

A comparative study of the response of fennel (*Foeniculum vulgare*, Mill) plants from Egypt and China to spraying with benzyladenine (BA).

ABSTRACT

Fennel plant (*Foeniculum vulgare*, Mill.) is one of most widely used plants in food and medicine. Our study aimed to show the response of fennel plants from Egypt and China planted in Egypt to foliar application of benzyladenine (BA) at various concentrations; control, 50, 100 and 150 ppm. The obtained results indicated that treated fennel plants with BA resulted in a significant increase in the vegetative growth, number of umbels/plant, fruit, yield and oil yield per plant and feddan as well as main components, total phenolic contents and antioxidant activity of essential oils compared to untreated plants. In general, the application of benzyladenine was more effective on Egyptian fennel plants than that of Chinese plants. Egyptian fennel plants gave higher values of growth, fruit yield, oil percentage and oil yield than Chinese plants. However, the major components, total phenolic contents (TPC) and antioxidant activity (DPPH %) had higher values in Chinese essential oil than Egyptian essential oil. The highest values of all studied parameters were obtained by using BA at 100 ppm. GC-MS analysis of the volatile oils showed that the major components were *trans*-anethole followed by fenshone, α - pinene and D-limonene, respectively for both essential oils. 100 ppm of BA gave the highest values of *trans*-anethole content and radical scavenging activities of oils. Therefore, we concluded that spraying fennel plants with 100 ppm BA can be used for obtaining higher vegetative growth, fruit and quantity and quality of oil.

Keywords: Benzyladenine, Egyptian fennel, Chinese fennel, Essential oil, GC-MS, Trans - anethole, Total phenolic content, DPPH%.

1. INTRODUCTION

Fennel plant (*Foeniculum vulgare*, Mill.) belongs to Apiaceae Family. It is native to the Mediterranean region [1]. The plant is considered one of the most important medicinal and aromatic plants which grows well in Egypt. It is also grown in Asia, Europe, Turkey, North and South America [2]. It is grown commercially in Russia, India, China and Japan [3].

Fennel plant is among the herbs of folk medicine. Traditionally, fennel seeds have been used as analgesic, anti-inflammatory, diuretic, digestive, and antispasmodic agents [4]. Fennel fruits have an economic value due to their essential oil content. Fennel essential oil is used to flavor foods such as beverages, breads, pickles, pastries, and cheeses. It is also used as an ingredient in cosmetics and pharmaceuticals [5]. The essential oils of fennel seed are reported to be *trans*-anethole, fenchone, estragole (methyl chavicol), and α -phellandrene. The relative concentrations of these compounds vary greatly depending on the phenological state and origin of fennel [6].

Our previous laboratory work in China [7] investigated the chemical composition of the essential oils of fennel seed from Egypt and China by GC–MS analysis. The results indicated that the chemical composition of fennel seed essential oils from two countries was very different. The main components of the Egyptian fennel oil were Estragole, Limonene, L-fenchone and *trans*-Anethole (51.04, 11.45, and 3.62% respectively). While the major constituents of Chinese fennel oil were *Trans*-Anethole, Estragole, L-fenchone and Limonene (54.26, 20.25, 7.36 and 2.41%, respectively).

On the other hand, Plant growth regulators are compounds that affect physiological processes of plants when applied in minor amounts. They play a significant role in the growth, development, and distribution of essential nutrients in plant systems. Cytokinins group is one of the most important plant growth promoters which play a crucial role in plant differentiation and development [8]. Benzyladenine is one of the most active cytokinins [9]. It has been identified as a natural cytokinin in number of plants [10].

Applications of benzyladenine have been shown to have positive effects for improvement quantity and/or quality of many plants. Talaat and Gamal El-Din [11] reported that number of branches and umbels per plant, fresh and dry weight of fennel plants were increased as a result of foliar application with kinetin. Jabbar and Hamza [12] indicated that spraying fennel plants with benzyladenine significantly increased

oil content and production of secondary metabolites. Khaswa et al. [13] reported that application of benzyladenine significantly improved growth, yield, yield attributes and quality of soybean. Same observation was obtained by Ayad and Gamal El-Din [14] who reported that exogenous application of BA on *Lupinus termis* plants significantly affected vegetative growth and biochemical constituents. Similarly, **Hussein** [15] on *Catharanthus roseus* plants noticed that spraying with BA significantly increased plant height, fresh and dry weight of plants. Also, Prins et al. [16] on *Lavandula dentate* and Hazzoumi et al. [17] on *Ocimum gratissimum*. They found that an increase in essential oil yield of leaves as a result of treatment with BA.

Many studies have been investigated the effect of benzyladenine on growth, yield and essential oil contents of Egyptian fennel and other plants from different origins. However, no abundant information on the impact of benzyladenine on Chinese fennel plants. Therefore, this study was conducted as a field experiment to comparatively investigate the effect of benzyladenine (BA) on growth, fruit, and oil yield and its main components of fennel plants from Egypt and China under Egyptian conditions.

