

## IMPACT OF SOME GROWTH STIMULANTS IN COOPERATION WITH ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH, PRODUCTIVITY AND CHEMICAL CONSTITUENTS OF DUTCH FENNEL PLANT

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**ABSTRACT:** Fennel is an important medicinal, and aromatic plant occupied the first in the list of Egyptian exports of herbs and spices. Dutch fennel is described by a large percentage of anethole and a lower percentage of estragole than the Egyptian fennel, therefore, it is used as an antidote to some viral diseases. The present research aims to investigate the influence of various growth stimulants (amino acids, brassinolide, humic acid, salicylic acid, seaweeds extract and tap water as control) and arbuscular mycorrhizal fungi as well as their combinations on vegetative growth, productivity and some chemical constituents of Dutch fennel plant (*Foeniculum vulgare* Mill. spp. *vulgare*). This study was conducted at the Experimental Farm and in the Laboratory at the Hort. Dept., Fac. Agric. at Moshtohor, Benha Univ., Egypt, during 2018/2019 and 2019/2020 seasons. The greatest significant values of all studied parameters were gained by the combined treatment of seaweed extract at 2 ml/l with superior for the inoculum mycorrhizal fungi in 1<sup>st</sup> and 2<sup>nd</sup> seasons. The largest seeds oil percentage (2.22 and 2.24) was recorded by the combined treatment between seaweed extract combined with inoculum arbuscular mycorrhizal fungi (AMF). The chemical constituents of Dutch fennel essential oil about ten components, the master components of essential oil constituents trans-anethole, estragole, and myrcene. In general, it could be recommended that the combinations of seaweed extract at 2 ml/l and inoculum arbuscular mycorrhizal fungi (AMF) could be achieved the greatest growth, productivity and chemical constituents of Dutch fennel.

**Key words:** Dutch fennel, *Foeniculum vulgare* Mill. spp. *vulgare*, growth stimulants, mycorrhiza, seed yield, oil productivity.

### INTRODUCTION

Fennel is an important medicinal and aromatic plant occupied the first in the list of Egyptian exports of herbs and spices. Family Apiaceae includes fennel, *Foeniculum vulgare* Miller, an herbaceous plant (Farrell, 1990; Wichtl and Bisset, 1994). Its origin is the Mediterranean and planted in many parts of the world i.e. Africa, Asia, Europe and some parts of South America (Blumenthal *et al.*, 2000). Assiut and Qena Governorates,

Egypt represent the largest area of fennel around 11000 feddans (Abd El-Aleem *et al.*, 2017). The fennel seeds and oils are used as an antidote to some viral diseases i.e. cancer, liver pain, colic in children and abdominal pains (Badgujar *et al.*, 2014; Rather *et al.*, 2012; Choi and Hwang, 2004; Koppula and Kumar, 2013). Dutch fennel (*Foeniculum vulgare* Mill. spp. *vulgare*) is a new strain of sweet fennel and imported from the Netherlands, as characterized by maximum

seeds and oil yields, a higher percentage of anethole and a lower percentage of estragole in comparison of the Egyptian fennel (Shalaby *et al.*, 2011).

In this context, major effort is applied as foliar spray of bio, organic fertilizers, natural biostimulators plus antioxidants. Here, amino acids more affect the activity of many enzymes, gene expression, and have a great vital role in plants. Brassinosteroids are among the safety biostimulators, they occur in free form and conjugated to sugars and fatty acids and are considered as highly promising, therefore, are considered one of the eco. safety growth stimulants. Humic acid (HA) has a large necessary for the plant and the soil as it works to enhance the properties of the soil and increase the rate of microbial activity as enhances the absorption of many nutrients in the soil. Salicylic acid (SA) antioxidant enzyme decreases generation of reactive oxygen species (ROS) and a signaling or messenger molecule in plants and induces plant tolerance against many biotic and abiotic stresses, so enhance plant tolerance to abiotic stress. Seaweeds comprise major and minor nutrients, amino acids, vitamins, cytokinins, auxin and abscisic acid such as growth promoting substances. Seaweeds reflexed the activity of enzymes, and consequently, and enhancement in biological processes in the plant. Finally, arbuscular mycorrhizal fungi (AMF) bio fertilizers, increase plant yield, enhance the plant uptake of immobile phosphate ions from the soil as well as N, P, K, Mg etc... (Rai, 2002; Taiz and Zeiger, 2002; Kang and Guo, 2011; Bera *et al.*, 2014; Berlyn and Russo, 1990; Horvath *et al.*, 2007; Metwally *et al.*, 2003; Crouch and Van Staden, 1994; EL-Boukhari *et al.*, 2020; Smith *et al.*, 2011 and Veresoglou *et al.*, 2011).

