeared to play an important role in the regulation of cell division, differentiation and organogenesis in developing plant (Skoog and Armstrong, 1970 and Hall 1973). These results are agreement with Shedeed *et al.*, (1990) on basil plant, Farooqi *et al.*, (1996) on *Artimisia arzura*.

Table 5: Effect of GA_3 and Kinetin on the number of leaves/plant of *Origanum majorana* L.

Treatments (mgL^{-1})	First Season		Second Season	
	First Cut	Second Cut	First Cut	Second Cut
Control	1803.60^{ns}	2473.50^{e}	1466.60^{bc}	2148.60^{c}
GA_3 50	1859.50^{ns}	3117.20^{cde}	1596.70^{abc}	2886.50^{bc}
GA_3 100	2218.10^{ns}	4255.60^{bcd}	2201.80^{a}	4333.80^{a}
GA_3 150	2914.50^{ns}	4058.50^{bcde}	2045.80^{abc}	3906.80^{ab}
GA_3 200	2073.10^{ns}	2777.90^{de}	1396.10^{c}	2645.30^{bc}
Kin. 10	3151.40^{ns}	2410.30^{bcd}	1476.10^{bc}	2678.90^{bc}
Kin. 20	3176.10^{ns}	4456.00^{bc}	1762.90^{abc}	3703.50^{ab}
Kin. 50	3306.70^{ns}	5600.30^{ab}	2105.10^{ab}	3777.40^{ab}
Kin. 100	3286.10^{ns}	6149.80^{a}	2181.50^{a}	3271.90^{abc}

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

1-5 Effect of GA_3 and Kinetin on the fresh weight of leaves/plant (gm) of *Origanum majorana* L.:

Generally, concentrations 50 and 100 mgL^{-1} of GA_3 gave the best results in the two cuts of both seasons compared with the other concentrations and control (table 6 and figure 5). These results may be due to the favorable effects of GA_3 on the vegetative growth, which lead to an increase in the photosynthesis efficiency and accumulation of dry matter in the leaves (EL-Nagaar and Sharaf 2002). The increase in leaves fresh

weight is a result of enhancing the growth of more lateral buds leading to more branches; more leaves number and exogenous cytokinins resulted in promotion of protein, RNA, lipid and starch synthesis in the plant (Mothes,1964). Similar trend of results was obtained by El-Sharkawy (1981) on *Origanum majorana* and Farooqi *et al.*, (1996) on *Artimisia arzura* and Balbaa (2002) on *Tagetes minata* plant.

1-6 Effect of GA_3 and Kinetin on the fresh weight of racemes (g) *Origanum majorana* L.:

It was cleared that, the GA_3 level up to 100/150 mgL^{-1} in both seasons presented the highest racemes fresh weight of *O. majorana* plant (table 7 and figure 6). This increase may be due to GA_3 which enhanced elongation of many cut flowers, promote bud break, thus producing more flowering shoots on some omamental plants (Halvey, 1995). Kinetin concentrations showed significant effect on racemes fresh weight of *O. majorana* plant in the first season as compared with the control treatment. The differences between kinetin treatments were not significant in both seasons. This data coincided with Shedeed *et al.*, (1990) on *Ocimum basilicum*.

1-7 Effect of GA_3 and Kinetin on the fresh weight of branches (g) of *Origanum majorana* L.:

The results in table 8 and figure 7 showed that all GA_3 treatments had a significant effect on increasing the fresh weight of branches per plant as compared with the control. GA_3 at the rate of 150 mgL^{-1} gave the highest value. This may be due to increase the vegetative growth, plant height and number of branches. This data coincided with El-Sharkawy (1981) on *Origanum majorana* and Farooqi *et al.*, (1996) on *Artimisia arzura*.