2. MATERIALS AND METHODS

The experiments study was conducted at the Horticulture Research Station Farm at Sides, Beni-Suef Governorate during the two successive seasons of 2020/2021 and 2021/2022. Egyptian sweet fennel seeds were obtained from Horticulture Research Station at Sides, Beni-Suef Governorate. Chinese sweet fennel seeds obtained from Bozhou City, Anhui Province, China. Fennel seeds were sown in a clay soil at the beginning of November for both seasons. Soil samples were obtained from a depth of 30 cm from the used soil surface and some physical and chemical properties of the soil were done according to the methods described by Jackson [18] Black et al. [19] as shown in Table 1

Table 1: Physical and Chemical properties of the experimental soil at 2020 and 2021

Season	Particle size distribution			Texture Grade	Chemical properties									
	Clay %	Silt %	Sand %		pH	Ec dS/m	OM %	Available (ppm)						
				N				P	K	Fe	Zn	Mn		
2020	48.30	33.50	17.20	Clay	7.7	1.15	1.70	40.00	15.10	261.40	2,50	0.27	0.60	
2021	49.60	32.40	17.00	Clay	7.8	1.20	1.80	39.00	13.60	246.22	2.25	0.30	0.65	

Randomized Complete Design (RCD) with three replicates was followed in this experiment, individually for each (Fennel of Egypt and China). Four concentrations; 0 (tap water), 50, 100 and 150 ppm of benzyladenine (BA) were used as treatments for both Chinese and Egyptian fennel. Fennel seeds were sown in 3 x 3.60 m contained five rows. The planting distance was 40 cm between plants. After 30 days from planting the plants were thinned to two plants per hill (50 plants/ plot and 18,518.51 plants/fed.).

Benzyladenine (6-Benzylaminopurine; BA; BAP) was obtained as commercial chemical substance from Agres Company, Egypt. Benzyladenine (BA) was applied as a foliar spray. Spraying was done to cover whole plant leaves. All used treatments were applied three times at 3 weeks interval and the first treatment was applied after 45 days from planting. All other agricultural practices were followed as recommended.

2.1 Vegetative growth and yield characteristics

Three plants at the end of the experiment, were randomly selected from each treatment replication to measure some morphological parameters per plant as follows: Plant height (cm), number of branches, stem diameter (cm) dry weight (g), Yield characteristics: Number of umbels/plant, fruit yield/plant and then the fruit yield/feddan was calculated.

2.2 Essential oil isolation:

Dried fruits of *F. vulgare* were crushed and subjected to hydrodistillation with sterile water for 3 h using a Clevenger-type apparatus as reported by Erich [20]. The obtained essential oil was dried over anhydrous sodium sulphate, the percentage of oils calculated and then the oil yield per plant and feddan was calculated.

2.3 GC-MS Analysis of essential oils

Fennel oil (First season, 2020/2021) analyzes were performed in a central laboratory network, National Research Centre, and Cairo, Egypt. The essential oils extract was carried out by GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A). Samples were diluted with hexane (1:19, v/v). The GC was equipped with HP-5MS column (50 m x 0.25 mm internal diameter and 0.25 µm film thickness). The analysis was performed

using helium as carrier gas, a flow rate of 1.0 ml/min, a split ratio of 1:10, an injection volume of 1 µl, and the following temperature program. 1 minute at 40 °C. Heat up to 150°C at 4°C/min and hold for 6 minutes. Ramp at 4°C/min to 210°C and hold for 1 minute. The injector and detector were maintained at 280°C and 220°C, respectively. Mass spectra were acquired by electron ionization (EI) at 70 eV. Use a spectral range of m/z 50-550 and a solvent delay of 5 min. The identities of the various components were determined by comparing the spectral fragmentation patterns with those stored in Wiley and NIST mass spectral library data.

2.4 Total phenolic content (TPC)

TPC in Fennel essential oils for the first season 2020 were determined spectrophotometrically with a slight modification of the Folin-Ciocalteu (FC) method described in Hourieh [21]. TPC was measured and expressed as mg Gallic acid equivalent (GAE/L) using the following standard curve as a reference. Mix 20 µl of extract (essential oil diluted 1:60 with methanol) with 1 ml of Folin-Ciocalteus phenol reagent, add 1 ml of saturated sodium Na₂CO₃ (20%) after 3 minutes, and adjusted to 980 µl with distilled water. After incubation for 100 minutes in the dark at room temperature, the absorbance at λ=765 nm was measured using UV-VIS. A standard curve was generated using Gallic acid. A standard reference curve for Gallic acid was constructed for the following concentrations: 0, 20, 40, 100, 160, and 200 mg L⁻¹, respectively.

