Effect of spraying with benzyl adenine, salicylic acid and phenylalanine on the yield of volatile oil of Fennel plant (*Foeniculum vulgare Mill*) and its content of some secondary compounds.

Ahmed Fahem Jabbar [1] **Ibrahim Abdullah Hamza[2]**

[1]Al-Qadisiyah University-College of Agriculture

[2]University of Baghdad - College of Agricultural Engineering Sciences

Email: Ibrahim.a@coagri.uobaghdad.edu.iq, ahmed.aljuboori@qu.edu.iq

Abstract

The field experiment for the current study was conducted at Experiment Station (A) of the Department of Field Crops - College of Agricultural Engineering Sciences - the University of Baghdad for the period from November 2018 to June 2019 in order to know the response to stimulating the production of volatile oil and secondary metabolism compounds of the Fennel plant using different concentrations Of the growth regulators [Benzyladenine (BA), Salicylic acid (SA) and Phenylalanine (Phe)]. The Randomized Complete Block Design (RCBD) was applied according to the order of factorial experiments with three replications. Growth regulators were used with the following concentrations: (0, 2.5, and 5.0 mg L^{-1} of BA, symbolized by BA0, 2.5BA, and 5BA, respectively, and 0, 150 and 300 mg L^{-1} of SA and Phe, symbolized by 0SA, 150SA, and 300SA). and 0 Phe, 150 Phe and 300 Phe respectively), the results of the current study showed the excelled and the different study factors with their bi and triple interactions in giving the highest averages of volatile oil content and production of secondary metabolites,The highest values were recorded in this study when spraying with growth regulator BA as well as their interactions with the rest of the factors. The spray treatment with a concentration of 2.5 mg L^{-1} achieved the highest average oil content of 25.73 ml. kg^{-1} with an increase of 45.2% compared to the control treatment, as well as It gave the highest values for the concentration of (t-Anethole, Fenchone, Limonene, and α-pinene) compounds, which were 1.231, 0.554, 0.866 and 0.857 µg ml^{-1} for the compounds, respectively, with an increase of 41.82, 26.48, 120.92 and 98.38%, respectively. It can be concluded from this study that the growth regulators used have a clear effect in stimulating the production of volatile oil and secondary metabolic compounds in the sweet bean plant.

Key words: secondary metabolites, Fennel, volatile oil, growth regulators

تأثير الرش بالبنزل ادنين وحامض السالسليك والفينيل الالانيين في حاصل الزيت الطيار لنبات الحبة الحلوة (*Foeniculum vulgare* Mill) ومحتواه من بعض المركبات الثانوية

م. احمد فاهم جبار
جامعة القادسية-كلية الزراعة
ahmed.aljuboori@qu.edu.iq

أ.م.د. ابراهيم عبدالله حمزة
جامعة بغداد- كلية علوم الهندسة الزراعية
Ibrahim.a@coagri.uobaghdad.edu.iq

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نفذت التجربة الحقلية للدراسة الحالية في محطة التجارب (A) التابعة لقسم المحاصيل الحقلية– كلية علوم الهندسة الزراعية – جامعة بغداد للمدة من شهر تشرين الثاني 2018 حتى حزيران 2019 وذلك بهدف معرفة الاستجابة لتحفيز انتاج الزيت الطيار و مركبات الايض الثانوي لنبات الحبة الحلوة باستخدام تراكيز مختلفة من منظمات النمو [البنزل ادنين (BA Benzyladenine) و حامض السالسيليك (SA Salicylic acid) والفينيل الالانين (Phe Phenylalanine)]. طبق تصميم RCBD تام التعشية وفق

ترتيب التجارب العاملية وبواقع ثلاثة مكررات، وقد استعملت منظمات النمو بالتراكيز الاتية: (0 و2.5 و 5.0 ملغم لتر$^{-1}$ من BA ورمز لها BA_0 و $BA_{2.5}$ و BA_5 على التتابع، و0 و150 و300 ملغم لتر$^{-1}$ من SA و Phe والتي رمز لها SA_0، SA_{150}، SA_{300} و Phe_0، Phe_{150}، Phe_{300} على التتابع)، أظهرت نتائج الدراسة الحالية تفوق عوامل الدراسة المختلفة مع تداخلاتها الثنائية والثلاثية في اعطاء اعلى متوسطات لمحتوى الزيت الطيار وانتاج مركبات الايض الثانوي، وقد سُجلت اعلى القيم في هذه الدراسة عند معاملة الرش بمنظم النمو BA فضلاً عن تداخلاتها مع بقية العوامل اذ حققت معاملة الرش بتركيز 2.5 ملغم لتر$^{-1}$ اعلى متوسط لمحتوى الزيت بلغ 25.73 مل كغم$^{-1}$ بنسبة زيادة بلغت 45.2% قياساً بمعاملة المقارنة، فضلاً عن اعطائها اعلى قيم لتركيز مركبات (t-Anethole و Fenchone و Limonene و α-pinene) اذ بلغت 1.231 و0.554 و0.866 و 0.857 ميكروغرام مل$^{-1}$ للمركبات على التتابع، محققة زيادة بنسبة 41.82 و 26.48 و 120.92 و 98.38 % على التتابع. يمكن الاستنتاج من هذه الدراسة بأن لمنظمات النمو المستخدمة اثر واضح في تحفيز انتاج الزيت الطيار ومركبات الايض الثانوي في نبات الحبة الحلوة.