Generally, data in table 10 and figure 7 clarified that branches weight of *O. majorana* increased by kinetin 50 and 100 mgL^{-1} in two seasons as compared with the other treatments of kinetin. This increase is a result of enhancing the growth of more lateral buds leading to more branches. The effect of kinetin can probably related to the action of cytokinin on the differentiation of areal parts since, the stimulation of shoot growth as a result of kinetin treatments has been occurred in *Asparagus officinalis* (Yang and Clore 1973) and Milbocker (1972) on poinsettia. These results were in agreement with the study of Zaltev (1979) on *Mentha piperita* and Farooqi *et al.*, (1998) on *Artimisia arzura*.

1-8 Effect of GA_3 and Kinetin on the total fresh weight (g) of *Origanum majorana* L.:

Data presented in table 9 and figure 8 showed that with increasing GA_3 concentration up to 150 mgL^{-1} , the total fresh weight of marjoram plant significantly increased in two cuts of the two seasons compared with the control. These increases in leaves fresh weight, racemes fresh weight, branches fresh weight and total fresh weight may be attributing to the increase of the number of both branches and leaves released from the apical dominance due to the GA_3 treatments (Shedeed *et al.*, 1990). This data coincided with Balbaa and Laila (2002) on *Lavendula officinalis* and Youssef (2004) on *Salvia officinalis*.

Generally, all kinetin treatments had a significant effect on marjoram total fresh weight per plant compared with the other treatments. These increases were a result of enhancing the growth of more lateral buds leading to more branches and more leaves number. The effect of kinetin can probably related to the action of cytokinin on the differentiation of areal parts (Yang and Clore 1973).These results were in agreement with Shedeed *et al.*, (1990) on Basil plant and Balbaa (2002) on *Tagetes minata* plant.

1-9 Effect of GA_3 and Kinetin on the leaf area (cm^2)/plant of *Origanum majorana* L.:

The data revealed in table 10, showed a significant effect of GA_3 treatments on leaf area of *Origanum majorana* L.. Whereas the highest value was obtained by GA_3 150 mgL^{-1} in the first cut of the both seasons (759.74, 546.19 cm^2). The Kinetin treatments had a highest effect on leaf area per plant of *Origanum majorana* L. and the values were increased in the second cut from each season. Generally, the highest values were obtained by using Kinetin at 50/100 mgL^{-1} in the two cuts of the both seasons. Cytokinins appeared to play an important role in the regulation of cell division, differentiation and organogenesis in developing plant (Skoog and Armstrong, 1970 and Hall 1973). These results were in agreement with the study of Shedeed *et al.*, (1990) on *Mentha piperita* and Mahmoud (1996) on *Ocimum basilicum*.

Table 6: Effect of GA_3 and Kinetin on the fresh weight of leaves (g) /plant of *Origanum majorana* L.

Treatment (mgL^{-1})	First Season		Second Season	
	First Cut	Second Cut	First Cut	Second Cut
Control	12.26^{c}	18.88^{def}	10.47^{de}	17.77^{ef}
GA_3 50	11.88^{c}	17.06^{ef}	10.86^{de}	18.66^{ef}
GA_3 100	13.65^{c}	25.16^{d}	14.38^{abc}	23.74^{cde}
GA_3 150	17.80^{b}	21.68^{de}	12.80^{bcde}	22.19^{ed}
GA_3 200	12.58^{c}	15.09^{f}	10.003^{e}	14.11^{f}
Kin. 10	22.23^{a}	32.84^{c}	12.28^{cde}	26.57^{bcd}
Kin. 20	23.37^{a}	33.16^{bc}	13.75^{bcd}	28.84^{bc}
Kin. 50	25.23^{a}	39.35^{ab}	17.40^{a}	35.15^{a}
Kin. 100	26.10^{a}	39.49^{a}	15.86^{ab}	30.58^{ab}

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

Table 7: Effect of GA_3 and Kinetin on the racemes fresh weight (g) / plant of *Origanum majorana* L.

