Annals Agric. Sci., Ain Shams Univ., Cairo, 50(1), 15-29, 2005

ENHANCEMENT OF THE CHEMICAL COMPOSITION AND THE YIELD OF ANISE SEED *(Pimpinella anisum L.)* OILS AND FRUITS BY GROWTH REGULATORS

[2]

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ABSTRACT

Field experiments for two seasons were conducted to study the effect of spraying the growth regulators GA_3 at 0, 50, 75 and 100 ppm and Kinetin at 0, 25, 50 and 75 ppm separately at pre-flowering and pre-setting stages, on the yield and oils quality of anise plant seeds. Fruit yield, essential and fixed oils percentage in addition to their physical and chemical properties were determined. The main components of seed oils were investigated by gas chromatography (GC). The results reveal that, GA_3 at 50 and 75 ppm and Kinetin at 25 and 50 ppm, increased the dry seed yield and the essential oil percentage significantly, while the total lipids percentages were slightly influenced. The acid number of anise seed fixed oil increased at the high doses of applied growth regulators, while the ester, saponification, iodine and peroxide were around control values. GC chromatographic analyses of anise fixed oil were not influenced by the growth regulator treatments. GA_3 at 100 ppm increased the saponification value of essential oil, while all concentrations of GA_3 in addition to kinetin at 25 and 50 ppm significantly lowered the acid value concomitant with an increase in ester and saponification values. GC analysis of anise essential oil manifested anethol and methyl chavicol as main constituents; also both the treatment with GA_3 at 50 ppm and kinetin at 25 and 50 ppm increased the major constituent anethol at different extents.

Key words: Anise (*Pimpinella anisum*), GA_3, Kinetin, Fixed oil, Essential oil, Gas chromatography (GC)

INTRODUCTION

Anise is one of the medicinal and aromatic plants cultivated in Egypt and some European countries, which reputed to posses several medicinal properties. It is an annual herb, with an erect, cylindrical, striated, smooth stem, arising to the height of 50 cm. The dried ripe fruits known as anise and/or aniseed are the part used in traditional medicine as they contain up to 3.5% essential oil, of which anethol is represent the major constituent (**Mahran, 1967**). Regulation of the con-

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(Received April 2, 2005)
(Accepted April 20, 2005)

ditions that limit the growth of plant should be useful in controlling terpenes accumulation (**Calvo and Sanchez, 1993**). Moreover, **Mahmoud *et al* (1996)** found that GA_3 application stimulate plant growth. Also, treated sweet basil with GA_3, IAA and kinetin individually at 50 ppm after two weeks of transplanting enhance plant growth. However, GA_3 treatment decreased oil percentage while IAA and Kinetin promote it. Also, **Bendari *et al* (1995)** noted that (*Ocimum basilicum L.*) plants when sprayed 3 times with 100 ppm GA_3, showed an increase in growth parameters and oil percentage. **Farooqi *et al* (1996)** observed that, the highest essential oil percentage and the yield resulted at flowering stage, were enhanced by the applications of kinetin (10 or 20 ppm) and GA_3 (50 ppm), in addition to a significant increase in dry weight production. Application of 250 ppm GA_3 to shade grown plant (Patchouli cv. Johor), improved most of growth Parameters, photosynthetic pigment contents and essential oil yield (**Misra, 1995**). **Kurapov *et al* (1999)** studied the phytohormonal balance and components of the essential oil in 5 varieties of peppermint (*Mentha piperita*) during vegetative growth over a 3-year period. They concluded that, there was a relationship between concentration of menthol, menthyl acetate and menthone in the essential oil on one hand and the content of GA_3 and ABA in the leaves on the other hand. Treatment of plants with the growth regulators, which inhibited endogenous gibberellins synthesis, stimulated the synthesis of menthol, menthyl acetate and/or ABA. **Binder and Abou Mandour, (2000)** treated *Melissa officinalis L.* with 10 mg benzyl adenine/liter and they found an increase in the oil percentage from 1.3-to 2.4-mg/g dry weight. They emphasized also that ABA treatment reduced the accumulation of monoterpenes and enhanced sesquiterpene synthesis. **Sarwat and El Hady, (2000)** found that, treatment with GA_3 at pre-flowering time followed by ABA at pre-setting time on caraway plants increased the essential oil percentage. Their application significantly increased the major constituent (carvone) of the essential oil by 29.01% (from 63.24% to 92.25%).

The objectives of this study are to evaluate the effect of GA_3 and kinetin by spraying them separately at different concentrations on anise plant (*Pimpinella anisum L.*) at pr-flowering and pre-setting times aiming to improve the content of both fixed and essential oils together with their chemical compositions in the anise seeds.

MATERIAL AND METHODS

1. Materials

1.1. *Plant Material:* Anise seeds (*Pimpinella anisum L.*) were obtained from the Medicinal and Aromatic Section, Horticulture Research Center, Dokki, Ministry of Agriculture, Cairo, Egypt.