الكلمات المفتاحية : مركبات الايض الثانوي, الحبة الحلوة , الزيت الطيار , منظمات النمو

Introduction

Fennel(*Foeniculum vulgare Mill*) has many names. Which belongs to the Umeblliferae family due to its spread in various countries of the world, and was known to the ancient Egyptians as Shamari and its fruits were found in their tombs (2).The uses of Fennel have numerous purposes as an appetite stimulant, diuretic, carminative, spice, intestinal activity, estrogenic, essential in cosmetics and perfumes, galactagogue, antioxidant, antimicrobial, anti-inflammatory, diarrheal, spasmolytic, nervous disorders, and hepatitis (32, 18). The Fennel plant has been used medicinally since ancient times where a natural source for the treatment of many pathological conditions because its fruits and oil contain many active substances. Its roots have therapeutic uses as well, where it is a diuretic and helps treat infections in the urinary tract. The volatile oil also has an effect in removing spasms in skeletal muscles in addition to smooth muscles (34). The oil is also useful in treating respiratory diseases and relieving asthma because it contains Anethole, which works to remove phlegm, in addition to its use in the treatment of urinary tract infections (38). The trans-Anethole compound is one of the main components of the volatile oil of the Fennel plant, which constitutes about 80%, as well as the Fenchone compound, which may reach 25% of the amount of the essential oil (8). Growth regulators are chemical compounds that are added to the nutrient medium in low concentrations and absorbed from the plant tissues and then moved to their sites of action as they bind to the receptor, thus activating the secondary transmission system to inhibit or stimulate the activity of cells (13). It also controls the physiological and biochemical processes through primary and secondary metabolism processes, and it uses growth regulators to a great extent to control the processes of maturation and aging in plants (23).(14) (2004) stated that SA stimulates gene expression related to the production of some classes of secondary metabolites in the plant and the activity of the enzyme (phenylalanine ammonia-lyase (PAL), which is an essential enzyme between primary and secondary metabolism and contributes to the pathway of phenylpropanoid production in secondary metabolism). The effect of stimuli such as SA was observed at a concentration of 0.25mM used as a spray on the Fennel plant, which gave the greatest economic value from the live mass and higher production of secondary metabolites (20). Also, benzyl adenine (BA) is one of the cytokinins that improve the yield of many plants as and/or species (29). Spraying Croton plants (*Codiaeum variegatum*) with different concentrations of BA led to a significant increase in most vegetative growth traits, fresh and dry weight, as well as an increase in chemical components such as carotenoids, total soluble sugars, total indoles and total soluble phenols compared to the control treatment (36). As for amino acids, such as (Phe), they are organic molecules that can influence the physiological activities related to plant growth and development directly or indirectly (11). Phe plays an important role in the production of aromatic compounds and antioxidants (3).In a study indicated that the use of amino acids (phe) at a concentration of 100 mg L^{-1} led to a significant increase in the percentage of essential oil and secondary metabolites (phenols and indoles) (15). It was also found (22) (2010) that spraying plants with Phe at a concentration of 300 mg L^{-1} gave the highest percentage of the essential oil of sweet cumin. Given the importance of the Fennel plant globally and locally and its role in many medical and food industries, part of which was mentioned above, and the few and limited studies that dealt with it in Iraq, and to know the effect of growth regulators on the content of volatile oil and secondary metabolic compounds in this plant, this study was conducted. Materials and methods:

The current study was conducted as a field experiment at the College of Agricultural Engineering Sciences, University of Baghdad, Al-Jadriya Complex, in Experiment Station (A) of the Field Crops Department for the period from November 2018 to June 2019 in order to know the response of volatile oil and secondary metabolites in the fruits of the Fennel plant using different concentrations. Growth Regulator (Benzyladenine (BA) and (Salicylic acid (SA) and (Phenylalanine (Phe)) of the local cultivar. The experimental land was prepared from conducting soil service operations such as tillage, smoothing, and division before cultivation, and the field was divided into three blocks, each replicates contains twenty-seven experimental units With dimensions of 2.5 x 2.5 m for one experimental unit, the seeds were sown on 5-11-2018 m inside the experimental units, with a distance of 70 cm between the lines and 25 cm between one plant and another. As the seeds were placed at a rate of 3-5 seeds per pit, the field was irrigated immediately after planting, and the process of controlling the weeds was conducted using pesticides, as well as removing the weeds manually whenever needed and completed the crop service operations of fertilization and irrigation and others.The different treatments were sprayed at the beginning of the flowering stage by preparing the spray solution for the growth regulators used in the study at levels (0, 2.5 and 5) ml L^{-1} of BA and (0, 150 and 300) mg L^{-1} of SA and Phe using 20-liter backbags prayers. The used sprinklers were calibrated before applying the treatments, taking into account that there was no interaction between the treatments when spraying using a cardboard (carton) and the used sprinklers were washed well after each use.

Studied traits:

After completing the above experiment, the required traits were measured as follows:

Oil content in fruits (ml kg^{-1}):

The volatile oil was extracted using the Water Distillation Method, according to the British Pharmacopoeia (10) and the method mentioned (12 and 4). A graduated Clevenger device was used connected to a 1 liter beaker. Where 25 g of fruits were weighed and milled with a ceramic mortar and then placed in the beakers of the device and 250 ml of plain water was added to it.The distillation process was conducted by heating the beaker, and the distillation process continued for three hours for each of the samples until the amount of volatile oil was extracted from the sample and the extracted oil quantity was recorded directly from the tube inserted in the device.

Detection of secondary metabolites in the Fennel fruits

Model type (LC-10A shimadzu) High Performance Liquid Chromatography (HPLC) was used to determine the quantity and quality of the active substances in the volatile oil of Fennel fruits. The device was injected with a quantity of the standard model to obtain a peak (peak) of the active substances to be measured, which are (Trans-anethole, Estragole, fenchone, limonene, α-pinene). Then the device was injected with the oil sample model from each of the different treatments and the peaks of the sample model were matched with the peaks of the standard model and then their quantities were calculated. The type of column in the device used in the detection was C-18 DB column with dimensions (50×2.0mm I.D) and minute size (3μM). The by-products of the sample extract were estimated by injecting 20 microliters into the column under the following conditions:

mobile phase solvent area 0.01M ammonium acetate

- Flow speed of 1.1 ml min-1.

The wavelength is 285 nm.

- The temperature is 40°C.

The readings were recorded at wavelengths and according to the Rt of the standard solutions and samples under study.

The concentrations of active substances were quantitatively estimated by comparing the area of the standard model bands with the area of the sample bands under the same conditions and based on the following equation:

Unknown substance concentration (μg mL^{-1})= $\frac{samples\ band\ area}{standard\ band\ area}\times$ Standard Concentration × Number of Dilutions

After recording all the data, the results were compiled and analyzed using the statistical program Genstat, and the averages were compared using the least significant difference test at a probability level of 0.05 (33).