2.5 DPPH radical scavenging assay

Free radical scavenging activity of Fennel seed oil was measured in terms of radical scavenging ability using the stable free radical DPPH [22]. Different concentrations (10l, 20l, 30 l, 40l and 50µl) of sample were taken and 50µl of 0.659 mM DPPH dissolved in methanol solution was added to make up to one using double distilled water. The tubes were incubated at 25°C for 20 minutes. The absorbance value was recorded at 510 nm using Shimadzu UV 1800 spectrophotometer. The same procedure was followed for control without the sample.

$$\text{DPPH Scavenging ability (\%)} = [A \text{ control} - A \text{ sample} / A \text{ control}] \times 100$$

2.6 Statistical analysis

The obtained data are, statistically, analyzed by using Statistic version 8.1 software [23]. Differences between means were determined using the Least Significant Difference (L.S.D._{0.05}) test.

3. RESULTS

3.1 Vegetative characteristics

The data presented in Table (2) clearly show that vegetative characteristics of fennel plants from Egypt and China were significantly affected by benzyladenine (BA) treatments. Foliar application of benzyladenine significantly increased plant height, stem diameter, number of branches/plant and plant dry weight with compared to control treatment. The highest values in both seasons of studied vegetative parameters were obtained by using 100 ppm of BA followed by 150 and 50 ppm of BA, respectively. However there were no significant deference in vegetative parameters between 150 and 50 ppm treatments.

Table 2: Effect of benzyladenine (BA) on vegetative characteristics of fennel during the 2020/2021 and 2021/2022 seasons.

Source	BA (ppm)	1st season 20/2021				2nd season 21/2022			
		Plant height (cm)	Stem diameter (cm)/plant	Number of branches/plant	Plant dry weight (g)	Plant height (cm)	Stem diameter (cm)/plant	Number of branches/plant	Plant dry weight (g)
Egypt	Cont.	124.33	1.60	7.00	171.57	132.07	1.67	7.33	183.93
	50	145.13	1.90	8.33	240.97	157.27	2.00	9.00	254.97
	100	152.47	2.33	9.33	264.40	165.47	2.43	9.89	275.20
	150	147.03	1.93	8.56	241.33	158.03	1.97	9.00	249.23
L.S.D._{0.05}		4.86	0.241	0.57	6.22	5.83	0.191	0.720	7.30
China	Cont.	64.99	0.85	4.67	81.17	69.83	0.91	4.89	84.70
	50	74.50	1.14	5.44	155.19	75.33	1.16	5.78	160.89
	100	85.00	1.41	7.00	180.12	87.57	1.41	7.11	181.90
	150	77.67	1.27	6.33	157.12	80.10	1.29	6.67	161.69
L.S.D._{0.05}		4.90	0.131	0.570	9.93	3.20	0.091	0.440	5.84

3.2 Fruit and yield characteristics

The averages of fruit and yield parameters including number of umbels per plant, fruit yield per plant and fruit yield per feddan for all spraying treatments during two seasons are shown in Table (3). Available results show the significant influence of benzyladenine treatments on the investigated traits of Egyptian and Chinese fennel plants in both seasons. In this respect foliar application of BA at various concentrations caused a significant increases in number of umbels/plant, fruit yield per plant and feddan of fennel plants as compared to control treatment. For Egyptian fennel plants the maximum values of investigated fruit and yield traits were obtained by using 100 ppm of BA followed by 150 and 50 ppm of BA, respectively. No

significant differences noted in fruit and yield parameters between 150 and 50 ppm treatments.

Regarding Chinese fennel plants fruit and yield parameters exhibited similar trends to fruit and yield parameters of Egyptian fennel plants as demonstrated in Table (3). The highest values of parameters were obtained from moderate treatment (BA at 100 ppm) followed by 150 and 50 ppm of BA, respectively. Also the results clear that a significant difference in number of umbels/plant between all BA treatments was observed in the first season.

Table 3: Effect of benzyladenine (BA) on Yield parameters of fennel during the 2020/2021 and 2021/2022 seasons.

Source	BA (ppm)	1st season 20/2021			2nd season 21/2022		
		Number of umbels/plant	fruit yield/plant (g)	fruit yield/feddan (kg)	Number of umbels/plant	fruit yield/plant (g)	fruit yield/feddan (kg)
Egypt	Cont.	21.42	53.35	987.96	22.33	53.57	991.97
	50	35.00	68.97	1277.16	35.15	70.83	1311.73
	100	39.78	75.23	1393.21	41.22	76.13	1409.88
	150	35.67	69.30	1283.27	36.11	70.11	1298.33
	L.S.D._{0.05}	2.38	3.26	60.38	2.40	4.09	75.85
China	Cont.	11.44	17.45	323.09	13.33	19.17	354.94
	50	17.67	21.55	399.14	18.67	23.17	429.14
	100	24.56	28.97	536.42	25.67	30.60	566.67
	150	19.89	23.50	435.12	20.78	23.92	443.02
	L.S.D._{0.05}	1.47	3.01	55.85	1.18	2.56	47.52

However, there have been no significant differences in other parameters between 150 and 50 ppm treatments in both two seasons. Generally, the results have confirmed that fennel plants from China showed lower values of vegetative and fruit parameters than that of fennel plant from Egypt.