1.2.*Chemical Material*: Gibberellic acid and Kinetin were obtained from SIGMA Chemical Company.

2. Methods

2.1. *Field Experiments*: The field experiments have been conducted at the experimental farm of the research center for tow seasons. The anise plants were cultivated in October after the field had

been prepared for planting as recommended by **El-Tantawy *et al* (1992)**. The plants were twice sprayed with GA_3 at 0, 50, 75 and 100 ppm and kinetin at 0, 25, 50 and 75 ppm at pre-flowering (December), followed by spraying with the same concentrations of growth regulators at pre-setting stage (February). Seeds were gathered at maturity stage (June) then subjected to chemical analysis.

2.2. *Chemical analysis*: Grains of anise were crashed; crude fat was extracted using diethyl ether through Soxhlet apparatus, while the essential oil was extracted by steam distillation according to **A. O. A. C. (1990).**

2.2.1. *Physical and chemical properties of oils*: The physical and chemical properties of the oil were measured as follow:

- Refractive index according to **Jacobs (1959).**
- Specific optical rotations were measured by Hilgerpolarimeter.
- Acid, Saponification, Ester, peroxide and Iodine values according to **A.O.A.C. (1990).**

Data represented means and standard deviation for three replications. Statistical analysis has been carried out according to **Snedecor and Cochran (1980).**

2.2.2. Gas Chromatographic analysis (GC) of oils

2.2.2.1. *Fatty acids elucidation and percentage*: Fatty acids methyl esters derived from crude oil were analysed with Shimadzu GC-14A Gas Chromatography on 30-meter (0.32-mm) glass capillary column coated with (OMEGA WAX 320) equipped with (FID) detector. Injection and detector temperature were 230°C, temperature program was 100 °C increased by 7 °C/min till 191 °C and still for 31 min. Fatty acids percentage were expressed as percentage of total fatty acid methyl esters. The authentic samples of fatty acids methyl esters were also injected under the same conditions for the identification of fatty acids.

2.2.2.2. Essential oil elucidation and percentage: A Varian Model 3700 gas chromatograph equipped with FID investigated the essential oil. Glass column OV 101-W-HP 80/100, coated with 15% PEGA was used. Oven temperature was increased by 15 °C/min. from 100 °C to 220 °C and then isothermally for 40 min. Detector and injector temperature were 250 °C and 230 °C respectively. Gases flow rate was 17, 30 and 300 ml/min. for N_2, H_2 and air respectively. The authentic samples of anethol, methyl chavicol, anisaldehyde, carvacrol, limonene, linalool and α-pinene were also injected under the same conditions for identification of constituents of the anise essential oil.

RESULTS AND DISCUSSION

1. Fruit yield, essential oil and crude lipids concentrations

Data listed in Table (1) demonstrate the effect of growth regulators GA_3 at (0, 50, 75 and 100 ppm) and kinetin at (0, 25, 50 and 75 ppm) on dry seed yield, essential oil concentration and total crude lipids percentage of anise crop.

1.1. Air dried fruit yield : It is obvious from the obtained data that the treatment with GA_3 at 50 ppm showed slight

Table 1. Effect of GA_3 and Kinetin on dry fruit yield, essential oil and total crude lipids percentage of anise (*Pimpinella anisum* L.)

Treatment	First Season			Second Season		
	Air Dried Fruit yield Kg/Fed. M ± SD	Essential oil % M ± SD	Total lipids % M ± SD	Air Dried Fruit yield Kg/Fed. M ± SD	Essential oil % M ± SD	Total lipids % M ± SD
Control	682.5 ± 75.7	3.40 ± 0.130	14.0 ± 0.19	696.3 ± 71.7	3.45 ± 0.083	14.3 ± 0.15
GA_3 50 ppm	690.5 ± 84.3	5.70 *** ± 0.15	19.20 *** ± 0.37	682.1 ± 82.5	5.73 *** ± 0.098	18.8 *** ± 0.27
GA_3 75 ppm	525 *** ± 90.8	5.27 *** ± 0.052	18.33 *** ± 0.45	611.0 ± 80.3	5.39 *** ± 0.075	18.7 *** ± 0.33
GA_3 100 ppm	546 ± 87.4	3.20 ± 0.082	12.90 * ± 0.028	553.3 *** ± 86.2	3.55 ± 0.101	14.5 ± 0.10
Kinetin 25 ppm	693 ± 69.7	4.60 ** ± 0.02	14.60 ± 0.023	733.5 ± 62.3	4.39 *** ± 0.030	15.2 * ± 0.16
Kinetin 50 ppm	745.5 ± 92.3	5.70 *** ± 0.043	14.50 ± 0.112	768.2 ± 87.1	5.71 *** ± 0.020	15.1 ± 0.011
Kinetin 75 ppm	656 ± 88.2	3.90 * ± 0.063	11.90 ** ± 0.028	676.3 ± 86.5	3.31 ± 0.55	12.4 *** ± 0.031

- GA_3 and Kinetin sprayed for two times at both pre-flowering and pre-setting.
- Values represented means of triplicate analysis.
- M ± SD = Mean and Standard Deviation ($P < 0.05$).

variations around control values while a marked decrease was observed at 75 ppm (23% for 1st season) and at 100 ppm (12.25% for 2nd season). Also at 100 ppm (19.9% for 1st season and 20.5.8% for 2nd season) were obtained. Kinetin treatment at 25 and 50 ppm showed a slight increase, which did not exceed than 9.23% 1st season and 10.32% 2nd season.