Treatments (mgL^{-1})	First Season		Second Season	
	First Cut	Second Cut	First Cut	Second Cut
Control	15.11^{c}	-	7.18^{b}	-
GA_3 50	21.18^{ab}	-	10.2^{ab}	-
GA_3 100	22.47^{a}	-	12.81^{a}	-
GA_3 150	22.59^{a}	-	12.79^{a}	-
GA_3 200	17.87^{bc}	-	10.12^{ab}	-
Kin. 10	16.66^{bc}	-	8.01^{b}	-
Kin. 20	19.33^{abc}	-	8.45^{b}	-
Kin. 50	19.66^{abc}	-	9.33^{b}	-
Kin. 100	19.81^{ab}	-	7.47^{b}	-

Means with the same letter in the same column are not significantly different (P<0.05).
(-): Racemes not formed in the second cut for each seasons.

Table 8: Effect of GA_3 and Kinetin on the fresh weight of branches (g) /plant of *Origanum majorana* L.

Treatments (mgL^{-1})	First Season		Second Season	
	First Cut	Second Cut	First Cut	Second Cut
Control	6.65^{e}	7.41^{c}	5.22^{d}	13.83^{d}
GA_3 50	8.60^{cde}	16.25^{bc}	11.85^{a}	20.40^{abc}
GA_3 100	9.64^{bcd}	21.42^{ab}	12.11^{a}	22.31^{ab}
GA_3 150	12.75^{a}	27.76^{a}	12.83^{a}	22.99^{a}
GA_3 200	10.74^{abc}	12.06^{c}	11.66^{a}	18.42^{abcd}
Kin. 10	7.82^{de}	9.26^{c}	4.97^{d}	15.75^{d}
Kin. 20	9.03^{cde}	12.01^{c}	5.30^{cd}	17.59^{cd}
Kin. 50	12.37^{ab}	12.91^{bc}	7.09^{bc}	21.16^{abc}
Kin. 100	9.96^{bcd}	14.90^{bc}	7.27^{b}	17.88^{bcd}

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

Table 9: Effect of GA_3 and Kinetin on the total fresh weight (g)/plant of *Origanum majorana* L.

Treatments (mgL^{-1})	First Season		Second Season	
	First Cut	Second Cut	First Cut	Second Cut
Control	34.03^{e}	26.29^{d}	22.88^{e}	33.60^{d}
GA_3 50	41.66^{d}	33.32^{cd}	32.91^{c}	39.07^{cd}
GA_3 100	45.77^{cd}	46.59^{ab}	39.32^{a}	46.06^{bc}
GA_3 150	53.15^{ab}	49.44^{ab}	38.42^{ab}	45.19^{bc}
GA_3 200	41.20^{d}	27.15^{d}	31.79^{cd}	32.54^{d}
Kin. 10	46.72^{c}	42.11^{bc}	25.26^{e}	42.32^{bc}
Kin. 20	51.74^{b}	45.17^{ab}	27.52^{de}	46.44^{bc}
Kin. 50	57.27^{a}	52.27^{ab}	33.83^{bc}	54.31^{a}
Kin. 100	55.88^{ab}	54.39^{a}	30.61^{cd}	48.47^{ab}

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

Table 10: Effect of GA_3 and Kinetin on the leaf area (cm^2)/plant of *Origanum majorana* L.

Treatments (mgL^{-1})	First Season		Second Season	
	First Cut	Second Cut	First Cut	Second Cut
Control	563.47^{c}	805.80^{ef}	439.27^{e}	743.60^{def}
GA_3 50	507.01^{c}	728.20^{ef}	463.67^{de}	796.40^{ef}
GA_3 100	582.47^{c}	1073.70^{d}	542.79^{cd}	1013.20^{bcde}
GA_3 150	759.74^{b}	925.30^{de}	546.19^{cd}	947.10^{cde}
GA_3 200	537.09^{c}	643.90^{f}	426.78^{e}	602.40^{f}
Kin. 10	928.15^{ab}	1370.80^{c}	501.79^{cde}	1085.40^{bcd}
Kin. 20	975.83^{a}	1384.10^{bc}	561.84^{bc}	1178.40^{bc}
Kin. 50	1053.40^{a}	1642.80^{ab}	710.86^{a}	1435.90^{a}
Kin. 100	1089.64^{a}	1648.60^{a}	648.16^{ab}	1249.40^{ab}