3.2 Essential oil percentage and oil yield

Data recorded in Table (4) clear that spraying fennel plants with benzyladenine (BA) at different concentration caused a significant increases in essential oil percentage, oil yield per plant and feddan compared to untreated plants in both seasons. For Egyptian fennel plants the highest percentage of essential oil was obtained by spraying with

100 and 150 ppm of BA (same percentage) in both seasons. However, the maximum oil yield per plant and feddan were obtained by using 100 ppm followed by 150 and 50 ppm of BA, respectively.

Table 4: Effect of benzyladenine (BA) on essential oil percentage, oil yield/plant and oil yield/feddan of fennel fruits during the 2020/2021 and 2021/2022 seasons.

Source	BA (ppm)	1st season 20/2021			2nd season 21/2022		
		Oil percentage	Oil yield/plant (ml)	Oil yield/feddan (liter)	Oil percentage	Oil yield/plant (ml)	Oil yield/feddan (liter)
Egypt	Cont.	1.41	0.75	13.97	1.42	0.76	14.09
	50	1.55	1.07	19.76	1.55	1.10	20.33
	100	1.68	1.26	23.41	1.69	1.28	23.78
	150	1.69	1.17	21.65	1.69	1.19	21.98
	L.S.D._{0.05}	0.057	0.074	1.43	0.042	0.074	1.35
China	Cont.	1.28	0.22	4.13	1.28	0.25	4.54
	50	1.36	0.29	5.43	1.37	0.32	5.86
	100	1.52	0.44	8.17	1.52	0.46	8.60
	150	1.50	0.35	6.53	1.50	0.36	6.66
	L.S.D._{0.05}	0.039	0.045	0.839	0.021	0.039	0.731

Concerning Chinese fennel, oil parameters exhibited similar trends to oil traits of Egyptian fennel plants as illustrated in Table (4). The maximum oil percentage, oil yield per plant and feddan were obtained by spraying BA at 100 ppm followed by 150 and 50 ppm of BA, respectively.

3.3 Main components of essential oil

The main components of essential oils for Egyptian and Chinese fennel fruits were presented in Table (5). Data clearly show that the major components of volatile oils were significantly affected by foliar applications with benzyladenine. The major components were trans-anethole followed by fenshone, α - pinene and D-limonene, respectively. The results indicate that spraying fennel plants with benzyladenine (BA) caused a slightly increase in these major components. For volatile oil of Egyptian fennel, the maximum value of trans-anethole resulted from plants treated with foliar

application of BA at 100 ppm followed by 150 ppm, respectively, as compared with the control.

Furthermore, the highest values of fenshone, α - pinene and D-limonene compounds were obtained from planted treated by 150 ppm followed by 100 ppm of BA treatments. The data also confirmed that there were no significant differences in trans-anethole and α - pinene compounds between 100 and 150 ppm spraying treatments. Regarding essential oil of Chinese fennel, the major components exhibited similar trends to the major components of Egyptian essential oil, the highest value of trans-anethole was obtained from planted treated with BA at 100 ppm followed by 150 ppm, respectively, relative to the control. Meanwhile, the highest values of fenshone, α - pinene and D-limonene compounds were obtained from planted treated by 150 ppm followed by 100 ppm of BA treatments. There were no significant differences in trans-anethole, fenshone and α - pinene compounds between 100 and 150 ppm treatments.

Table 5: Effect of benzyladenine (BA) on Main components % of essential oils during the 2020/2021 season.

Source	BA (ppm)	Main components %			
		Trans-anethole	Fenshone	α - Pinene	D-Limonene
Egypt	Cont.	30.11	9.31	8.24	6.68
	50	31.29	9.54	8.68	7.11
	100	35.16	10.42	9.75	8.04
	150	34.63	11.45	10.08	8.66
	L.S.D._{0.05}	2.49	0.72	0.37	0.39
China	Cont.	36.25	10.26	10.49	7.77
	50	38.69	10.93	10.93	9.57
	100	40.87	11.91	11.47	10.21
	150	39.84	12.01	11.84	10.83
	L.S.D._{0.05}	1.24	0.45	0.41	0.52

3.4 Total phenolic contents of essential oils

The total phenolic contents (TPC) for essential oils from fennel plants of Egypt and China are presented in Table (6). The results have confirmed that total phenolic contents in both essential oils were significantly affected by spraying BA at various

concentrations. For Egyptian essential oil, spraying fennel plants with benzyladenine (BA) caused an increase in TPC as relative to untreated plants. In among BA treatments, the maximum value of TPC was obtained from plants treated with foliar application of BA at 150 ppm followed by 100 and 50 ppm, respectively. However, there were no significant differences in TPC between 100 and 50 ppm spraying treatments.

Table 6: Effect of benzyladenine (BA) on antioxidant activities of fennel essential oils during the 2020/2021 season.