1.2. Essential oil percentage: It is clear from the data presented in Table (1) that there was a significant increase in the essential oil percentage induced by GA_3 treatment at 50 ppm (67.6% for 1st season and 66% for 2nd season) also at 75 ppm (55% for 1st season and 56.23% for 2nd season). The same trend was observed with kinetin treatment at 50 ppm (67.6% for 1st season and 65.5% for 2nd season).

1.3. Total crude lipids percentage: Concerning with total crude lipids concentrations, data reveal that the treatment with GA_3 at 50 ppm showed an increment (37.14% for 1st season and 31.46% for 2nd season) also at 75 ppm (30.92% for 1st season and 30.76% for 2nd season). On

the other hand, kinetin treatment at both 25 and 50 ppm resulted in no significant increment, while at 75 ppm kinetin treatment decreased the total lipids percentage (18.5% for 1st season and 18.4% for 2nd season). However, these results are in agreement with those obtained by **Mahmoud *et al*** (1996) who found that GA_3, IAA and kinetin at 50 ppm stimulated sweet basil (*Osimum basilicum L.*) growth. Also **Bendari *et al*** (1995) stated that GA_3 treatment increased the growth parameter and oil percentage of the same plant. On the other hand **Farooqi *et al*** (1996) found that kinetin treatment at (10 or 20 ppm) on *Artemisia annua L,* increased essential oil percentage and yield. They also fond that GA_3 at 50 ppm induced an increase in essential oil yield and a significant increase in dry weight production.

It could be deduced from these obtained data that GA_3 at both concentrations (50 and 75 ppm) also kinetin at (25 and 50 ppm) increased the dry seed yield and the essential oil percentage significantly, while the total lipids percentage was slightly affected.

2. Effect of growth regulators on the chemical properties and fatty acids composition of anise seed fixed oil

2.1. Chemical properties of anise seed fixed oil : Data presented in Table (2) show that the acid number of anise seed fixed oil increased significantly due to application of GA_3 at 100 ppm (1.80 vs 1.10 for 1st season and 1.87 vs 1.15 for 2nd season) while kinetin showed around control values. No general trend could be concluded concerning the role of the applied growth regulators on the acid value of anise seed fixed oil. It could be deduced that the growth regulators under investigation affected the secondary metabolism of the plant, co-related to the total percentage of the short chain free fatty acids as shown in Table (3 and 4). At the same time, the obtained data listed in Table (2) show also that, the ester, the saponification, the iodine and the peroxide values were not significantly changed by the application of the investigated growth regulators for the two seasons.

The present study concentrated on the evaluation of the quality of anise fixed and essential oils in the second season yield, since, data obtained from the 1st season were confirmed by those obtained from the 2nd season.

2.2. Fatty acids composition : One of the criteria for the determination of the fat quality is the percentage of the essential fatty acids (poly unsaturated fatty acids) i.e. linoleic, arachidic and lignoceric acids as well as oleic as unsaturated fatty acid which represent the common unsaturated fatty acids in oils. The importance of these essential fatty acids are arising from their role in diet to prevent fatty acids deficiency, which related to skin dryness, poor hair growth and its low rate growth (**Kinsella, 1987**).

GC of fatty acids methyl ester derived from anise seed fixed oil, are reported in Table (3 and 4) including the identified fatty acids existing in the anise seed fixed oil in relative concentrations. The GC analysis revealed that, the predominant fatty acids in anise seed fixed oil are oleic, linoleic and palmitic acids. Based on the data obtained from the GC analysis (Table 3), other fatty acids, myristic, stearic and arachidic acids were recognized as a minor constituents of anise seed fixed oil in the untreated and treated

Table 2. Effect of GA_3 and Kinetin on the Chemical Properties of Anise (*Pimpinella anisum L.*) Seed Fixed Oil in Two Seasons