The effect of some safety growth stimulant treatments and arbuscular mycorrhizal fungi on vegetative growth, yield and oil productivity in several studied of medicinal and aromatic plants was revealed by Mohamed *et al.* (2015) on

*Ocimum basilicum*, L. cv. Genovese plant, El-Khateeb (2017) on *Majorana hortensis*, Ibrahim and Helaly (2017) on fertilized fenugreek, Mahdy *et al.* (2019) on roselle, Mansori *et al.* (2019) on *Salvia officinalis*, Moghith (2019) on chia, Ghatas (2020) on *Coriandrum sativum* L. and also Mady (2020) on Dutch fennel.

The aim of this work was to appraise the benefits of supplementing some growth stimulating substances i.e. amino acids, brassinosteroids, humic acid, salicylic acid and seaweeds extract in the presence of arbuscular mycorrhizal fungi on vegetative growth, seed yield, oil production and the chemical constituents of Dutch fennel (*Foeniculum vulgare* Mill. spp. *vulgare*) plant.

## MATERIALS AND METHODS

This study was executed successfully at the Experimental Farm and in the Laboratories of Horticulture Departments, Faculty of Agriculture at Moshtohor, Benha Univ., during 2018/2019 and 2019/2020 seasons to study the effect of some growth stimulant treatments i.e. amino acids, brassinolide, humic acid, salicylic acid and seaweeds extract and arbuscular mycorrhizal fungi as well as their combinations on vegetative growth, essential oil productivity and some chemical constituents of Dutch fennel (*Foeniculum vulgare* Mill. spp. *vulgare*) plant.

Dutch fennel seeds were obtained from Floriculture Farm, Horticulture Department, Faculty of Agriculture, Benha Univ. Seeds of Dutch fennel (1000 seed weight 12.77 g) were sown in clayey loam soil on mid-October in both seasons in plots (1×1 m) containing two rows (50 cm in between) every row has two hills (50 cm apart) and six weeks later, the plants were thinned, leaving only two seedling/hill.

Physical and chemical analysis of the experimental soil were determined according to Jackson (1973) and Black *et al.* (1982), respectively. The obtained results of soil analysis are presented in Table (1).

**Table 1. Mechanical and chemical analysis of the experimental soil.**

Mechanical properties			Chemical analysis		
Parameters	Values		Parameters	Values	
	2018/2019	2019/2020		2018/2019	2019/2020
Coarse sand	3.96 %	4.08 %	Organic matter	1.82%	1.94 %
Fine sand	16.33 %	15.88 %	CaCO <sub>3</sub>	0.78 %	0.84 %
Silt	25.01 %	26.42 %	Available nitrogen	0.87 %	0.95%
Clay	54.70%	53.62%	Available phosphorus	0.49 %	0.53%
Textural class	Clayey loam	Clayey loam	Available potassium	0.71 %	0.68 %
			pH	7.66	7.52
			EC (dS/m)	0.69	0.77

The layout of the experiment was a complete randomized block design with two factors in three replicates. The first factor involving six growth stimulants treatments, whereas the second factor was devoted to two mycorrhizal fungi treatment (uninoculum and inoculum). Therefore, the experiment included 12 treatments with three replicates, each replicate contained 20 plants i.e. 60 plants in each treatment.

**Safety growth stimulants treatments:**

1. The control treatment (spray with tap water).
2. Amino acids as amino power at 2 ml/l for each as foliar spray, is a commercial product from Union for Agriculture Development (UAD) contain 20% free L-amino acids, 40% total amino acids, 3% mix of vitamins, 3.5% potassium citrate and some micro nutrients such as 1500 ppm Fe, 500 ppm Zn and 500 ppm Mn.
3. Brassinolide at 50 mg/l for each as foliar spray, was obtained from the Union for Agriculture Development Co. (UAD) Egypt.
4. Humic acid at 5 ml/l for each as a foliar spray, 80% soluble potassium humate. It is a commercial product by Leili Agrochemistry Co. Ltd. It includes: humic acid (80%), K<sub>2</sub>O (11-13%) and Moisture (5-7%).
5. Salicylic acid: salicylic acid solution sprayed at 200 ppm for each as foliar spray.
6. Seaweeds extract at 2 ml/l for each as foliar spray, seaweeds extract produced by Orbital for Agrochemicals, Egypt. The

used extract contains minerals as Fe, Zn, Cu, Mn and Mo, vitamins, enzymes, amino acids, sugars, and plant hormones, (i.e. auxins, cytokinins and gibberellins).

The growth stimulants amino acids, brassinolide, humic acid, salicylic acid and seaweed extracts were applied as a foliar spray on plant leaves 4 times, the first one was added after 30 days from transplanting. The second time was after 15 days from the first, while the third was applied after 15 days from the second and the fourth was after 15 days from the third. Spraying was done to the run off the plant foliage,

Using bio-film at 1 ml/l as a wetting agent, was added to all tested solutions including the control. Treated plants were sprayed with a hand pump mister till run off, whereas control plants were sprayed with tap water. Common agricultural practices (irrigation, fertilization, manual weed control, ... etc.) were carried out when needed.