BA (ppm)	Egyptian fennel essential oil		Chinese fennel essential oil	
	TPC (mg GAE/100 g)	DPPH (Inhibition %)	TPC (mg GAE/100 g)	DPPH (Inhibition %)
Cont.	422.90	26.40	610.94	31.02
50	431.32	31.05	619.51	35.49
100	434.73	34.55	626.14	37.55
150	470.55	33.35	642.31	35.71
L.S.D._{0.05}	5.49	2.77	2.51	2.15

For Chinese essential oil, the TPC exhibited same line to TPC of Egyptian essential oil as illustrated in Table (6). All benzyladenine treatments caused a significant increase in TPC as compared to the control. The highest value of TPC was obtained from plants treated with foliar application of BA at 150 ppm followed by 100 and 50 ppm, respectively.

3.5 DPPH radical scavenging assay of fennel essential oils

The effect of benzyladenine treatments on free radical scavenging activities of the fennel essential oils are shown in Table (6). Data demonstrated that essential oils extracted from plants treated with BA produced high values of inhibition of DPPH radical activity compared with untreated plants. For Egyptian essential oil, BA treatments caused a significant increase in inhibition of DPPH radical activity. 100 ppm of BA gave the highest value of inhibition of DPPH followed by 150 and 50 ppm, respectively. However, no significant differences between 100 and 150 ppm treatments. Also no significant difference between 150 and 50 ppm treatments was observed.

Concerning to Chinese essential oil, inhibition of DPPH radical activity significantly increased by BA treatments as illustrated in table 6. The highest value of

inhibition of DPPH obtained from plants treated with foliar application of BA at 100 ppm followed by 150 and 50 ppm, respectively, without significant differences between them. In general, the results have confirmed that Chinese essential oil exhibited higher contents of phenolic and radical scavenging activity than that of Egyptian essential oil.

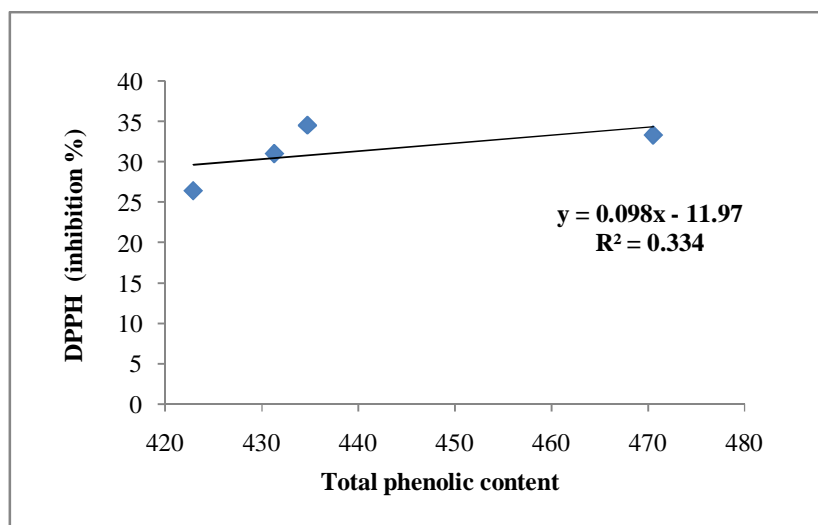


Fig. 1. Linear correlation between the antioxidant activity data from DPPH assays and the total phenolic content of Egyptian essential oil.

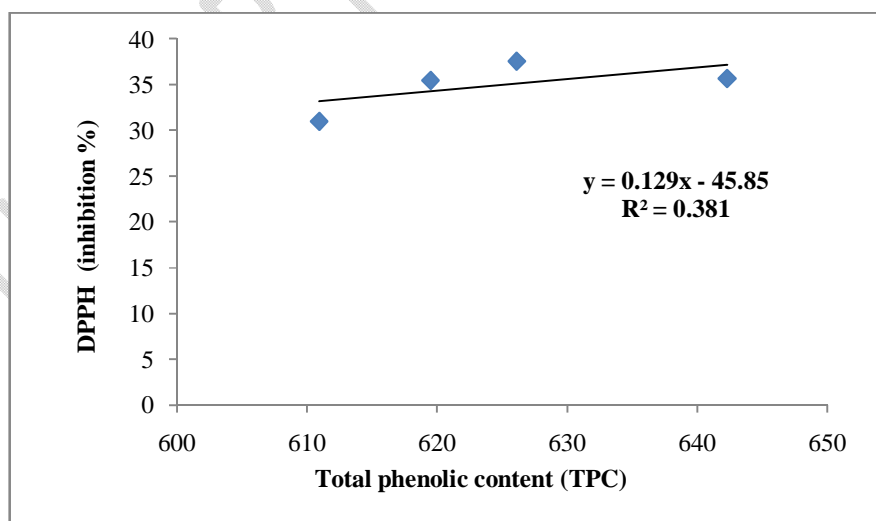


Fig. 2. Linear correlation between the antioxidant activity data from DPPH assays and the total phenolic content (TPC) of Chinese essential oil.

4. DISCUSSION

Spraying fennel plants from Egypt and China with benzyladenine as foliar application had positive effect on vegetative growth, number of umbels/plant, fruit, yield and oil yield per plant and feddan as well as main components, total phenolic contents and antioxidant activity of essential oils compared to untreated plants.