Treatment	First Season					Second Season				
	Acid value M ± SD	Ester value M ± SD	Sapon. value M ± SD	Iodine value M ± SD	Peroxide value M ± SD	Acid value M ± SD	Ester value M ± SD	Sapon. value M ± SD	Iodine value M ± SD	Peroxide value M ± SD
Control	1.10	177.16	178.26	87.0	7.60	1.15	179.73	178.59	89.23	7.91
	±	±	±	±	±	±	±	±	±	±
	0.000051	1.625	1.243	1.437	0.00031	0.000031	1.598	1.387	1.277	0.000088
GA_3	1.20 *	177.14	178.34	88.5	7.71 *	1.21	176.15	177.47	87.66	7.85
50 ppm	±	±	±	±	±	±	±	±	±	±
	0.00003	1.312	1.258	1.298	0.00032	0.000038	1.234	1.511	1.452	0.00044
GA_3	1.30 **	176.99	178.59	90.5 **	7.82 **	1.53 ***	175.87 *	178.60	89.93	7.75 **
75 ppm	±	±	±	±	±	±	±	±	±	±
	0.00028	0.731	1.101	1.375	0.00020	0.00034	1.351	1.623	1.337	0.000073
GA_3	1.80 ***	177.01	178.81	93.3	7.82 **	1.87 ***	179.34	179.73	95.40 ***	7.83
100 ppm	±	±	±	±	±	±	±	±	±	±
	0.00013	1.243	0.981	1.285	0.0020	0.000151	1.587	1.223	1.226	0.00037
Kinetin	1.40 **	176.07	177.47	95.8 ***	7.90 ***	1.24	175.34 *	177.43	83.75 ***	7.88
25 ppm	±	±	±	±	±	±	±	±	±	±
	0.00032	1.253	1.015	1.363	0.00021	0.000022	1.187	1.278	1.663	0.00054
Kinetin	0.99 **	177.10	178.09	87.0	7.50	1.23	176.33**	176.32	89.22	8.32 ***
50 ppm	±	±	±	±	±	±	± 1.445	±	±	±
	0.00001	1.113	1.187	1.255	0.00036	0.000077		1.488	1.276	0.000037
Kinetin	1.20 *	176.38	177.58	99.0 ***	8.94 ***	1.44 ***	178.57	179.37	103.59 ***	8.44 ***
75 ppm	±	±	±	±	±	±	±	±	±	±
	0.00011	1.258	1.231	1.511	0.0010	0.000037	1.543	1.337	1.556	0.00019

- Values represented means of triplicate analysis
- M ± SD = Mean and Standard Deviation ($P < 0.05$)

Table 3. Relative amount of anise seed fixed oil constituents from GC of control and treated plants with GA_3 at pr-flowering and pre-setting times (data obtained from the second season)

Peak No	Components	Control	GA_3 50 ppm	GA_3 75 ppm	GA_3 100 ppm
1	**Caprylic acid**	0.00	1.04	1.66	0.40
2	Unknown (a)	0.00	0.28	0.00	0.00
3	Capric acid	0.23	1.06	0.00	0.00
4	Lauric acid	0.56	4.99	0.00	1.95
5	Myristic acid	2.32	6.08	2.73	1.86
6	Unknown (b)	0.12	0.00	0.00	0.00
7	Unknown (c)	0.51	0.50	0.99	0.00
8	Unknown (d)	0.13	0.00	0.00	0.00
9	Palmitic acid	13.5	9.42	13.16	14.47
10	Unknown (e)	0.88	0.00	0.00	0.00
11	Unknown (f)	0.51	0.00	0.30	1.72
12	Stearic acid	2.10	0.00	1.68	1.90
13	Oleic acid	55.74	54.74	54.40	53.17
14	Linoleic acid	19.86	21.19	24.28	24.53
15	Arachidic acid	0.60	0.70	0.80	0.00
16	Lignoceric acid	2.94	0.00	0.00	0.00

Table 4. Relative amount of anise seed fixed oil constituents from GC of control and treated plants with Kinetin at pr-flowering and pre-setting times (data obtained from the second season)

Peak No	Components	Control	Kinetin 25 ppm	Kinetin 50 ppm	Kinetin 75 ppm
1	**Caprylic acid**	0.00	0.00	0.00	0.00
2	**Capric acid**	0.23	0.00	0.00	0.00
3	Lauric acid	0.56	0.00	1.06	0.00
4	Myristic acid	2.32	0.40	2.07	1.40
5	Unknown (a)	0.12	0.00	0.00	0.00
6	Unknown (b)	0.51	0.00	0.00	0.00
7	Unknown (c)	0.13	0.00	0.00	0.00
8	Palmitic acid	13.5	5.18	13.20	8.87
9	Unknown (d)	0.88	0.00	0.00	0.00
10	Unknown (e)	0.51	1.77	0.74	0.97
11	Stearic acid	2.10	0.00	2.98	0.88
12	Oleic acid	55.74	67.51	58.83	64.88
13	Linoleic acid	19.88	25.14	19.37	22.70
14	Arachidic acid	0.60	0.00	0.00	0.30
15	Lignoceric acid	2.94	0.00	1.75	0.00

plants with GA_3, while capric and lignoceric fatty acids were disappeared. The same trend was observed in the data obtained in Table (4) dealing with the plants treated with kinetin

The effect of exogenous GA_3 on the fatty acid compositions could be observed in Table (3) where at 50 ppm the relative amount of capric, lauric, myristic and linoleic fatty acids increased, while palmitic, stearic and lignoceric fatty acids were decreased. On the other hand, at 75 and 100 ppm of GA_3 treatment the amounts were normal around the control values. A same trend was deduced from the data presented in Table (4) when the plants were treated with kinetin.