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

2- Effect of GA_3 and Kinetin on the total chlorophyll (a and b) content (SPAD unit) of *Origanum majorana* L.leaves :

The results in table 11 and figure 10 showed that there were no significant effects between the GA_3 treatments on the total chlorophyll content of *Origanum majorana* L. at each cut in two seasons compared with the control. In addition, the results showed that there were significant effects of Kinetin treatments on the total chlorophyll content of leaves. Generally, the differences between all kinetin treatments within each cut were not significant.

Cytokinins appeared to play an important role in the regulation of cell division and increase plastids number, differentiation and organogenesis in developing plant (Skoog and Armstrong, 1970 and Hall 1973). These results are in agreement with Ahmed (1982) on chamomile plants.

3- Effect of GA_3 and Kinetin on oil percentage in *Origanum majorana* L.:

3-1 Effect of GA_3 and Kinetin on oil percentage in the leaves.

Oil yields from the leaves of *Origanum majorana* L.expressed in relation to fresh weight of the leaves were shown in table 12 and figure 11

as a percentage. GA_3 significantly increased the essential oil percentage and oil yield. Generally, GA_3 at the rate of 100 mgL^{-1} gave the highest oil percentage in both seasons. 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. It was found by Yousyida and Tamai (1958) that the volatile oil production increased by the increase in number of oil glands per unit area. Also, GA_3 increased the oil glands number (El-Sahhar *et al.*, 1984).

Similar results were found by Singh *et al.*, (1993) on *Rosmarinus officinalis*, Balbaa and Laila (2002) on *Lavendula officinalis*.

Data presented in table 12 and figure 11, cleared that, all kinetin treatments in two cuts of the two seasons significantly increased the oil percentage. The highest oil percentage found by kinetin 50 mgL^{-1} in the first season with the mean of 0.522- 0.387% and kinetin at the rate of 100 mgL^{-1} in the second season with the mean of 0.467- 0.431%. The increase in oil yield may be due to increase the number of leaves, leaf area and leaves fresh weight/plant and to the role of cytokinins in increasing monoterpene biosynthesis (El-keltawi and Croteau, 1987). Similar results were found by Hafez (1990) on marjoram plants and Mahmoud (1996) on *Ocimum basilicum*.

3-2 Effect of GA_3 and Kinetin on the oil percentage in the racemes of *Origanum majorana* L.:

The data in table 13 and figure 12 showed that oil percentage of *Origanum majorana* L. racemes significantly increased by GA_3 treatment. The highest percentage was obtained by using GA_3 at the rate of 100 mgL^{-1}. This increase may be due to the effect of GA_3 on elongation of cut flowers and promote bud break, thus producing more flowering shoots (Halvey 1995). This data coincided with Shedeed *et al.*, (1990) on *Ocimum basilicum*.

Data in table 13 and figure 12 showed that oil percentage of racemes significantly increased by using kinetin treatments. The highest percentage was obtained by using kinetin at the rate of 10 mgL^{-1}. In addition, the highest value was presented by using kinetin at the rate of 10 mgL^{-1} (0.656%).

Table 11: Effect of GA_3 and Kinetin on the total chlorophyll of *Origanum majorana* L. Leaves.

Treatments (mgL^{-1})	First Season		Second Season	
	First Cut	Second Cut	First Cut	Second Cut
Control	31.90^{b}	37.03^{b}	46.03^{bc}	51.26^{cd}
GA_3 50	35.73^{b}	36.60^{b}	48.26^{bc}	43.70^{e}
GA_3 100	37.66^{b}	39.56^{b}	49.10^{b}	45.76^{de}
GA_3 150	37.40^{b}	34.40^{b}	45.40^{bc}	54.90^{de}
GA_3 200	32.53^{b}	34.00^{b}	42.13^{c}	49.00^{cde}
Kin. 10	50.66^{a}	53.03^{a}	51.56^{ab}	54.40^{bc}
Kin. 20	51.23^{a}	53.83^{a}	51.66^{ab}	55.30^{bc}
Kin. 50	51.33^{a}	59.90^{a}	57.23^{a}	59.53^{ab}
Kin. 100	54.00^{a}	56.10^{a}	56.73^{a}	63.60^{a}

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

Table 12: Effect of GA_3 and Kinetin on the oil percentage of *Origanum majorana* L. Leaves.