This increment in vegetative parameters by using benzyladenine could be attributed to stimulatory effects of BA on cell division, cell elongation, shoot initiation and growth, nutrient mobilization, delay the processes of senescence and apical dominance alternation in plants [24,25]. These results are in a similar pattern with those of Abdel-Rahman and Abdel-Kader [26] they observed that foliar application of BA had a significant increase in plant height, number of branches/plant and plant dry weight. The highest values of measurements were obtained by using 100 ppm of BA. Likewise, Talaat and Gamal El-Din [11] reported that spraying fennel plants with kinetin significantly increased number of branches per plant, fresh and dry weight. Similar results were obtained by Ayad and Gamal El-Din [14] on *Lupinus termis*, Scravoni et al. [27] on *Mentha piperita*, Reda et al. [28] on *Chamomile recutite*. They observed that the vegetative growth parameters were increased as a result of foliar application with BA.

On the other hand our results indicate that spraying fennel plants from Egypt and China with high level 150 ppm of BA resulted in a significant decrease in the plant growth compared with moderate level (100 ppm). These results are in harmony with the findings of Abdel-Rahman and Abdel-Kader [26] they found that spraying fennel plants with high concentration of BA caused a reduction in plant growth parameters. Also, Srivastava [29] who observed that exogenously applied cytokinins in high concentrations results in growth inhibition.

Regarding to fruit and yield parameters, the reason for increment in fruit and yield parameters of Egyptian and Chinese fennel is that benzyladenine treatment may stimulate the synthesis of nucleic acids and proteins and help transport nutrients and hormones, including auxins, to the treated area [30]. In addition to the role of cytokinin in stimulating division, cell elongation, growth of lateral buds, and breaking dormancy of buds, it is one of the main determinants of fruit growth and reaching the final size, as the addition of synthetic cytokinins increases the effectiveness of cytokinin like compounds in plant tissues, which stimulates the growth of fruits in some plants [31, 32].

These results are in consistent with Abdel-Rahman and Abdel-Kader [26] on fennel, Al-Taey *et al.* [33] on *Vicia faba* and Al-Hamdani and Al-Jubouri [34] on orange. Furthermore, our experiment identified a strong relationship between the vegetative and fruit parameters in Egyptian and Chinese fennel. Spraying plants with BA at 100 ppm improved plant height and branches number which in turn led to an increase in number of umbels, fruit yield per plant and total yield per feddan. The variation in values of vegetative and fruit parameters may be due to varied in climatically and geographical conditions of Egypt than that of China.

The positive results of BA applications on oil parameters may be due to physiological role of cytokinins such as benzyladenine (BA), zeatin and kinetin in stimulating cell divisions and regulation of biochemical processes [35]. Moreover, the stimulative effect of these treatments on increasing essential oil percentage and oil yield might be attributed to their enhancing effect on vegetative growth characteristics and plant chemical composition such as nitrogen, phosphorus, potassium and total carbohydrates of fennel plants, which can influence the fruit and oil yield. In addition, the increases in the oil yield per plant and feddan as a result of BA applications could be attributed to the increase in the volatile oil percentage and fruit yield production.

In addition, the increase in the essential oil yield with benzyladenine application attributed to the increase in number of glandular hairs in the leaves and fruits of several species [36, 37, 16]. Our results are in agreement with the findings of [26, 12] on fennel.

Main components of essential oil also increased due to application of BA. The significant increase in the concentration of secondary metabolites in this study can be explained by the role of growth regulators such as benzyladenine in stimulating and activating different enzymes that have a direct relationship in the synthesis of secondary metabolites. These results are in accordance with those obtained by Jabbar and Hamza [12] who found that spraying fennel plant with benzyladenine gave the highest values for the concentration of (*trans*-Anethole, Fenchone, Limonene, and α -pinene) compounds. Li *et al.* [38] and Hazzoumi *et al.* [17] who reported that monoterpenes of *Ocimum gratissimum* and *Ocimum basilicum* are highly influenced by plant growth regulators treatments due to the genes regulation which cause an increase in enzyme numbers related to the metabolic pathways of these compounds.

Weak correlation ($R^2= 0.334$) was found between antioxidant activity data from DPPH assays and total phenolic contents of Egyptian essential oils (Fig.1). Also

we observed that a weak correlation ($R^2= 0.381$) between antioxidant activity data from DPPH assays and total phenolic contents of Chinese essential oils (Fig. 2). This weakness correlation between the antioxidant activity and the total content of phenols in the volatile oils reflects the fact that the high percentage of antioxidant activity is not only attributable to the volatile oil content of phenols. It may also come from the presence of other antioxidant secondary metabolites.

The increment in radical scavenging activity of essential oils was probably due to an increase of *trans*-anethole content [39, 40, 7]. In Our experiment Chinese essential oil from plants treated with 100 ppm of BA recorded higher *trans*-anethole content than that of Egyptian essential oil (table 5). This could be an explanation for the increasing value of radical scavenging activity of Chinese essential oil.