**Arbuscular mycorrhizal fungi:**

Arbuscular mycorrhizal fungi inoculum consisted of roots, hyphae, spores and growth media from a pot culture of onion plants colonization with *Glomus mosseae* NRC31 and *Glomus fasciculatum* NRC15 originally isolated from Egyptian soils and multiplied on sterilized peat:vermicolite:perlite (Badr El-Din *et al.*, 1999). Arbuscular mycorrhizal fungi were obtained from Agricultural Microbiology Department, National Research Center. Inoculum material contained 275 spores g<sup>-1</sup> oven dry bases in addition to the colonization roots pieces (the infectivity 104 propagola). Mycorrhizal

fungi inoculation was done by mixing 20 g of it with 5 g of fennel seed before cultivation and then after 10 days by injection into the soil in roots area of seedlings from four sides at 3.5 g/hill of inoculum material.

#### **Harvesting:**

The plants were harvested on May, 1<sup>st</sup> in the first and second seasons.

#### **Data measurements and recorded:**

The vegetative and yield parameters were measured and recorded at harvesting time on May, 1<sup>st</sup> 2019 and 2020 as follows: the vegetative parts were cut about 1 cm above the soil surface. Measurements of the following traits were collected:

1. Vegetative characteristics at the beginning of flowering: height of plant (cm), branches No./plant and fresh and dry weights of the plant (g/plant).
2. Seeds yield parameters: after harvesting time plants were removed to station for sampling and other measurements, the following measurements were taken; umbels No./plant, 1000 seeds weight (g), weight of seeds/plant (g) and weight of seeds/feddan (ton).
3. Essential oil parameters:
  - a. Essential oil percentage was calculated as ml of oil/100 grams of seeds using the following equation:  
$$\text{Essential oil percentage} = \frac{\text{oil volume in the graduated tube}}{\text{dry weight of samples}} \times 100$$
  - b. Essential oil yield/plant (ml).
  - c. Essential oil yield/feddan (l).

Essential oil percentage of fennel fruits was determined by hydro distillation for 3 h using the method of Guenther (1961), the oil percentage was used to calculate essential oil yield/plant (ml), and feddan (l). The extracted volatile oil was dehydrated over anhydrous sodium sulphate and stored in a refrigerator until GC/MS analysis.

#### 4. Chemical constituents:

- a. Photosynthetic pigments: chlorophyll a, b and carotenoids were colorimetrically determined in leaves of fennel according to the method described by Inskeep and Bloom (1985) and calculated as mg/g fresh weight.
- b. Nitrogen, phosphorus, potassium and total carbohydrates % were determined in dried fennel leaves according to the methods described by Horneck and Miller (1998), Hucker and Catroux (1980), Horneck and Hanson (1998) and Chaplin and Kennedy (1994), respectively.

#### **GLC analysis of essential oil:**

The GLC analysis for oil samples from the second season only from the first experiment was carried out at the Medicinal and Aromatic Plant Laboratory, Dokki, Egypt. The gas liquid chromatography was analyzed according to Guenther (1961) and British Pharm. (1980).

#### **Statistical analysis:**

The design of the experiment was factorial experiment in a complete randomized block design, the differences between the mean values of various treatments were compared by Duncan's multiple range test (Duncan's, 1955) as given by Snedecor and Cochran (1989) using MSTAT-C statistical software package.

## **RESULTS AND DISCUSSION**

### **Effect of some growth stimulant treatments, arbuscular mycorrhizal fungi and their combination treatments on:**

#### **1. Vegetative growth measurements:**

Concerning the effect of foliar spray of some growth stimulants treatments, data presented in Tables (2 and 3) declared that, the foliar spray of each of amino acid (amino power) at 2 ml/l, brassinolide at 50 mg/l, humic acid at 5.0 ml/l, salicylic acid (SA) at 200 ppm and seaweed extract at 2 ml/l

**Table 2. Effect of some growth stimulants, mycorrhizal fungi and their combination treatments on plant height and number of branches/plant of Dutch fennel (*Foeniculum vulgare* Mill.), during 2018/2019 and 2019/2020 seasons.**