Results and discussion :

Effect of different treatments (BA, SA and Phe) on the oil content of fruits (ml kg^{-1})

The volatile oil is one of the most important traits that indicate the plant's response to many different treatments such as adding nutrients, growth regulators, stimuli, initiators and other natural and industrial chemicals where they are metabolites produced accidentally in different parts of the plant and in different conditions. It is known that secondary metabolites do not directly contribute to plant growth and development, but play a key role in plant defense, survival and interaction with the environment, such as defense against predators and diseases, plant protection from abiotic stresses, and also have a role in attracting pollinators and spreading seeds (27). The results in Table (1) show that there are significant effects of the different treatments on the oil content of the fruits. The spray treatment with growth regulator BA at a concentration of 2.5 mg L^{-1} recorded the highest average oil content of 25.73 ml kg^{-1} with an increase of 45.2%, followed by the spraying treatment with a concentration of 5 mg L^{-1}, which amounted to 23.29 ml kg^{-1} with an increase of 31.43% compared with control treatment, which recorded the lowest average oil content, it was 17.72 ml kg^{-1}.The same applies to spraying treatments with SA. The spray treatment with a concentration of 300 mg L^{-1} achieved the highest average oil content in the fruits, which amounted to 24.22 ml kg^{-1}, with an increase of 17.52%, followed by the spraying treatment with a concentration of 150 mg L^{-1}, which amounted to 21.91 ml kg^{-1}, with an increase of 6 .31% compared to the control treatment, which recorded the lowest average oil content in the fruits of 20.61 ml kg^{-1}. As for the bi-interactions, the combinations of different treatments significantly affected the oil content in the fruits. For the interaction treatments between BA and SA, the treatment of the combination BA2.5 with SA150 achieved the highest average oil content of 27.47 ml kg^{-1} with an increase of 100.67% and excelled on the treatment of the combination that It was followed by BA2.5 with SA300, which

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amounted to 25.60 ml kg^{-1}. It is worth noting that all combinations of the BA2.5 treatment clearly excelled on all the rest of the combinations, and we note that there is a relative decrease in all combinations of the BA5 treatment, but it excelled on control treatment that The lowest levels of the average oil content were recorded as 13.69 ml kg^{-1}.The interaction between BA and Phe also behaved like the interactions between BA and SA, as they significantly excelled on their effect in this trait. The treatment of the combination BA2.5 with Phe150 achieved the highest average oil content in the fruits of 26.40 ml kg-1 with an increase of 83.33% It was followed by the treatment of the combination BA2.5 with Phe0, which amounted to 25.87 ml $kg^{-1,}$ with an increase of 79.63% compared to the control treatment, which recorded the lowest average for this trait of 14.40 ml kg^{-1}. It is noticeable that the combinations of the growth regulator BA2.5 treatment with all levels of Phe were significantly excelled on the fact that the combinations of the treatment of growth regulator BA5 with all levels of Phe decreased relatively despite their significantly excelled in the effect compared to the control treatment. As for the interaction treatments between SA and Phe, it achieved a significant effect in this trait, where the treatment of the combination SA300 with Phe150 recorded the highest average oil content in the fruits amounting to 24.53 ml kg^{-1} with an increase of 21.05%. Followed by the treatment of the combination SA300 with Phe0, which amounted to 24.267 ml kg^{-1}, with an increase of 19.74% compared to the control treatment, which amounted to 20.27 ml kg^{-1}. The behavior here was different from the previous bi-interactions, where the combinations of SA300 treatment with all levels of Phe significantly excelled on the rest of the treatments and their combinations. As for the triple interactions between the study factors, the data in the same table showed that there were significant differences between the combinations of treatments for the different factors under study in the trait of the oil content of the fruits. The treatment of the combination BA2.5 with SA150 with Phe300 achieved the highest significant difference in the average oil content in the fruits. It reached 31.20 ml kg^{-1}, with an increase of 178.57%, excelled on all the rest of the treatments, followed by the treatment of the combination BA2.5 with SA300 with Phe150, in which the average oil content was 28.40 ml kg^{-1} with an increase of 153.57% compared to the control treatment, which recorded the lowest average of these the trait was 11.20 ml kg^{-1}. It is noted that all the combinations were significantly excelled on the control treatment, and this may indicate the positive role played by the study factors in achieving the moral increase in the volatile oil of Fennel, which may reflect the positive effect of the interaction between the levels of different growth regulators and stimuli in promoting and increasing the controlling vital processes. On the manufacture of oil, and growth regulators control the physiological and biochemical processes through the processes of primary and secondary metabolism (23).The above results were in agreement with some researchers that the use of cytokinins (BA) led to a significant increase in the oil content in Fennel plant (37 and 1), lupine plant (Lupinus termis) (7) and basil plant (21).The positive results may be due to the physiological role of cytokinins in stimulating cells and regulating biochemical processes (9) and thus was a reason for the positive effect of the increase in the oil content in the fruits. The important role of SA, which it plays in many metabolic processes in the plant, may also be a reason for the significant effects in the trait under study, where the activity and activity of SA is prominent in the growth and development of the plant through its physiological role in the plant, so it can be considered a plant hormone (24).SA also affects physiological processes in different methods, it may stimulate some processes and inhibit others, depending on its concentration, environmental conditions and plant species (16).

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Studies also show that the use of SA on plants can enhance vital processes through its important role in plant signaling systems and in stimulating the action of some enzymes, which explains its catalytic role for plant-produced substances in medicinal and food plant species (19).The results were in agreement with several studies conducted by researchers confirming the positive role of SA in the oil content of the plant, where it was stated (6) that the use of SA led to a significant increase in the oil content in different genotypes of the Fennel plant. It was also shown (20) (2017) that spraying Fennel plants with SA led to a significant increase in the oil content. This increase may be due to the direct effect of amino acids on many vital processes in the plant, such as photosynthesis, which leads to an increase in the products of metabolic processes, including oil. Several studies have shown that the use of Phe on plants has a significant effect in increasing the oil content.