Treatments (mgL^{-1})	First Season		Second Season	
	First Cut	Second Cut	First Cut	Second Cut
Control	0.292^{cd}	0.281^{ns}	0.340^{d}	0.241^{bcd}
GA_3 50	0.368^{bcd}	0.265^{ns}	0.309^{bcd}	0.254^{abcd}
GA_3 100	0.454^{ab}	0.286^{ns}	0.507^{a}	0.296^{abcd}
GA_3 150	0.491^{ab}	0.368^{ns}	0.397^{abcd}	0.182^{cd}
GA_3 200	0.248^{d}	0.248^{ns}	0.258^{cd}	0.156^{d}
Kin. 10	0.385^{bcd}	0.207^{ns}	0.441^{abc}	0.385^{ab}
Kin. 20	0.407^{abc}	0.232^{ns}	0.448^{abc}	0.388^{ab}
Kin. 50	0.522^{a}	0.387^{ns}	0.467^{ab}	0.431^{a}
Kin. 100	0.481^{ab}	0.292^{ns}	0.483^{ab}	0.343^{abc}

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

Table 13: Effect of GA_3 and Kinetin on the Oil Percentage of *Origanum majorana* L. Racemes.

Treatments (mgL^{-1})	First Season		Second Season	
	First Cut	Second Cut	First Cut	Second Cut
Control	0.388^{c}	-	0.313^{ab}	-
GA_3 50	0.473^{bc}	-	0.051^{c}	-
GA_3 100	0.549^{ab}	-	0.437^{a}	-
GA_3 150	0.510^{abc}	-	0.378^{ab}	-
GA_3 200	0.502^{abc}	-	0.300^{ab}	-
Kin. 10	0.656^{a}	-	0.298^{ab}	-
Kin. 20	0.621^{ab}	-	0.267^{ab}	-
Kin. 50	0.618^{ab}	-	0.240^{b}	-
Kin. 100	0.594^{ab}	-	0.234^{b}	-

Means with the same letter in the same column are not significantly different ($P<0.05$).
(-): Racemes not formed in the second cut for each seasons

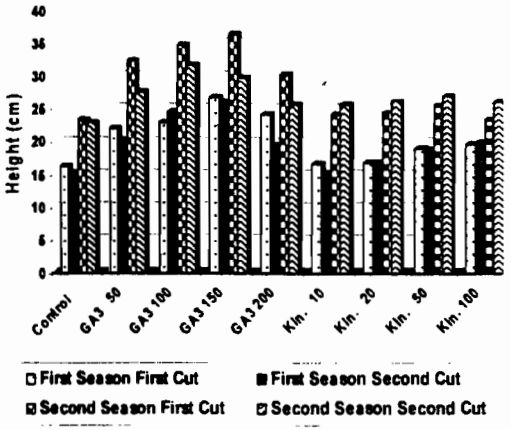


Figure 1. Effect of GA_3 and Kinetin treatments on *O. majorana* L.height (cm).

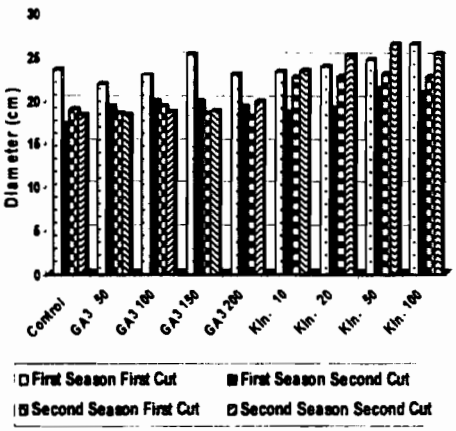


Figure 2. Effect of GA3 and Kinetin treatments on O. majorana L.diameter (cm).