Growth stimulants	Height of plant (cm)			Branches No./plant		
	First season					
	Uninoculum AMF	Inoculum AMF	Mean (A)	Uninoculum AMF	Inoculum AMF	Mean (A)
Control (tap water)	59.39 l	63.40 k	61.40 f	12.33 f	15.67 e	14.00 e
Amino power at 2ml/l	90.00 f	93.47 d	91.74 c	19.33 d	21.33 c	20.33 c
Brassinolide at 50 mg/l	71.37 i	75.32 g	73.34 d	16.33 e	18.33 d	17.33 c
Humic acid at 5.0 ml/l	92.03 e	96.09 c	94.06 b	19.33 d	23.33 b	21.33 b
SA at 200 ppm	67.39 j	73.32 h	70.35 e	15.33 e	18.00 d	16.67 d
Seaweeds extract at 2 ml/l	99.53 b	106.3 a	102.9 a	23.33 b	26.00 a	24.67 a
Mean (B)	79.95 b	84.65 a		17.67 b	20.44 a	
	Second season					
Control (tap water)	59.50 h	66.31 g	62.90 f	12.67 f	16.33 e	14.50 d
Amino power at 2ml/l	90.74 d	95.04 c	92.89 c	20.33 c	22.33 b	21.33 b
Brassinolide at 50 mg/l	70.71 f	75.34 e	73.03 d	15.67e	19.33 cd	17.50 c
Humic acid at 5.0 ml/l	94.03 c	97.81 b	95.92 b	19.33 cd	23.00 b	21.17 b
SA at 200 ppm	69.48 f	74.03 e	71.76 e	16.33 e	18.33 d	17.33 c
Seaweeds extract at 2 ml/l	97.00 b	107.4 a	102.2 a	22.33 b	26.67 a	24.50 a
Mean (B)	80.24 b	86.00 a		17.78 b	21.00 a	

AMF = Arbuscular mycorrhizal fungi; SA= Salicylic acid

**Table 3. Effect of some growth stimulants, mycorrhizal fungi and their combination treatments on herb fresh and dry weights (g/plant) of Dutch fennel (*Foeniculum vulgare* Mill.), during 2018/2019 and 2019/2020 seasons.**

Growth stimulants	Herb fresh weight (g/plant)			Herb dry weight (g/plant)		
	First season					
	Uninoculum AMF	Inoculum AMF	Mean (A)	Uninoculum AMF	Inoculum AMF	Mean (A)
Control (tap water)	168.1 k	171.9 j	170.0 f	54.62 l	61.03 k	57.83 f
Amino power at 2ml/l	457.3 e	462.3 d	459.8 c	165.8 f	168.4 e	167.1 c
Brassinolide at 50 mg/l	410.5 g	417.0 f	413.8 d	151.4 h	153.9 g	152.6 d
Humic acid at 5.0 ml/l	462.3 d	469.2 c	465.8 b	171.0 d	174.6 c	172.8 b
SA at 200 ppm	359.8 i	369.0 h	364.4 e	135.9 j	140.3 i	138.1 e
Seaweeds extract at 2 ml/l	479.4 b	487.4 a	483.4 a	177.9 b	181.3 a	179.6 a
Mean (B)	389.6 b	396.1 a		142.8 b	146.6 a	
	Second season					
Control (tap water)	170.3 k	172.7 j	171.5 f	56.56 l	61.33 k	58.95 f
Amino power at 2ml/l	459.3 e	464.4 d	461.9 c	165.7 f	171.0 e	168.4 c
Brassinolide at 50 mg/l	412.5 g	417.1 f	414.8 d	151.7 h	154.9 g	153.3 d
Humic acid at 5.0 ml/l	466.1 d	473.0 c	469.6 b	172.4 d	175.4 c	173.9 b
SA at 200 ppm	362.5 i	372.7 h	367.6 e	136.7 j	141.9 i	139.3 e
Seaweeds extract at 2 ml/l	481.3 b	490.0 a	485.6 a	180.0 b	181.7 a	180.8 a
Mean (B)	392.0 b	398.3 a		143.8 b	147.7 a	

AMF = Arbuscular mycorrhizal fungi; SA= Salicylic acid

during the two growing seasons increased all measured vegetative growth parameters of Dutch fennel (*Foeniculum vulgare* Mill.) expressed as plant height, number of branches, herb fresh and dry weights/plant (g) compared with the control (tap water) treatment. In this connection, seaweed extract at 2 ml/l reflected the highest values of all studied vegetative growth traits followed by humic acid and amino acids in descending order.

On the other side, all vegetative growth mentioned above were increased by using arbuscular mycorrhizal fungi (AMF) when compared to untreated (uninoculum mycorrhizal fungi).

As for the interaction between some growth stimulants treatments and mycorrhizal fungi treatments, the same data in Tables (2 and 3) reveal that the highest values in all measured growth traits were recorded as a result of using seaweed extract combined with arbuscular mycorrhizal fungi (AMF) (uninoculum and inoculum) with superior for the inoculum mycorrhizal fungi. In this context, the second values of these parameters were recorded by using the combined treatment between humic acid at 5 ml/l and inoculum mycorrhizal, followed descendingly by the combined treatment between amino acid (amino power) at 2 ml/l and mycorrhizal fungi in 1<sup>st</sup> and 2<sup>nd</sup> seasons.