Based on the obtained data, it could be concluded that, the chemical properties and fatty acid composition of anise seed fixed oil from untreated and treated plants with growth regulators under investigation were rather analogous to each other in the major essential components confirming the role of growth regulators on the secondary metabolism of the plants.

3. Effect of growth regulators on physical and chemical properties of anise seed Essential oil and its composition

3.1. Physical and chemical properties of anise seed essential oil: Table (5) represents the investigated physical and chemical properties of anise seed essential oil. The saponification value of the essential oil extracted from seeds of treated plants with 100 ppm GA_3 showed an increment while the acid value of GA_3 treatment at 50, 75 and 100 ppm in addition to kinetin treatment at 25 and 50 ppm were significantly lower than those of the untreated anise plants concomitant with an increment in ester and saponification values as shown by all GA_3 treatments and kinetin at 75 ppm treatment. Meanwhile, no significant differences were observed in the refractive index. On the contrary, significant differences were observed especially at 75 ppm and 100 ppm for GA_3 and at 50 ppm and 75 ppm for kinetin.

3.2. Composition of anise seed essential oil: Data summarized in Table (6) show the effect of growth regulators under investigation (GA_3 and kinetin) on the terpenoids percentage of anise seed essential oil. By following the major constituents of anise seed essential oil e.g. anethol, methyl chavicol and anisaldehyde, the data obtained expressed a relative increment of anethol at 50-ppm concentration of GA_3 and kinetin at 50 ppm also.

However our results are in agreement with those of **Ghosh *et al* (1990)** findings, when they treated *Palma rosa* grass with GA_3 at 50 ppm. They found that GA_3 enhanced essential oil synthesis and accelerated the synthesis of geraniol. Also, **El-Khateeb *et al* (1991)** emphasized that GA_3 treatment decreased terpenols percentage in *Ruta graveolens L.* plants. A relationship was found between the concentration of menthol, menthyl acetate and menthone in the essential oil on one hand and the percentage of GA_3 and ABA in the leaves of peppermint plant on the other hand. Treatment with GA_3 on peppermint plants inhibited endogenous gibberellins synthesis and stimulated the synthesis of menthol, menthyl acetate and menthone and/or ABA (**Kurapov *et al* 1999**).

Table 5. Effect of growth regulators GA_3 and Kinetin treatment at pre-flowering and pre-setting times stages on the physical and chemical properties of anise seed essential oil

Treatment	Optical Rotation	Refractive Index	Acid Value	Ester Value	Sapon. Value
Control	+ 1.25	1.556	4.89	23.5	28.39
M ± SD	± 0.00063	± 0.004	± 0.076	± 0.0042	± 0.031
GA_3 50 ppm	+ 1.18 *	1.553	2.97 ***	27.6 ***	30.59 **
M ± SD	± 0.00082	± 0.003	± 0.065	± 0.0078	± 0.081
GA_3 75 ppm	+ 1.20 **	1.551	2.98 ***	27.9 **	30.88 **
M ± SD	± 0.00031	± 0.0023	± 0.014	± 0.0078	± 0.080
GA_3 100 ppm	+ 1.33 ***	1.550	3.21 **	30.01 ***	33.22 ***
M ± SD	± 0.0024	± 0.00098	± 0.056	± 0.0069	± 0.025
Kinetin 25 ppm	+ 1.26	1.555	3.01 **	23.21	26.22 **
M ± SD	± 0.0087	± 0.0011	± 0.034	± 0.0057	± 0.067
Kinetin 50 ppm	+ 1.22 ***	1.554	3.12 **	21.55 **	23.67 ***
M ± SD	± 0.00097	± 0.0029	± 0.03	± 0.0077	± 0.032
Kinetin 75 ppm	+ 1.38 ***	1.554	5.66 ***	32.09 ***	36.75 ***
M ± SD	± 0.00087	± 0.0076	± 0.021	± 0.0066	± 0.072

($P < 0.05$)

The effect of growth regulators under investigation on anise seed essential oil percentage could be summarized as follow:

Spraying GA_3 at 50 ppm gave an increment of anethol percentage (10.4%) concomitant with decrement of anisaldehyde percentage (68.5%) and slight effect on methyl chavicol. The same trend could be observed by the treatment of kinetin at 50 ppm, which gave an increment of anethol percentage (17.2%) concomitant with a decrement (63.6%) of anisaldehyde percentage and methyl chavicol too. Hence, the two investigated growth regulators under the applied conditions (twice and separately sprayed on anise plants at pre-flowering time and at pre-setting stages) are resulted in the increment of the major constituent of anise seed essential oil (anethol) on the account of the two other major constituents (anisaldehyde and methyl chavicol).