5. CONCLUSION

The obtained results showed that spraying fennel plants with BA caused a significant increase in the vegetative growth, yield characteristics and oil yield as well as main components, total phenolic contents and antioxidant activity of essential oils compared to untreated plants. Application of benzyladenine was more effective on Egyptian fennel plants than that of Chinese plants. The highest values of all studied parameters were obtained by using BA at 100 ppm. GC–MS analysis of the volatile oils showed that the major components were *trans*-anethole followed by fenshone, α -pinene and D-limonene, respectively for both essential oils. 100 ppm of BA gave the highest values of *trans*-anethole content and radical scavenging activities of oils. Therefore, we recommended that spraying fennel plants with 100 ppm BA can be used for obtaining higher vegetative growth, fruit and quantity and quality of oil.

References

1. **Barros L, Carvalho AM, Ferreira ICFR.** The nutritional composition of fennel (*Foeniculum vulgare*): shoots, leaves, stems and inflorescences. *LWT—Food Sci. Technol.* 2010; 43: 814–818.
2. **Brender T, Gruenwald J, Jaenicke C.** Herbal Remedies. Phytopharm Consulting Institute for Phytopharmaceuticals. Second ed. Schaper and Brummer GmbH and Co. Salzgitter, Berlin, Germany 1997
3. **Damjanovic B, Lepojevic Z, Zivkovic V, Tolic A.** Extraction of fennel (*Foeniculum vulgare* Mill.) seeds with supercritical CO₂: comparison with hydrodistillation. *Food Chem.* 2005; 92: 143–149.
4. **Oktay M, Gülcin İ, Küfrevioğlu ÖI.** Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *LWT—Food Sci. Technol.* 2003; 36: 263–271.
5. **Piccaglia R, Marotti M.** Characterization of some Italian types of wild fennel (*Foeniculum vulgare* mill.). *J. Agric. Food Chem.* 2001; 49: 239–244.
6. **Diaaz-Maroto MC, Pearez-Coello MS, Esteban J, Sanz J.** Comparison of the volatile composition of wild fennel samples (*Foeniculum vulgare* Mill.) from Central Spain. *J. Agric. Food Chem.* 2006; 54: 6814–6818.
7. **Ahmed AF, Shi M, Liu C, Kang W.** Comparative analysis of antioxidant activities of essential oils and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Egypt and China. *Food Sci. Hum. Wellness.* 2019; 8: 67–72.
8. **Yeh SY, Chen HW, Ng CY, Lin CY, Tseng TH, Li WH, Ku MS.** Down-regulation of cytokinin oxidase 2 expression increases tiller number and improves rice yield. *Rice.* 2015; 8: 36.
9. **Buban T.** The use of benzyl adenine in orchard fruit growing: A mini review. *Plant Growth Reg.* 2000; 32: 381-390.
10. **Van Staden J, Crouch R.** Benzyladenine and derivatives – their significance and inter conversion in plants. *Plant Growth Reg.* 1996; 19: 153-175.
11. **Talaat IM, Gamal El-Din KM.** Physiological effect of indole acetic acid and kinetin on the growth, yield and chemical constituents of fennel (*Foeniculum vulgare* Mill) plants. *Annals of Agricultural Science, Moshtohor.* 1998; 36: 187–196.

12. **Jabbar AF, Hamza IA.** 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. Euphrates Journal of Agriculture Science. 2021; 13: 53-71
13. **Khaswa SL, Dubey RK, Singh S, Tiwari RC.** Growth, productivity and quality of soybean (*Glycine max* (L.) Merr.) under different levels and sources of phosphorus and plant growth regulators in Subhumid Rajasthan. Afr. J. Agric. Res. 2014; 9:1045–1051.
14. **Ayad HS, Gamal El-Din KM.** Effect of atonik and benzyladenine on growth and some biochemical constituents of lupine plant (*Lupinus termis* L.). American-Eurasian J. Agric & Environ Sci. 2011; 10: 519–524.
15. **Hussein HAA.** Biochemical and Physiological Studies on some Alkaloids. PhD Thesis. Faculty of Agriculture Cairo University. 2005
16. **Prins CL, Vieira IJC, Freitas Braz SP.** Growth regulators and essential oil production. Braz. J. Plant Physiol. 2010; 22: 91–102.
17. **Hazzoumi Z, Moustakime Y, Joutei KA.** Effect of gibberellic acid (GA), indole acetic acid (IAA) and benzylaminopurine (BAP) on the synthesis of essential oils and the isomerization of methyl chavicol and trans-anethole in *Ocimum gratissimum* L. Springer plus 2014; 3: 321–327.
18. **Jackson ML.** Soil Chemical Analysis. Prentic-Hall. Inc, Englewood, Cliffs, U.S.A. 1973.
19. **Black CA, Evans DD, NHITE JI, Ensminger LE, Clark FE.** Methods of soil analysis. American Society of Agronomy Inc. Madison, Wisconsin, U.S.A. 1965; 1-770.
20. **Erich K.** Handbook of Laboratory Distillation with an Introduction to Pilot Plant Distillation. Published in co-edition with VEB Deutmher Verlag der Wissenechaftan, Berlin. 1982
21. **Hourieh A.** Determination of Chemical Composition, Antioxidant activity, and Antimicrobial activity of essential oils of Damask *Ocimum Basilicum* L. J. Mater. Environ. Sci. 2021; 12: 919-928.
22. **Jain PK, Agrawal RK.** Antioxidant and free radical scavenging properties of developed mono and poly herbal formulations. Asian J Exp Sci 2008; 22: 213-220.