## 2. Seeds yield parameters:

Data presented in Tables (4 and 5) illustrated that, all the four used growth stimulants treatments progressively increased the seeds yield parameters i.e. number of umbels/plant, weight of 1000 seed (g), seeds yield/plant (g) and seeds yield/feddan (ton) of Dutch fennel (*Foeniculum vulgare* Mill.) as compared with control in both seasons. Hence, in the two consecutive seasons of this study the highest values of these parameters were obtained from the seaweed extract at 2 ml/l, followed by humic acid at 5.0 ml/l which scored the second highest values with the exception of number of umbels/plant.

Whereas the second highest values of number of umbels/plants was resulted by amino acid (amino power) at 2 ml/l. Irrespective of control, the lowest values of aforementioned parameters were scored by salicylic acid (SA) at 200 ppm and brassinolide at 50 mg/l, in ascending order. On the other hand, inoculated the Dutch fennel plant with mycorrhizal fungi significantly increased these parameters when compared to the untreated (uninoculum mycorrhizal fungi). Additionally, data presented in Tables (4 and 5) recorded that all the interactions between growth stimulants and arbuscular mycorrhizal fungi (AMF) treatments statistically improved the aforementioned seed parameters, Dutch fennel plant when compared to control in both seasons especially, the combination between seaweed extract combined and inoculum arbuscular mycorrhizal fungi (AMF) significantly produced the highest values of these parameters in the first and second seasons. Also, seaweed extract combined with uninoculum arbuscular mycorrhizal fungi (AMF) or the combined treatment between amino acid at 2 ml/l and inoculum mycorrhizal fungi recorded highly increments of these parameters in both seasons. The minimum values of abovementioned parameters were obtained by control plants (tap water) or salicylic acid (SA) at 200 ppm especially uninoculum mycorrhizal fungi, in an ascending order and in the both seasons.

The obtained results of some safety growth stimulants treatments and arbuscular mycorrhizal fungi on vegetative growth, yield and in several studied parameters of medicinal and aromatic plants was revealed by Mohamed *et al.* (2016) on basil plant, El-Khateeb (2017) of *Majorana hortensis*, Ibrahim and Helaly (2017) on fenugreek plants. Mansori *et al.* (2019) demonstrated that, the application of seaweeds extracts enhances the vegetative growth of *Salvia officinalis* plant. Moghith (2019) declared that, plants inoculum of mycorrhizal fungi scores the highest significant increases of all

**Table 4. Effect of some growth stimulants, mycorrhizal fungi and their combination treatments on number of umbels/plant and weight of 1000 seeds (g) of Dutch fennel (*Foeniculum vulgare* Mill.), during 2018/2019 and 2019/2020 seasons.**

Growth stimulants	Umbels No. /plant			1000 seeds weights (g)		
	First season					
	Uninoculum AMF	Inoculum AMF	Mean (A)	Uninoculum AMF	Inoculum AMF	Mean (A)
Control (tap water)	58.33 k	67.67 j	63.00 f	11.89 g	12.38 f	12.13 e
Amino power at 2ml/l	80.00 e	86.67 c	83.33 c	14.72 c	15.11 b	14.91 b
Brassinolide at 50 mg/l	71.00 h	76.67 f	73.83 d	13.34 e	13.89 d	13.61 d
Humic acid at 5.0 ml/l	82.67 d	86.33 c	84.50 b	14.10 d	14.99 bc	14.55 c
SA at 200 ppm	69.33 i	74.00 g	71.67 e	13.10 e	13.79 d	13.44 d
Seaweeds extract at 2 ml/l	92.00 b	97.33 a	94.67 a	14.90 bc	15.55 a	15.23 a
Mean (B)	75.56 b	81.44 a		13.68 b	14.28 a	
Second season						
Control (tap water)	60.00 h	69.00 g	64.50 e	11.99 e	12.26 e	12.12 d
Amino power at 2ml/l	81.67 d	87.00 c	84.33 c	14.96 a	14.84 a	14.90 a
Brassinolide at 50 mg/l	70.33 g	77.00 e	73.67 d	13.37 cd	13.90 bc	13.63 c
Humic acid at 5.0 ml/l	83.67 d	89.00 c	86.33 b	14.11 b	14.89 a	14.50 b
SA at 200 ppm	70.33 g	74.00 f	72.17 d	13.17 d	13.74 b-d	13.46 c
Seaweeds extract at 2 ml/l	93.33 b	98.33 a	95.83 a	15.11 a	15.44 a	15.27 a
Mean (B)	76.56 b	82.39 a		13.78 b	14.18 a	

AMF = Arbuscular mycorrhizal fungi; SA= Salicylic acid

**Table 5. Effect of some growth stimulants, mycorrhizal fungi and their combination treatments on seeds yield/plant (g) and seeds yield/feddan (ton) of Dutch fennel (*Foeniculum vulgare* Mill.), during 2018/2019 and 2019/2020 seasons.**