Concerning the effect of growth regulators under investigation on anise seed essential oil (terpenic compounds) percentage and quality, it could be deduced the following aspects:

Table 6. Relative amount of anise seed essential oil constituents from GC of untreated and treated plants with GA_3 and kinetin at pre-flowering and pre-setting stages (data obtained from the second season)

Peak No	Components	Control	GA_3			*Kinetin*		
			50 ppm	75 ppm	100 ppm	25 ppm	50 ppm	75 ppm
1	α-pinene	0.19	0.50	1.55	0.57	0.39	0.30	0.28
2	Limonene	1.31	1.29	1.24	2.14	2.12	3.39	0.28
3	Linalool	1.90	1.17	0.93	0.54	0.78	0.92	0.05
4	Methylchavicol	21.13	20.75	22.6	24.6	22.80	13.80	8.63
5	Anethol	65.00	71.80	63.49	52.84	68.80	76.23	61.87
6	Anisaldehyde	5.50	1.73	4.04	3.57	1.04	2.00	8.63
7	Unknown (a)	0.60	0.34	0.31	0.28	0.26	0.30	0.28
8	Unknown (b)	0.77	0.51	0.93	0.57	0.52	0.77	1.72
9	Unknown (c)	0.72	0.51	0.56	0.85	0.38	0.30	4.60
10	Carvacerol	2.88	2.40	4.35	5.01	2.91	1.99	13.66

A- The important actions of the **gibberellins** are the initiation of the synthesis of the various hydrolytic and proteolytic enzymes upon which seed germination and seeding establishment depend. The growth effect of gibberellins arises by cell elongation in the sub-apical meristem regions where young internodes are developing in addition to changing of the number of glandular hairs. While gibberellins are concerned largely with cell enlargement **Kinetin** has more specific effect on cell division and also regulate the pattern and frequency of organ production as well as position and shape (**Runeckles, 1974**).

B- Terpenic compounds are essential part of the secondary metabolism in the medicinal plants, which are responsible for defense against predators and pathogens and for attraction of pollinators and seed disperse. Biosynthesis of terpenic compounds is done in plastids while the most important is done in glandular trichomes in leaves and in glandular epidermis of flower petals by certain types of enzymes (**Rodney *et al* 2000**).

C- Based on the above mentioned, It could be ascribe the changing of the anise seed essential oil percentage and composition to the effect of gibberellins on the number of the glandular hairs i.e. one of the two places for the biosynthesis of terpenic compounds in addition to its effect of the enzymes. Also, the effect of kinetin on the essential oil could attribute to its effect on the cell division and increasing the cell number, which lead to the increasing of the plastids content. Hence, the essential oil content influence dramatically by increasing the plastids number i.e. the second place for the biosynthesis of terpenic compounds. In addition to the effect of kinetin on organs formation i.e. leaves and flowers which consider the factories for plant materials including terpenoids.

Conclusion: Anise is one of the medicinal plants cultivated in Egypt, which is reputed to posses several medicinal properties which are useful in traditional medicine and confirmed by modern medicine. A decoction of the dried ripe fruits is taken as a cure for emmenia (menses) withheld, for cough whooping cough, for pneumonia, for kidney insufficiency and for the intestinal colic (Medical Encyclopedia). The used part is the dried ripe fruits, which contain up to 3.5% essential oil, of which anethol is represent the major constituent (**Mahran, 1967**). Hence, the obtained results, which achieved the objectives of this study, could be summarized as follow:

1- Significant increment in the essential oil percentage expressed by GA_3 treatment at 50 ppm 67.64% for 1st season and 66% for 2nd season also at 75 ppm 55% for 1st season and 56.23% for 2nd season. The same trend was observed with kinetin treatment at 50 ppm.

2- A relative increment of anethol, the major constituent of anise seed essential oil was obtained in case of GA_3 at 50 ppm (10.4%) and kinetin at 50 ppm (17.2%) application.

3- The treatment with kinetin at 50 ppm give increment of the fruit yield (10%) while the treatment with GA_3 at 50 ppm showed around control values (has no effect).

4- GA_3 application at 50 ppm increased the total crud lipid significantly (37%) in addition to better quality by increasing the percentage of the essential fatty acid (linoleic 22% at 75 ppm and 23.5% at 100 ppm), while, the kinetin application at 25 ppm gave the highest content of the linoleic acid (26.4%) in addition to high increment of oleic acid (21.1%).