Table 1. The effect of different treatments (BA, SA and Phe) on the volatile oil content in fruits (ml kg^{-1})

average Phe	BA X Phe	SA ($mg.L^{-1}$)			BA ($mg.L^{-1}$)	Phe ($mg.L^{-1}$)
		300	150	0		
21.47	14.40	19.60	12.40	11.20	**0**	**0**
	25.87	27.60	27.60	22.40	**2.5**	
	24.13	25.60	19.60	27.20	**5**	
22.83	19.96	22.80	22.40	14.67	**0**	**150**
	26.40	28.40	23.60	27.20	**2.5**	
	22.13	22.40	24.40	19.60	**5**	
22.44	18.80	26.40	14.80	15.20	**0**	**300**
	24.93	20.80	31.20	22.80	**2.5**	
	23.60	24.40	21.20	25.20	**5**	
0.12	**0.21**	**0.36**			**$L.S.D_{0.05}$**	
average BA		24.22	21.91	20.61	**average SA**	
		0.12			**$L.S.D_{0.05}$**	
17.72		22.93	16.53	13.69	**0**	**SA X BA**
25.73		25.60	27.47	24.13	**2.5**	
23.29		24.13	21.73	24.00	**5**	
0.12		**0.21**			**$L.S.D_{0.05}$**	
		24.27	19.87	20.27	**0**	**SA X Phe**
		24.53	23.47	20.49	**150**	
		23.8	22.40	21.07	**300**	
		0.21			**$L.S.D_{0.05}$**	

Effect of different treatments on t-Anethole concentration in fruit oil (µg ml^{-1}).

The results in Table (2) show that the different factors in the study had a significant effect on the concentration of t-Anethole. The treatments of adding the growth regulator BA excelled by giving the highest average concentration of the compound, where treatment BA5 recorded the highest significant increase with an average of 1.231 µg ml^{-1}, followed by the BA2.5 treatment,

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which amounted to 0.905 μg ml^{-1}, with an increase of 41.82 and 4.26% for the two treatments, respectively. Compared to the control treatment, which recorded the lowest mean for the trait, which was 0.868 μg ml^{-1},As for the SA addition treatments, the SA300 treatment was excelled by giving the highest average concentration of the compound, which amounted to 1.083 μg ml^{-1}, followed by the SA150 treatment, which amounted to 0.982 μg ml^{-1}, with an increase of 15.34 and 4.58% for the two treatments respectively, compared to the control treatment, which recorded the lowest average of 0.939 μg ml^{-1}. The treatment of adding Phe also achieved a significantly excelled in the concentration of the compound, as the Phe300 treatment recorded the highest significant increase in the concentration of the compound, reaching 1.148 μg ml^{-1}, followed by the Phe150 treatment, which amounted to 0.940 μg ml^{-1}, with an increase of 25.19 and 2.51% for the two treatments respectively, compared to the control treatment the lowest average was 0.917 μg ml^{-1}. As for the bi-interactions, the results in the same table showed that there were significant differences in the treatment of the bi-interactions, and the BA and Phe interactions treatment achieved the highest significant increase in the bi-interactions. The treatment of the combination BA5 with phe300 achieved the highest mean t-Anethole concentration of 1.310 μg ml^{-1}, with an increase of 84.25% compared to the comparison treatment, which recorded the lowest average concentration of the compound amounted to 0.711 μg ml^{-1}.It is worth noting that the high values of the averages in this interaction were in all the combinations containing the BA5 treatment. As for the interaction between BA and SA, it also achieved a significantly excelled in the concentration of the compound, where the treatment of the combination BA5 with SA300 recorded the highest significant increase, reaching 1.265 μg ml^{-1}, with an increase of 58.13% compared to the control treatment, while the treatment of the combination BA2.5 with SA0 recorded the lowest The mean for the trait was 0.767 μg ml^{-1}, recording a decrease of 4.13% compared to the control treatment. It can also be seen that the highest values were recorded in the parameters of the combinations containing BA5. While there was a significant increase in the interaction between SA and Phe. The SA300 combination treatment with Phe300 had the highest significant increase in average concentration of the compound, which amounted to 1.254 μg ml^{-1}, with an increase of 45.31% compared to the control treatment, which recorded the lowest mean of this traits amounted to 0.863 μg ml^{-1}. It is worth noting that the highest values were recorded in the combinations that contain Phe300 and that the increase was gradual with the increase in the level of factors in this interaction. As for the triple interaction, the results in the same table showed that the interaction between the three study factors had a significant effect on the concentration of the t-Anethole compound. BA2.5 with SA0 with Phe300 the highest average concentration of the compound was 1.416 μg $ml^{-1}$with an increase of 92.65% compared to the control treatment,While the lowest value of the average concentration of the compound when treating the combination BA0 with SA150 with Phe0 was 0.659 μg ml^{-1} with a decrease of 10.34% compared to the control treatment. It should be noted that the lowest values were recorded for the combinations containing BA0.

Table 2. Effect of different treatments on the concentration of t-Anethole in fruit oil (µg ml^{-1}).

average Phe	BA X Phe	SA (mg.L^{-1}) 300	150	0	BA (mg.L^{-1})	Phe (mg.L^{-1})
0.917	0.711	0.739	0.659	0.735	**0**	**0**
	0.880	0.992	0.955	0.693	**2.5**	
	1.159	1.304	1.012	1.161	**5**	
0.940	0.787	0.773	0.893	0.695	**0**	**150**
	0.810	0.813	0.866	0.751	**2.5**	
	1.223	1.369	1.131	1.169	**5**	
1.148	1.107	1.248	1.103	0.969	**0**	**300**
	1.026	1.390	0.831	0.858	**2.5**	
	1.310	1.123	1.390	1.416	**5**	
0.008	0.014	0.025			**L.S.D**	
average BA		1.083	0.982	0.939	**average SA**	
		0.008			**L.S.D**	
0.868		0.920	0.885	0.800	**0**	**SA X BA**
0.905		1.065	0.884	0.767	**2.5**	
1.231		1.265	1.178	1.249	**5**	
0.008		0.014			**L.S.D**	
		1.012	0.875	0.863	**0**	**SA X Phe**
		0.985	0.963	0.872	**150**	
		1.254	1.108	1.081	**300**	
		0.014			**L.S.D**	

Effect of different treatments on estragole concentration in fruit oil (µg ml^{-1}).