Growth stimulants	Seeds yield/plant (g)			Seeds yield/feddan (ton)		
	First season					
	Uninoculum AMF	Inoculum AMF	Mean (A)	Uninoculum AMF	Inoculum AMF	Mean (A)
Control (tap water)	30.29 k	35.29 j	32.79 f	1.02 l	1.19 k	1.10 f
Amino power at 2ml/l	69.40 d	73.04 c	71.22 b	2.33 e	2.45 c	2.39 b
Brassinolide at 50 mg/l	61.00 g	64.33 f	62.66 d	2.05 h	2.16 g	2.11 d
Humic acid at 5.0 ml/l	66.09 e	69.89 d	67.99 c	2.22 f	2.35 d	2.28 c
SA at 200 ppm	43.41 i	48.79 h	46.10 e	1.46 j	1.64 i	1.55 e
Seaweeds extract at 2 ml/l	74.89 b	77.56 a	76.23 a	2.51 b	2.60 a	2.56 a
Mean (B)	57.51 b	61.48 a		1.93 b	2.07 a	
Second season						
Control (tap water)	31.09 i	37.02 h	34.05 f	1.05 i	1.24 h	1.15f
Amino power at 2ml/l	70.40 c	73.02 b	71.71 b	2.37 c	2.45 b	2.41 b
Brassinolide at 50 mg/l	62.11 e	64.77 d	63.44 d	2.08 e	2.18 d	2.13 d
Humic acid at 5.0 ml/l	65.81 d	69.23 c	67.52 c	2.21 d	2.33 c	2.27 c
SA at 200 ppm	44.01 g	47.62 f	45.82 e	1.48 g	1.60 f	1.54 e
Seaweeds extract at 2 ml/l	74.08 b	77.86 a	75.97 a	2.49 b	2.58 a	2.53 a
Mean (B)	57.92 b	61.59 a		1.94 b	2.06 a	

AMF = Arbuscular mycorrhizal fungi; SA= Salicylic acid

vegetative growth and seed yield characteristics as compared of uninoculated plants (control) of chia (*Salvia hispanica* L.) plants. Also, Ghatas (2020) indicated that, salicylic acid as foliar spray at 150 ppm which gave slightly increases in the growth, seeds yield of *Coriandrum sativum* L. plant.

Amino acids contribute to the tolerance of plants against biotic and abiotic stresses either directly or indirectly by serving as precursors to secondary compounds and hormones. Additionally, the functions in the synthesis of other organic compounds, i.e. protein, amines, purines and pyrimidines, alkaloids, vitamins, enzymes, terpenoids and others (Behzad, 2011). The effect of brassinolide on growth vegetative parameters may be due to improve the cells growth, differentiation, enlargement and division, change membrane potentials and the metabolism of nucleic acids and proteins (Müssig, 2005 and Prins *et al.*, 2010).

In this concern, humic acid has a great importance for the plant in terms of its effect on growth in addition to its clear importance to the soil as it works to improve the properties of the soil and increase the rate of microbial activity as it enhances the absorption of many nutrients as a chelating agent. It is known that humic materials increase the activity of root growth such as auxins, which in turn is reflected in the increased growth of the plant in various stages of growth, and leaf pigments (Berlyn and Russo, 1990; Frankenberger and Arshad, 1995; Graves *et al.*, 2004; Pereiral *et al.*, 2019).

Salicylic acid (SA) plays a significant role in physiological and biological processes in plants and can be used to enhance the growth of plant under unfavorable environmental conditions (Karalija and Parić, 2017). Furthermore, the obtained increase of these traits could be seaweed products promote plant growth when applied in small quantities and are also referred to as metabolic enhancers. Seaweed extract contained components such as macro- and micro element nutrients, amino acids,

vitamins, cytokinins and auxins like growth substances in which affect cellular metabolism in treated plants leading to enhanced growth and crop yield (Zhang and Schmidt, 1997). Using of seaweeds lead to the activity of enzymes, and consequently, and increase in biological processes within the plant, which results in an increase in the growth and yield of the plant. (El-Boukhari *et al.*, 2020).