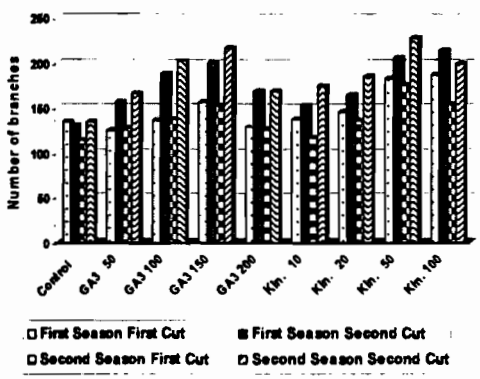


Figure 3. Effect of GA_3 and Kinetin treatments on number of branches of *O. majorana* L.

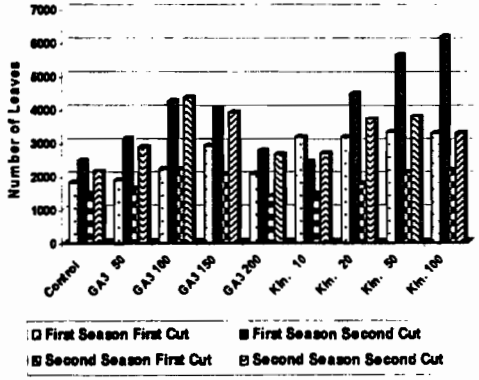


Figure 4. Effect of GA_3 and Kinetin treatments on number of leaves/plant of *O. majorana* L.

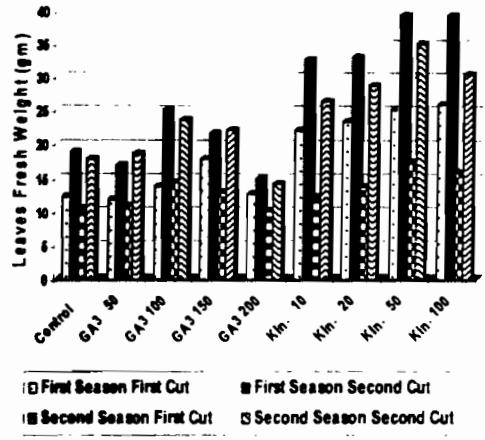


Figure 5. Effect of GA_3 and Kinetin treatments on fresh weight of leaves (gm)/plant *O. majorana* L.

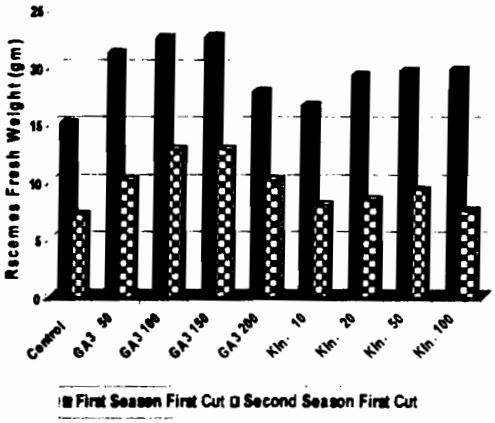


Figure 6. Effect of GA_3 and Kinetin treatments on fresh weight of racemes (gm) of *O. majorana* L.

Figure 7. Effect of GA_3 and Kinetin treatments on branches fresh weight (gm) of *O. majorana* L.