The results in Table (3) show that all study factors with bi and triple interactions had a significant effect on Estragole concentration. The SA addition treatments excelled by giving the highest value for the average concentration of the compound.The SA300 treatment recorded the highest significant increase compared to the rest of the treatments, which amounted to 0.522 µg ml^{-1}, followed by the SA150 treatment, which amounted to 0.403 µg ml^{-1}, with an increase of 60.62 and 24.00% for the two treatments respectively compared to the control treatment, which recorded the lowest average concentration of the compound amounted to 0.325 µg ml^{-1}. The same applies to the growth regulator BA treatments that achieved a significantly excelled in increasing the concentration of this compound. The BA5 treatment recorded the highest significant increase in the average concentration of the compound, reaching 0.483 µg ml^{-1}, followed by the BA2.5 treatment, which amounted to 0.434 µg ml^{-1}, achieving an increase of 45.05. And 30.33% for the two treatments respectively, compared to the control treatment, which recorded the lowest mean concentration of the compound was 0.333 µg ml^{-1}.While the Phe addition treatments did not behave the same as in the previous compound, but they were significantly excelled in their effect on this trait. The Phe300 treatment recorded the highest significant increase in the mean concentration of the compound, reaching 0.436 µg ml^{-1}, with an increase of 5.57% compared to the control treatment, which did not differ significantly for the treatment of Phe150, which recorded the lowest value of the average concentration of the compound was 0.401 µg ml^{-1}.As for the two interactions, all the two interactions achieved significantly excelled when compared to the control treatment. The interactions between BA and SA recorded the highest significant increase in the concentration of Estragole.The treatment of the combination BA5 with SA300 achieved the highest value of significant increase for the averages, as it reached 0.625 µg ml^{-1}, achieving an increase in the concentration of the compound by 141.31% compared to the control treatment, which recorded the lowest average concentration of the compound amounted to 0.259 µg ml^{-1}.It is noticeable here that the highest values of the averages were recorded for the combinations containing SA300 in this interaction. As for the interaction between SA and Phe, most of the treatments achieved significantly excelled in increasing the concentration of the compound in the volatile oil in the fruits. The treatment of the combination SA300 with Phe150 recorded the highest average concentration of 0.536 µg $ml^{-1}$and did not differ significantly from the treatment of the combination SA300 with Phe300, which recorded a significant increase of 54.47% compared to the control treatment, while the treatment of the combination SA0 with Phe150 recorded the lowest value for the average concentration of the compound, which reached 0.280 µg ml^{-1}, thus recording a decrease of 19.31% compared to the control treatment. There was also a significant effect of the interaction treatments between BA and Phe, as the treatment of the combination BA5 with Phe0 achieved a significant increase in the average concentration of Estragole compound, superior to the rest of the treatments in this interaction, which amounted to 0.528 µg ml^{-1}, achieving a significant increase of 61.96% compared to the control treatment, which recorded the lowest The mean value of the compound concentration was 0.326 µg ml^{-1}. It is noteworthy that the highest values in this interaction were recorded for the combinations containing BA5.As for the triple interaction, the results show that there is a significant effect of the interaction between the three study factors (BA, SA and Phe). Most of the combination treatments were significantly excelled in increasing the concentration of estragole in the volatile oil of the fruits.Whereas, the treatment of the combination BA5 with SA300 with Phe150 achieved the highest significant increase in the concentration of the compound, reaching 0.668 µg ml^{-1}, with an increase of 267.03% compared to the control treatment, which recorded the lowest value of the average concentration in this interaction, which was 0.182 µg ml^{-1}.It is worth noting that the highest values of the averages were recorded when the combination containing BA5 with SA300, and also that the increase in the mean values was not linear with the increase of the combinations, there was an increase with the increase in the concentrations of factors (BA and SA), while the increase in the averages was by adding the second level of phenylalanine (Phe), then the values were decreased by adding the third level (Phe300).

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Table 3. Effect of different treatments on Estragole concentration in fruit oil (µg ml^{-1}).

average Phe	BA X Phe	SA (mg.L^{-1})			BA (mg.L^{-1})	Phe (mg.L^{-1})
		300	150	0		
0.413	0.326	0.401	0.394	0.182	**0**	**0**
	0.385	0.503	0.432	0.220	**2.5**	
	0.528	0.584	0.361	0.638	**5**	
0.401	0.315	0.380	0.326	0.240	**0**	**150**
	0.431	0.561	0.451	0.282	**2.5**	
	0.455	0.668	0.380	0.317	**5**	
0.436	0.358	0.388	0.331	0.356	**0**	**300**
	0.485	0.590	0.519	0.346	**2.5**	
	0.465	0.623	0.429	0.343	**5**	
0.021	0.036	0.062			**L.S.D$_{0.05}$**	
average BA		0.522	0.403	0.325	**average SA**	
		0.021			**L.S.D$_{0.05}$**	
0.333		0.390	0.350	0.259	**0**	**SA X BA**
0.434		0.551	0.468	0.283	**2.5**	
0.483		0.625	0.390	0.433	**5**	
0.021		0.036			**L.S.D$_{0.05}$**	
		0.496	0.396	0.347	**0**	**SA X Phe**
		0.536	0.386	0.280	**150**	
		0.534	0.426	0.348	**300**	
		0.036			**L.S.D$_{0.05}$**	

Effect of different treatments on Fenchone concentration in fruit oil (µg ml^{-1}**).**

The results in Table (4) show that there are significant differences in the use of all study factors in the production and concentration of the compound Fenchone. The individual treatments with bi and triple interactions differed significantly in this trait, where the treatments of adding the growth regulator BA achieved the highest values of the averages.