#### Oil yield parameters:

According to data listed in Tables (6 and 7) it could be showed that, essential oil (%), essential oil yield/plant (ml) and essential oil yield/feddan (l) of Dutch fennel plant were more affected by using growth stimulants and arbuscular mycorrhizal fungi (AMF) as well as their combinations treatments as compared to control plants in the first and second seasons. In this respect, in 1<sup>st</sup> and 2<sup>nd</sup> seasons seaweed extract at 2 ml/l or inoculum mycorrhizal fungi significantly gained the maximum values of theses parameters. However, the highest seeds oil percentage (2.22 and 2.24%), the highest essential oil yield/plant (1.72 and 1.75 ml) and the maximum essential oil yield/feddan (57.90 and 58.69 l) were recorded by the combined treatment between seaweed extract combined and inoculum arbuscular mycorrhizal fungi (AMF) in the first and second seasons, respectively. Also, the combined treatment between seaweed extract combined and uninoculum arbuscular mycorrhizal fungi (AMF) ranked the second values in this context. Furthermore, using the combined treatment of amino acids or humic acid and inoculum arbuscular mycorrhizal fungi occupied the third values with non-significant differences between them in most cases. The minimum values of abovementioned oil parameters were obtained by control plants (tap water) especially uninoculum mycorrhizal fungi. In this concern, the results similar to those obtained by Maryam *et al.* (2014) on *Ocimum basilicum*, L., Mohamed *et al.* (2015) on *Ocimum basilicum*, L. cv. Genovese, Mohamed *et al.* (2016) on

**Table 6. Effect of some growth stimulants, mycorrhizal fungi and their combination treatments on essential oil (%) and essential oil yield/plant (m) of Dutch fennel (*Foeniculum vulgare* Mill.), during 2018/2019 and 2019/2020 seasons.**

Growth stimulants	Essential oil (%)			Essential oil yield/plant (ml)		
	First season					
	Uninoculum AMF	Inoculum AMF	Mean (A)	Uninoculum AMF	Inoculum AMF	Mean (A)
Control (tap water)	1.25 j	1.35 i	1.30 e	0.38 k	0.47 j	0.43 f
Amino power at 2ml/l	1.88 f	1.91 e	1.90 c	1.31 d	1.39 c	1.35 b
Brassinolide at 50 mg/l	1.79 h	1.82 g	1.81 d	1.09 g	1.17 f	1.13 d
Humic acid at 5.0 ml/l	1.94 d	1.98 c	1.96 b	1.28 e	1.38 c	1.33 c
SA at 200 ppm	1.79 h	1.82 g	1.80 d	0.77 i	0.89 h	0.83 e
Seaweeds extract at 2 ml/l	2.03 b	2.22 a	2.13 a	1.52 b	1.72 a	1.62 a
Mean (B)	1.78 b	1.85 a		1.06 b	1.17 a	
Second season						
Control (tap water)	1.29 j	1.37 i	1.33 e	0.40 j	0.51 i	0.45 e
Amino power at 2ml/l	1.91 ef	1.94 de	1.93 c	1.34 d	1.43 c	1.38 b
Brassinolide at 50 mg/l	1.81 gh	1.86 fg	1.84 d	1.13 f	1.21 e	1.17 c
Humic acid at 5.0 ml/l	1.96 cd	2.03 b	1.99 b	1.29 d	1.40 c	1.35 b
SA at 200 ppm	1.80 h	1.82 gh	1.81 d	0.79 h	0.87 g	0.83 d
Seaweeds extract at 2 ml/l	2.01 bc	2.24 a	2.13 a	1.49 b	1.75 a	1.62 a
Mean (B)	1.80 b	1.88 a		1.07 b	1.19 a	

AMF = Arbuscular mycorrhizal fungi; SA= Salicylic acid

**Table 7. Effect of some growth stimulants, mycorrhizal fungi and their combination treatments on essential oil yield/feddan (l) and chlorophyll a mg/g f.w. of Dutch fennel (*Foeniculum vulgare* Mill.), during 2018/2019 and 2019/2020 seasons.**

Growth stimulants	Essential oil yield/feddan (l)			Chlorophyll a mg/g f.w.		
	First season					
	Uninoculum AMF	Inoculum AMF	Mean (A)	Uninoculum AMF	Inoculum AMF	Mean (A)
Control (tap water)	12.77 k	15.79 j	14.28 e	0.698 l	0.713 k	0.705 f
Amino power at 2ml/l	44.02 d	46.82 c	45.42 b	0.898 f	1.010 e	0.954 c
Brassinolide at 50 mg/l	36.74 g	39.31 f	38.03c	0.824 i	0.865 h	0.844 d
Humic acid at 5.0 ml/l	43.01 e	46.59 c	44.80 b	1.030 d	1.100 c	1.065 b
SA at 200 ppm	25.98 i	29.79 h	27.89 d	0.796 j	0.878 g	0.837 e
Seaweeds extract at 2 ml/l	51.07 b	57.90 a	54.49 a	1.157 a	1.142 b	1.149 a
Mean (B)	35.60 b	39.37 a		0.900 b	0.951 a	
Second season						
Control (tap water)	13.44 k	17.03 j	15.23 f	0.708 e	0.717 de	0.713 b
Amino power at 2ml/l	45.02 d	47.94 c	46.48 b	0.994 a-d	0.699 e	0.846 b
Brassinolide at 50 mg/l	37.86 g	40.54 f	39.20 d	0.835 b-e	0.870 a-e	0.853 b
Humic acid at 5.0 ml/l	43.46 e	47.15 c	45.31 c	1.043 a-c	1.102 ab	1.073 a
SA at 200 ppm	26.54 i	29.12 h	27.83 e	0.813 c-e	0.884 a-e	0.849 b
Seaweeds extract at 2 ml/l	49.95 b	58.69 a	54.32 a	1.122 a	1.146 a	1.134 a
Mean (B)	36.4 b	40.08 a		0.919 a	0.903 a	