23. **Analytical software** Statistix 8.1 for Windows analytical software. Tallahassee, Florida. 2005
24. **Duck MW, Gregg BM, Fernandez RT, Royal DH, Cardoso FF.** Height control of *Picea spp.* and *Chamaecyparis lawsoniana* with uniconazole and 6-enzyladenine. J. Enviro Hort. 2004; 22: 165–169.
25. **Taiz L, Zeiger E.** Plant Physiology. 4th ed. Sinauer Associates Inc. Publishers, Massachusetts. 2006, USA.
26. **Abdel-Rahman SSA, Abdel-Kader AAS.** Response of Fennel (*Foeniculum vulgare*, Mill) plants to foliar application of moringa leaf extract and benzyladenine (BA). South African Journal of Botany. **2020**; 129:113–122.
27. **Scravoni J, Vasconcellos MC, Valmorbida J, Ferri AF, Marques MOM, Ono EO, Rodrigues JD.** Rendimento e composição química do óleo essencial de *Mentha piperita* L. submetida a aplicações de giberelina e citocinina. Revista Brasileira de Plantas Medicinaias 2006; 8: 40–43.
28. **Reda F, Tarraf SH, Abd El Rahim EA, Afify AS, Ayad HH.** The response of growth and some chemical constituents of chamomile plant to benzylaminopurine (BAP). The Journal of Agricultural Science, Mansoura University. 1999; 24: 2209–2222.
29. **Srivastava LM 2002.** Plant Growth and Development. Academic Press, San Diego. 2002: 303–379.
30. **Wilkins MB.** The physiology of plant growth and development. Mc Graw – Hill , London. 1979: 695.
31. **Al-Taey DKA, Majid ZZ.** Study Effect of Kinetin, Bio-fertilizers and Organic Matter Application in Lettuce under Salt Stress. Journal of Global Pharma Technology. 2018; 10:148-164.
32. **AL-Taey DKA, Saadoon AHS, ALAzawi SSM.** Study of Kinetin treatment on growth and activity of some antioxidant enzymes of Spinach under salt stress. Current Trends in Natural Sciences. 2021; 10:457-465.
33. **Al-Taey DKA, Mijwel AK Al-Azawy SS.** Study efficiency of poultry litter and kinetin in reduced effects of saline water in *Vicia faba*. Research J. Pharm. and Tech. 2018; 11: 294-300.
34. **Al-Hamdani KAS, Al-Jubouri MNH.** Effect of Benzyladenine , Urea , Iron, Boron and Nu film-17 Antitranspiration Spraying on Fruit Set, Dropping and Some Growth Characteristics of Vegetative in Orange (*Citrus seninsis*) Tikrit

University Journal of Agricultural Sciences - a special issue of the proceedings of the third specialized conference-plant production. 2014:28-35.

35. **Borkowska B.** Cytokininy. In: Jankiewicz, L.S. (Ed.), *Regulatory wzrostu i rozwoju roślin*. Wyd. Nauk. PWN, Warszawa, 1997: 60–71.
36. **Gershenzon J.** Metabolic costs of terpenoid accumulation in higher plants. *J Chem Ecol.* 1994; 20, 1281–1328.
37. **Erbelgin N, Krokene P, Christiansen E, Gazmend Z, Gershenzon J.** Exogenous application of methyl jasmonate elicits defenses in Norway spruce (*Picea abies*) and reduces host colonization by the bark beetle *Ips typographus*. *Oecologia.* 2006; 148: 426–436.
38. **Li Z, Wang X, Chen F, Kim HJ.** Chemical changes and overexpressed genes in sweet basil (*Ocimum basilicum* L.) upon methyl jasmonate treatment. *J Agric Food Chem.* 2007; 55: 706–713.
39. **Viuda-Martos M, Mohamady MA, Fernández-López J, Abd ElRazik KA, Omer EA, Pérez-Alvarez JA, Sendra E.** In vitro antioxidant and antibacterial activities of essential oils obtained from Egyptian aromatic plants. *Food Control.* 2011; 22: 1715–1722.
40. **Senatore F, Oliviero F, Scandolera E, Tagliatalata-Scafati O, Roscigno G, Zaccardelli M, De Falco E.** Chemical composition, antimicrobial and antioxidant activities of anethole rich oil from leaves of selected varieties of fennel [*Foeniculum vulgare* Mill. ssp. *vulgare* var. *azoricum* (Mill.) Thell]. *Fitoterapia.* 2013; 90: 214–219.