Whereas, the BA5 treatment recorded the highest significant increase in the concentration of the compound, reaching 0.554 µg ml^{-1}, followed by the addition treatment BA2.5, which amounted to 0.463 µg ml^{-1}, with an increase of 26.48 and 5.71% for the two treatments, respectively, compared to the control treatment, which recorded the lowest average concentration the compound was 0.438 µg ml^{-1}.There was also a significant effect of the treatments using SA in increasing the concentration of the compound, where the SA300 treatment achieved the highest significant increase for this trait, which amounted to 0.520 µg ml^{-1}, followed by the SA150 treatment, which amounted to 0.498 µg ml^{-1}, with an increase of 18.99 and 13.96% for the two treatments respectively, compared to the treatment of the comparison that recorded the lowest mean concentration of the compound was 0.437 µg ml^{-1}. While the treatments of adding Phe achieved a significant decrease in the concentration of the Fenchone compound in the volatile oil of the fruits, and thus it behaved opposite to that of its counterparts from the treatments in the previous compounds. Compared to the control treatment, it was followed by the Phe150 treatment, which amounted to 0.494 µg ml^{-1}, which did not differ significantly from the control treatment. As for the bi interactions, significant effects were achieved in the concentration of Fenchone in the fruit oil, where the interaction between BA and SA recorded the highest values for the average concentration of the compound in the two interactions, where the treatment of the combination BA5 with SA300 gave the highest average concentration of the compound as it reached 0.635 µg ml^{-1}With an increase of 67.99% compared to the control treatment, which recorded the lowest average of 0.378 µg ml^{-1}. It is worth noting that the increase in the mean values was gradual with the increase in the concentrations of factors in the combinations, but the highest values appear in the combinations containing BA5. Also, the interaction between SA and Phe had a significant effect in this trait, where the combination treatment SA300 with Phe150 achieved the highest average concentration of the compound, reaching 0.580 µg ml^{-1}, and thus it gave the largest significant difference with an increase of 25% compared to the control treatment. While the treatment of the combination SA0 with Phe150 recorded the lowest value for the average concentration of the compound was 0.398 µg ml^{-1}. As for the interaction treatments between BA and Phe, they achieved a significantly excelled in this trait and varied in the nature of the response. The treatment of the combination BA5 with Phe0 recorded the highest significant increase in the average concentration of the compound in this interaction, which amounted to 0.569 µg ml^{-1}, with an increase of 29.02% compared to the control treatment, while the lowest value of the average concentration was recorded when treating the mixture BA2.5 with Phe300, which amounted to 0.376 µg ml^{-1}, achieving a decrease in the concentration of the compound by 14.34% compared to the control treatment. It is worth noting that the highest values were recorded for the combinations containing BA5. As for the triple interaction, the results showed that there were significant differences between the interaction of the study factors (BA, SA and Phe), where the treatment of the combination BA5 with SA300 with Phe300 was excelled on the rest of the triple interaction treatments by giving it the highest average concentration of the Fenchone compound, which amounted to 0.731 µg ml^{-1}. A significant increase of 110.66% compared to the control treatment, The lowest value of the average concentration of the compound was recorded when treating the combination BA2.5 with SA300 with Phe300, which amounted to 0.058 µg ml^{-1}, with a decrease of 83.29% compared to the control treatment. It is also noted that most of the high mean values of the compound concentration were when the mixtures containing BA. As for the bi interactions, significant effects were achieved in the concentration of Fenchone in the fruit oil, where the interaction between BA and SA recorded the highest values for the average concentration of the compound in the two interactions, where the treatment of the combination BA5 with SA300 gave the highest average concentration of the compound as it reached 0.635 µg ml^{-1} With an increase of 67.99% compared to the control treatment, which recorded the lowest average of 0.378 µg ml^{-1}. It is worth noting that the increase in the mean values was gradual with the increase in the concentrations of factors in the combinations, but the highest values appear in the combinations containing BA5. Also, the interaction between SA and Phe had a significant effect in this trait, as the combination treatment SA300 with Phe150 achieved the highest average concentration of the compound, reaching 0.580 µg ml-1, and thus it gave the largest significant difference with an increase of

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25% compared to the control treatment. While the treatment of the combination SA0 with Phe150 recorded the lowest value for the average concentration of the compound was 0.398 µg ml^{-1}. As for the interaction treatments between BA and Phe, they achieved a significantly excelled in this trait and varied in the nature of the response. The treatment of the combination BA5 with Phe0 recorded the highest significant increase in the average concentration of the compound in this interaction, which amounted to 0.569 µg ml^{-1}, with an increase of 29.02% compared to the control treatment , while the lowest value of the average concentration was recorded when treating the mixture BA2.5 with Phe300, which amounted to 0.376 µg ml^{-1}, achieving a decrease in the concentration of the compound by 14.34% compared to the control treatment. It is worth noting that the highest values were recorded for the combinations containing BA5.As for the triple interaction, the results showed that there were significant differences between the interaction of the study factors (BA, SA and Phe), where the treatment of the combination BA5 with SA300 with Phe300 was excelled on the rest of the triple interaction treatments by giving it the highest average concentration of the Fenchone compound, which amounted to 0.731 µg ml^{-1}. A significant increase of 110.66% compared to the control treatment,

Table 4. Effect of different treatments on the concentration of Fenchone in fruit oil (µg ml^{-1}).

average Phe	BA X Phe	SA (mg.L^{-1}) 300	150	0	BA (mg.L^{-1})	Phe (mg.L^{-1})
0.495	0.441	0.516	0.459	0.347	**0**	**0**
	0.474	0.556	0.505	0.362	**2.5**	
	0.569	0.558	0.465	0.683	**5**	
0.494	0.416	0.493	0.389	0.367	**0**	**150**
	0.537	0.631	0.550	0.431	**2.5**	
	0.528	0.617	0.571	0.397	**5**	
0.466	0.456	0.519	0.430	0.419	**0**	**300**
	0.376	0.058	0.583	0.487	**2.5**	
	0.566	0.731	0.529	0.439	**5**	
0.011	0.019		0.033		**L.S.D$_{0.05}$**	
average BA		0.520	0.498	0.437	**average SA**	
			0.011		**L.S.D$_{0.05}$**	
0.438		0.509	0.426	0.378	**0**	**SA X BA**
0.463		0.415	0.546	0.427	**2.5**	
0.554		0.635	0.522	0.506	**5**	
0.011			0.019		**L.S.D$_{0.05}$**	
		0.543	0.476	0.464	**0**	**SA X Phe**
		0.580	0.503	0.398	**150**	
		0.436	0.514	0.448	**300**	
			0.019		**L.S.D$_{0.05}$**	

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Effect of different treatments on the concentration of Limonene in fruit oil (µg ml^{-1}).