5- Applying of GA_3 and/or kinetin at 50 ppm are economically profitable where they increased the fruit yield in addition to highest content of essential and fixed oils moreover high quality in return for low concentration of them **as we recommend as follow:**

5.1. For the fruit yield increment; kinetin application at 50 ppm is the best.

5.2. For highest content of essential oil in addition to better quality by increasing anethol percentage; kinetin and/or GA_3 at 50 ppm is recommended.

5.3. The highest quantity of fixed oil in addition to increment of the essential fatty acid linoleic moreover oleic could be recommended by the application of GA_3 at 50 ppm and kinetin at 25 ppm respectively.

REFERENCES

A.O.A.C. (1990). Officials Methods of Analysis, ***Association of Official Analytical Chemists 15th Ed.*** Arlington, Virginia, U.S.A.

Bendari, H. A. L.; M. S. Aly and F. A. Nagwan (1995). Effect of Foliar Application of GA_3 and Zn on *Osimum basillicum* L. Growth in Different Soil Types. ***Egyptian-Journal of Physiological Sciences, 18 (2): 365-380.***

Binder, G. and A. A. Abou Mandour (2000). Regeneration of Plants and Production of Volatiles from Callus Cultures of *Melissa Officinalis* L. Investigations on the Subspecies Altissima. ***Angewandte Botanik, 74 (1-2): 26-31.***

Calvo, M.C. and G.M. Sanchez (1993). Accumulation of Monoterpenes in Shoot-Proliferation Cultures of *Lavandula latifolia Med.* ***Plant Science Limerick, 19 (2): 207-212.***

El-Khateeb, M.A.; E.M. Badawy and N.A.E. Boselah (1991). Effect of Some Growth Regulators on Growth, Chemical Composition and Essential Oil of *Ruta graveolens L.* plants. ***Bulletin of Faculty of Agriculture, Cairo University, 42(3): 829-848.***

El-Tantawy, A.; M.S. Hanafy and S.M. Selim (1992). Effect of Nitrogen Fertilization and Gibberellic Acid on the Growth of Caraway (*Carum Carvi L.*) Plants. ***J. Agric. Res. Tanta Univ., 18 (4): 728-740.***

Farooqi, A.H.A.; S. Abha; S. Srikant-Sharma; K. Asifdulla; A. Shukla; S. Sharma and A. Khan (1996). Effect of Plant Age and GA_3 on Artemisinin and Essential Oil Yield in ***Artemisia annua L. Journal of Herbs, Spices and Medicinal Plants, 4 (1): 73-80.***

Ghosh, M.L.; S.K. Chatterjee; S.C. Bhattacharyya; N. Sen and K.L. Sethi (1990). Developmental and Metabolic Control of Essential Oil Synthesis Under Varying Physical and Chemical Treatments in *Palma Rosa* Grass. ***Proceedings of the 11th International Congress of Essential Oils, Fragrances and Flavors. New Delhi, India, 3: 119-126.***

Jacobs, M. B. (1959). ***The Chemical and Technology of Food Production. 2: 245-251.*** Inter. Science Pub., New York.

Kinsella, J.E. (1987). ***Sea Foods and Fish Oils in Human Health and Disease.*** **p 311.** Marcel Dekker Inc., New York.

Kurapov, P.B.; S.S. Shain; V.L. Dmitrieva; L.B. Dmitrieva; N.Y. Shelaeva; I.V. Skorobogatova; A.G. Siusheva; E.I. Sal'nikova and A.A. Terkhin (1999). Secondary Metabolism and Hormonal Balance in Plants Under the Influence of Exogenous Growth Regulators. ***Sel'skokhozyaistvennaya Biologiya, 3: 73-78.***

Mahmoud, S.E.D.M.; L.E. Craker; L. Nolan and K. Shetty (1996). Response of Growth and Essential Oil Content of Sweet Basil (*Osimum basilicum L.*) to Some Natural Hormones. International Symposium on Medicinal and Aromatic Plants, Amherst, Massachusetts, USA. 27-30 Aug. 1995. ***Acta-Horticulture, 426: 629-634.***

Mahran, G. (1967). Family Umbelliferae or Parsley Family. ***Medicinal Plants.*** **1st Ed. 355-356.** Anglo-Egyptian Bookshop, Cairo.

Misra, M. (1995). The Effect of Gibberellic Acid on the Growth, Photosynthetic Pigment Content and Oil Yield of Patchouli (*Pogostemon Cablin Benth.*) Plants Grown in Shade Condition. ***Acta Physiologica Plantarum, 17 (4): 367-370.***

Rodney, C.; M. Toni and G. Norman (2000). Biochemistry & Molecular Biology of Plants. Eds. © 2000, ***American Society of Plant Physiologists, 3: 265-269.***

Runeckles, V.C. (1974). Chemistry and Biochemistry of Plant Hormones, ***Recent Advances in Phytochemistry, 7: 78-82.***

Sarwat, M.I. and S. El-Hady (2000). Chemical Constituents of Caraway Seed Oils from Plants Treated with Gibberellic Acid Sequenced by Abscisic Acid. 8th ***Conf. Agric. Dev. Res., Fac. Agric., Ain Shams Univ. Cairo, Egypt, 1: 17-27.***

Snedecor, G.W. and W.G. Cochran (1980). ***Statistical Methods,*** **7th Ed. 281-305.** Ames., Iowa, Iowa State Univ. Press, U.S.A.