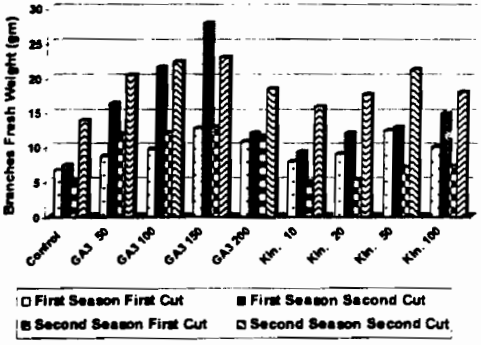
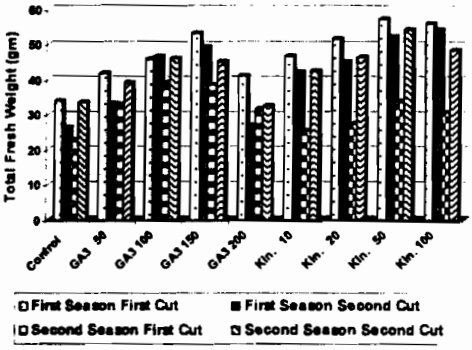


Figure 8. Effect of GA_3 and Kinetin treatments on total fresh weight (gm)/plant of *O. majorana* L



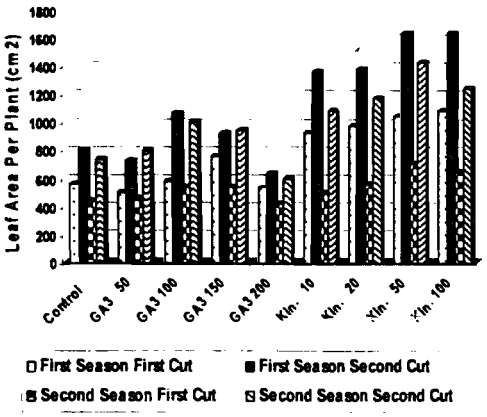


Figure 9. Effect of GA_3 and Kinetin treatments on leaves area/plant of *O. majorana* L.

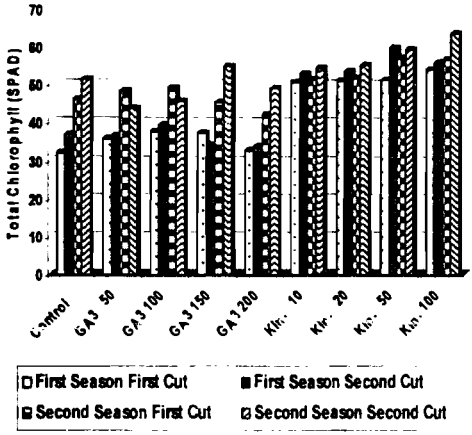


Figure 10. Effect of GA_3 and Kinetin treatments on total chlorophyll content of fresh weight of *O. majorana* L.

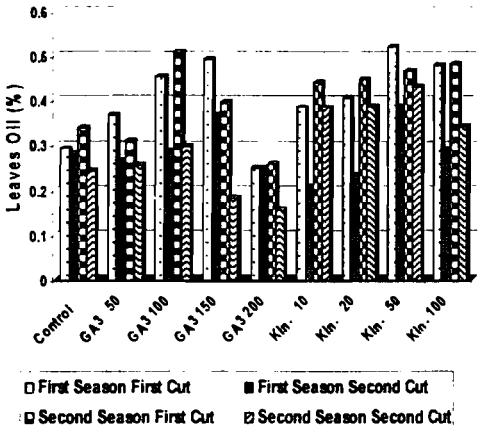


Figure 11. Effect of GA_3 and Kinetin treatments on oil percentage of *O. majorana* L.Leaves.

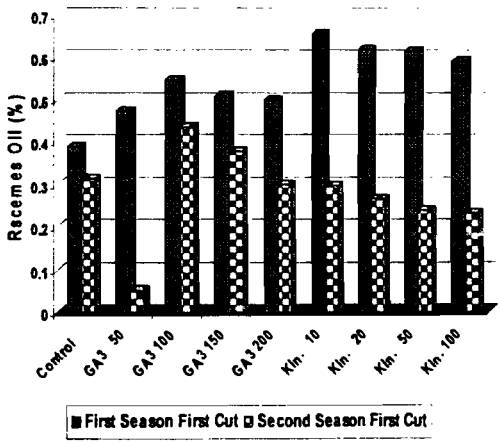


Figure 12. Effect of GA_3 and Kinetin treatments on oil percentage of *O. majorana* L. racemes.