The results in Table (5) show that there is a significant effect of the different study factors as well as the bi and triple interactions between the factors in the concentration of the limonene compound. The treatments of adding the growth regulator BA excelled in giving the highest values for the average concentration of the compound, as the BA5 treatment achieved the highest significant difference compared to the rest of the treatments It was 0.866 µg ml^{-1}, recording a significant increase of 120.92%, followed by the BA2.5 treatment, which amounted to 0.530 µg ml^{-1}, with an increase of 35.20% compared to the control treatment, which recorded the lowest average concentration of the compound, which amounted to 0.392 µg ml^{-1}. The addition of SA treatments significantly increased the concentration of the compound, as the SA300 treatment recorded the highest mean of 0.714 µg ml^{-1}, followed by the SA150 treatment, which amounted to 0.577 µg ml^{-1}, with an increase of 43.66 and 16.10% for the two treatments respectively compared to the control treatment, which recorded the lowest average The concentration of the compound was 0.497 µg ml^{-1}. While the significant effect of the Phe addition treatments was only in the Phe300 treatment, which achieved the highest mean concentration of the compound, reaching 0.630 µg ml^{-1}, achieving a significant increase of 9.57% compared to the comparison treatment, which recorded the lowest value of the average compound concentration of 0.575 µg ml^{-1}, which did not differ significantly about the treatment of the additive Phe150. As for the bi-interactions between the study factors, it achieved a significant effect in the concentration of the limonene compound, the interaction between BA and SA were significantly excelled by giving the highest values of the average concentration of the compound in the volatile oil of the fruits, where the treatment of the combination BA5 with SA300 excelled by giving the highest value of the significant difference in concentration. The compound in this interaction, which amounted to 1.021 µg ml^{-1}, was excelled on the rest of the treatments of the combinations, recording a significant increase of 239.20% compared to the control treatment, which recorded the lowest value of the average concentration of the compound amounted to 0.301 µg ml^{-1}. It is worth noting that the highest values of the averages were for all treatments of the combinations containing BA5, and the increase in the values of the means increased gradually towards the increase of the levels of the two interference factors (BA and SA). As for the interaction treatments between BA and Phe, they were significantly excelled in recording the highest values of the average concentration of the compound, where the treatment of the combination BA5 with Phe300 excelled in recording the highest significant increase in the concentration of the compound, reaching 0.898 µg ml^{-1}, superior to an increase of 138.83% compared to the control treatment, which recorded the lowest The value of the mean concentration of the compound was 0.376 µg ml^{-1}, and it did not differ significantly from the treatment of the combination BA5 with Phe0. It is noticeable that the increase in the mean values was gradual with the increase in the levels of the factors, and that the highest values were recorded at the treatment of the combinations that contain BA5 in this interaction. The interaction treatments between SA and Phe had a significant effect on increasing the concentration of the compound. The treatment of the combination SA300 with Phe300 was excelled by giving the highest value of the average concentration of the compound in the fruit oil, which amounted to 0.761 µg ml^{-1}, with an increase of 69.11% compared to the comparison treatment, which recorded the lowest The mean concentration of the compound was 0.450 µg ml^{-1}.It is noted from the results in the same table that the highest values were concentrated in the combinations containing SA300, despite that, the increase was gradual in the mean values with the increase in the level of the study factors in this interaction. As for the triple interaction between all the factors of the study, it had a significant effect in increasing the concentration of Limonene in the volatile oil of the fruits. The treatment of the combination BA5 with SA300 with Phe0 recorded the highest average concentration of the compound with the largest significant difference, reaching 1.043 µg ml^{-1}, achieving an increase of 305.83% compared to the comparison treatment, which recorded the lowest value of the average concentration of the compound, and it is noted from the results in the table that the combinations containing both BA5 and SA300 together regardless were characterized by recording the highest values, while the lowest values were in most of the combinations containing BA0 in this interaction.

Table 5. Effect of different treatments on the concentration of Limonene in fruit oil (µg ml^{-1}).

average Phe	BA X Phe	SA (mg.L^{-1})			BA (mg.L^{-1})	Phe (mg.L^{-1})
		300	**150**	**0**		
0.575	0.376	0.488	0.384	0.257	**0**	**0**
	0.470	0.553	0.576	0.281	**2.5**	
	0.878	1.043	0.780	0.812	**5**	
0.583	0.364	0.447	0.347	0.298	**0**	**150**
	0.565	0.605	0.654	0.435	**2.5**	
	0.821	1.011	0.766	0.687	**5**	
0.630	0.435	0.548	0.409	0.348	**0**	**300**
	0.556	0.725	0.396	0.548	**2.5**	
	0.898	1.009	0.878	0.808	**5**	
0.011	0.019	0.033			**L.S.D0.05**	
average BA		0.714	0.577	0.497	**average SA**	
		0.011			**L.S.D0.05**	
0.392		0.494	0.380	0.301	**0**	**SA X BA**
0.530		0.628	0.542	0.421	**2.5**	
0.866		1.021	0.808	0.769	**5**	
0.011		0.019			**L.S.D0.05**	
		0.695	0.580	0.450	**0**	**SA X Phe**
		0.688	0.589	0.473	**150**	
		0.761	0.561	0.568	**300**	
		0.019			**L.S.D0.05**	

Effect of different treatments on a-Pinene concentration in fruit oil (µg ml^{-1}).