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تحسين التركيب الكيميائى وكمية المحصول لزيت بذرة اليانسون بواسطة منظمات النمو

[٢]

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تم اجراء تجربة حقلية لمحصول اليانسون خلال موسمين زراعيين متتاليين عوملت فيهما النباتات بمنظمات نمو نباتية (بالرش) وهى حمض الجبريليك (٥٠ و٧٥ و١٠٠ جزء فى المليون) والكينيتين (٢٥ و٥٠ و٧٥ جزء فى المليون) كل على حدة ولعدد مرتين من الرش الأولى فى مرحلة ما قبل الأزهار والثانية فى مرحلة ما قبل العقد. ولتقييم الجزء الاقتصادى تم دراسة كمية المحصول ، % للزيت العطرى مع دراسة مكوناته التربينية ، % لليبيدات الكلية مع دراسة مكوناتها من الأحماض الدهنية ، الخواص الطبيعية والكيماوية لهما وأيضا مكوناتهما الاساسية باستخدام GC.

أتضح من النتائج المتحصل عليها أن :

المعاملة بالهرمونات تحت الدراسة، بالتركيزات المستخدمة تزيد كمية المحصول حيث أدت المعاملة بالكينيتين الى زيادة قدرها ١٠% وذلك باستخدام التركيز ٥٠ جزء فى المليون.

- الزيت الطيار والذى يمثل الأهمية الطبية لليانسون حدثت له زيادة معنوية قدرها ٦٧% وذلك باستخدام الجبريليك بالتركيز ٥٠ جزء فى المليون وأدت المعاملة بالكينيتين الى نفس الزيادة عند نفس التركيز.
- أدت المعاملة بالجبريليك أيضا الى زيادة فى نسبة الليبيدات الكلية بنسبة ٣٧% عند التركيز ٥٠ جزء فى المليون فى حين لم تكن هناك زيادة معنوية عند المعاملة بالكينيتين.
- الخواص الكيماوية للزيت الغير طيار فى جميع المؤشرات تحت الدراسة ثابتة وعند استخدام GC لتحليل مكونات الزيت الثابت اتضح أن الأحماض الدهنية السائدة هى: الأوليك، اللينوليك، البالمتيك وقد زادت كمية الأوليك واللينوليك زيادة معنوية مع اختلاف تركيزات الهرمونات المستخدمة ونوعها.
- الخواص الطبيعية والكيماوية للزيت الطيار حدثت لها تغيرات مع اختلاف تركيزات الهرمونات المستخدمة ونوعها.

وعند استخدام GC لتحليل مكونات الزيت العطرى اتضح أن المركبات السائدة هى الأنيثول والميثيل شافيكول و الأنيسالدهيد وقد حدثت زيادة معنوية فى نسبة الأنيثول مصحوبة بانخفاض معنوى فى نسبة الأنيسالدهيد عند التركيزات ٥٠ جزء فى المليون من كلا من الجبريليك و الكينيتين بالاضافة الى انخفاض معنوى فى نسبة الميثيل شافيكول عند التركيز ٥٠ جزء فى المليون من الكينيتين.

ولقد أدى ذلك ألى احداث تأثير معنوى فى:
زيادة كمية محصول اليانسون (١٠%) باستخدام الكينيتين قبل الازهار و قبل العقد على التوالى بجرعة متوسطة (٥٠ جزء فى المليون).
- زيادة كمية المادة الفعالة (الأنيثول) بالزيت العطري بنسبة تصل الي ١٠%

مع زيادة نسبة الزيت العطرى ككل بنسبة تصل الى ٦٧% باستخدام الجرعة المنخفضة (٥٠ جزء فى المليون) من حمض الجبريليك والكينيتين أيضا بنفس الجرعة .

من هذه الدراسة وبناء على النتائج المتحصل عليها: يمكن أعتبارأستخدام منظمات النمو وخاصة الجبريليك والكينتين وبجرعات بسيطة مفيد أقتصاديا نظرا للزيادة المعنوية والتى أوضحها الرش بهذه المنظمات وخاصة نسبة المحصول (١٠%) وأيضا نسبة الزيت العطرى (٦٧%) بالأضافة الى الأهمية الأقتصادية العالية للزيت العطرى بزيادة المادة الفعالة فيه (١٠%) وكل هذا مقابل جرعات بسيطة من منظمات النمو لاتصل تكلفتها أطلاقا الى ما حدث فى محصول اليانسون من تحسين كمى ونوعي.

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