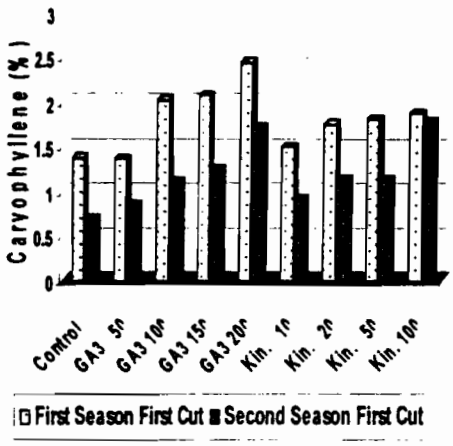


Figure 13. Effect of GA_3 and Kinetin treatments on caryophyllene percentage of *M. hortensis* leaves.

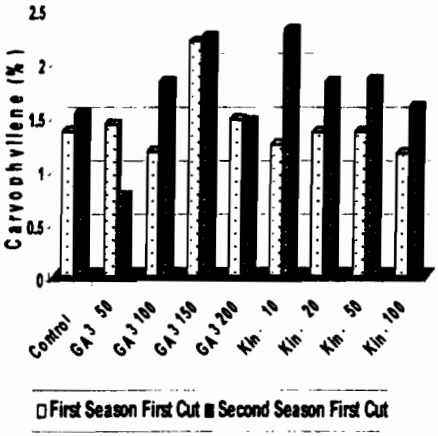


Figure 14. Effect of GA_3 and Kinetin treatments on caryophyllene percentage of *M. hortensis* racemes.

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الملخص العربى

دراسات فسيولوجية على نباتات البردقوش

١- تأثير حامض الجبيريلليك والكينيتين على النمو وإنتاج الزيت

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قسم الزهور ونباتات الزينة وتنسيق الحدائق

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اجري هذا البحث في حدائق أبحاث الزهور ونباتات الزينة بقسم الزهور ونباتات الزينة وتنسيق الحدائق كلية الزراعة-جامعة الإسكندرية خلال الأعوام ٢٠٠٥/ ٢٠٠٦ ويهدف هذا البحث الى دراسة تأثير تركيزات مختلفة من منظمات النمو (حامض الجبريللين بتركيزات ٥٠- ١٠٠ - ١٥٠- ٢٠٠ مجم/لتر و الكينيتين بتركيزات ١٠- ٢٠-٥٠ - ١٠٠ مجم/لتر) على النمو الخضري والزيت العطري لنباتات البردقوش وتم رش نباتات المقارنة بماء مقطر. ومن أهم النتائج المتحصل عليها وجد أن استخدام تركيز ١٠٠و١٥٠ مجم/لتر من الجبريللين أعطي نباتات أكثر ارتفاعا وكذلك اكبر عدد من الأفرع/نبات في كلا الموسمين و اكبر عدد من الأوراق وأثقل الأوزان الطازجة لكل نبات وأشارت النتائج الى أن أعلى نسبة زيت في الأوراق والأزهار حصل عليها برش النباتات بتركيز ١٠٠ مجم/لتر من الجبريللين في كل من الموسمين. كذلك وجد أن استخدام الكينيتين بتركيز ٥٠ و ١٠٠ مجم/لتر أعطي اكبر قطر للنبات و اكبر عدد من الأفرع لكل نبات و اكبر عدد من الأوراق لكل نبات و أثقل وزن للنورات و أثقل وزن كلى طازج و اكبر مساحة ورقية واكبر محتوى كلى للكلوروفيل في حين أن أعلى نسبة زيت في النورات حصل عليها باستخدام كينيتين ١٠ مجم/لتر في كل من الموسمين.