The results in Table (6) show that there is a significant effect of the different study factors (BA, SA and Phe), as well as their bi and triple interactions in the concentration of the a-pinene compound in the volatile oil of the fruits. The BA5 treatment had the highest average, as it reached 0.857 µg ml^{-1}, followed by the BA2.5 treatment, which amounted to 0.618 µg ml^{-1}, with an increase of 98.38 and 43.06% for the two treatments, respectively, compared to the control treatment, which recorded the lowest average concentration of the compound, which amounted to 0.432 µg ml^{-1}.Also, the addition of SA treatments significantly increased the concentration of the compound. The SA300 treatment achieved the highest value of the average concentration, reaching 0.694 µg ml^{-1}, followed by the SA150 treatment, which amounted to 0.659 µg ml^{-1}, with an increase of 25.05 and 18.74% for the two treatments respectively compared to the control treatment, which recorded The lowest average concentration of the compound was 0.555 µg ml^{-1}. While the behavior of the Phe addition treatments was different from the behavior of the two previous factors, that the Phe300 treatment was significantly excelled by giving the highest mean concentration of the compound, which amounted to 0.685 µg ml^{-1}, with an increase of 10.48% compared to the control treatment. However, the concentration of a-pinene decreased by adding the first level of the factor (Phe150) to 0.602 µg ml^{-1}, with a decrease of 2.90% compared to the control treatment. As for the bi-interactions, the results in the same table showed that there is a significantly excelled of these interactions in increasing the concentration of a-pinene compound, where the interaction treatments between BA and SA were significantly excelled by giving the highest values of the average concentrations of the compound, as the treatment of the combination BA5 with SA300 achieved the highest significant increase in the treatments The bilateral interaction was 0.902 µg ml^{-1}, with an increase of 128.93% compared to the control treatment, which recorded the lowest mean value of the concentration, which was 0.394 µg ml^{-1}.It is worth noting that the highest values recorded were for the combinations containing BA5, and the increase in the average values was gradual with the increase in the levels of the above factors in the combinations. As for the interaction treatments between BA and Phe, it achieved a significant superiority in increasing the concentration of the compound, as the treatment of the combination BA5 with Phe300 recorded the highest value of the average concentration of the compound amounted to 0.891 µg ml^{-1}, achieving a significant increase of 114.18% compared to the control treatment that the highest values recorded were with the combinations containing BA5 and the lowest values were with the combinations containing the BA0 treatment. Also, the interaction treatments between SA and Phe had a significant effect on this trait, as the treatment of the combination SA300 with Phe300 significantly excelled on the rest of the treatments in this interaction by recording the highest value of the mean concentration of the compound, which amounted to 0.735 µg ml^{-1}, achieving an increase of 47.89% compared to the control treatment. Which recorded the lowest value for the average concentration of the compound, which amounted to 0.497 µg ml^{-1}. It should be noted here that the highest values of this interaction were recorded when the mixtures containing SA300. As for the triple interaction between the factors of the study, the results in the same table showed that the different combinations in this interaction had a significant effect in increasing the concentration of the a-pinene compound, and the treatments behaved similarly to the treatments in the triple interaction of the previous compound (Limonene), Where the treatment of the combination BA5 with SA300 with Phe0 achieved the highest mean concentration of the compound with the largest significant difference, reaching 0.977 µg ml^{-1}, recording a significant increase of 227.85% compared to the control treatment, which recorded the lowest value of the average concentration of the compound, which amounted to 0.298 µg ml^{-1}.

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Table 6. Effect of different treatments on the concentration of a-Pinene in fruit oil (µg ml^{-1}).

average Phe	BA X Phe	SA ($mg.L^{-1}$) 300	SA 150	SA 0	BA ($mg.L^{-1}$)	Phe ($mg.L^{-1}$)
0.620	0.416	0.439	0.511	0.298	**0**	**0**
	0.584	0.698	0.581	0.473	**2.5**	
	0.859	0.977	0.881	0.720	**5**	
0.602	0.415	0.404	0.448	0.394	**0**	**150**
	0.570	0.670	0.602	0.438	**2.5**	
	0.821	0.850	0.819	0.794	**5**	
0.685	0.464	0.495	0.406	0.491	**0**	**300**
	0.701	0.831	0.721	0.551	**2.5**	
	0.891	0.880	0.959	0.834	**5**	
0.011	0.019		0.033		**$L.S.D_{0.05}$**	
average BA		0.694	0.659	0.555	**average SA**	
			0.011		**$L.S.D_{0.05}$**	
0.432		0.446	0.455	0.394	**0**	**SA X BA**
0.618		0.733	0.635	0.487	**2.5**	
0.857		0.902	0.886	0.783	**5**	
0.011			0.019		**$L.S.D_{0.05}$**	
		0.705	0.658	0.497	**0**	**SA X Phe**
		0.641	0.623	0.542	**150**	
		0.735	0.695	0.625	**300**	
			0.019		**$L.S.D_{0.05}$**	

The reason for the significant increase in the concentration of secondary metabolites in this study may be the role of growth regulators in stimulating and activating different enzymes that have a direct relationship in the synthesis of secondary metabolites. The results in this study agreed with the results of several researchers, as (35) (2020) indicated that the use of 1 mg L^{-1} of BA led to a significant increase in the production of t-Anethole compound in the laboratory. SA also initiates or increases the biosynthesis of secondary metabolites in plants (19), It was found (5) (2014) that the use of SA at a concentration of 200 mg L^{-1} led to a significant increase in the production of secondary metabolites of Marigold plant. It was shown (17) (2017) that the addition of Phe at a concentration of 5, 10 and 15 mg L^{-1} can cause a significant increase in the accumulation of secondary metabolites in the callus of the mint plant. As the presence of phe may increase the activity of the enzyme PAL (Phenylalanine Amonia Lyase), which is a key to the activity of the shamrock pathway and the phenylpropanoid pathway, which produces phenolic compounds (28).The use of Phe at a concentration of 3 mg L^{-1} stimulated the production of flavonoids in the callus of Hydrocotyle bonariensis (26).It can be concluded from the current study that the growth regulators affected significantly in increasing the volatile oil content in the fruits of the Fennel, as well as the production of secondary metabolites, the increase in it was significant and significantly, especially when using the growth regulator benzyl adenine, as well as the different combinations of growth regulators had the greatest significant effect In increasing the above traits due to achieving the highest values of oil content and various metabolic compounds, which reflects the important and positive role of the study factors in the production and accumulation of essential oils and their contents.

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