AMF = Arbuscular mycorrhizal fungi; SA= Salicylic acid

*Ocimum basilicum*, L. cv. Genovese demonstrated that, essential oil percentage in leaves gave the greatest values when plants treated with the combined treatments of seaweeds extract (2 ml/l) or amino acids (1 g/l) and boron (100 ppm). Mansori *et al.* (2019) demonstrated that, the application of seaweeds extracts enhances the oil parameters of *Salvia officinalis* plant. Mady (2020) on Dutch fennel (*Foeniculum vulgare* Mill.) suggested that, the lowest values were obtained with plants treated by T5 (biofertilizers only) as compared to the other treatments in both seasons.

#### Chemical composition determinations:

Data presented in Tables (7, 8, 9 and 10) demonstrated that chemical composition i.e. (chlorophyll a, b and carotenoids, N, P, K and total carbohydrates %) were increased by foliar spray of all growth stimulants when compared to untreated (tap water). However, the maximum chemical composition values were registered by seaweed extract at 2 ml/l treatment in the two seasons. Moreover, in both seasons, humic acid at 5.0 ml/l and

amino acid at 2 ml/l score the second and third values of these parameters in both seasons, respectively. Regarding mycorrhizal fungi treatments, data in the same Tables suggested that inoculum mycorrhizal fungi treatment significantly increased these chemical parameters describe above of Dutch fennel plant when compared to uninoculum mycorrhizal fungi with the exception of chlorophyll a in the second season only. Whereas, the highest slightly increases of chlorophyll a (mg/g f.w.) was registered by uninoculum mycorrhizal fungi as compared inoculum mycorrhizal fungi with non-significant differences between them. Additionally, the highest values of aforementioned chemical parameters were recorded by using the combined treatment between seaweed extract with inoculum arbuscular mycorrhizal fungi (AMF) with the exception of chlorophyll a (mg/g f.w.) in the first season only. Whereas, the first season the highest values of chlorophyll a (mg/g f.w.) was scored by seaweed extract with uninoculum arbuscular mycorrhizal

**Table 8. Effect of some growth stimulants, mycorrhizal fungi and their combination treatments on chlorophyll b (mg/g f.w.) and carotenoids (mg/g f.w.) of Dutch fennel (*Foeniculum vulgare* Mill.), during 2018/2019 and 2019/2020 seasons.**

Growth stimulants	Chlorophyll b mg/g f.w.			Carotenoids mg/g f.w.		
	First season					
	Uninoculum AMF	Inoculum AMF	Mean (A)	Uninoculum AMF	Inoculum AMF	Mean (A)
Control (tap water)	0.305 j	0.322 i	0.313 f	0.136 j	0.146 i	0.141 f
Amino power at 2ml/l	0.455 e	0.470 d	0.463 c	0.261 e	0.267 d	0.264 c
Brassinolide at 50 mg/l	0.440 f	0.457 e	0.449 d	0.250 f	0.256 e	0.253 d
Humic acid at 5.0 ml/l	0.488 c	0.495 b	0.492 b	0.269 cd	0.273 c	0.271 b
SA at 200 ppm	0.390 h	0.399 g	0.394 e	0.192 h	0.200 g	0.196 e
Seaweeds extract at 2 ml/l	0.496 b	0.515 a	0.505 a	0.282 b	0.290 a	0.286 a
Mean (B)	0.429 b	0.443 a		0.232 b	0.239 a	
Second season						
Control (tap water)	0.310 j	0.325 i	0.318 f	0.140 j	0.149 i	0.144 f
Amino power at 2ml/l	0.460 e	0.472 d	0.466 c	0.261 e	0.270 d	0.266 c
Brassinolide at 50 mg/l	0.448 f	0.460 e	0.454 d	0.248 f	0.258 e	0.253 d
Humic acid at 5.0 ml/l	0.489 c	0.500 b	0.495 b	0.269 d	0.278 c	0.274 b
SA at 200 ppm	0.396 h	0.405 g	0.400 e	0.198 h	0.208 g	0.203 e
Seaweeds extract at 2 ml/l	0.492 c	0.520 a	0.506 a	0.283 b	0.293 a	0.288 a
Mean (B)	0.432 b	0.447 a		0.233 b	0.243 a	

AMF = Arbuscular mycorrhizal fungi; SA= Salicylic acid

**Table 9. Effect of some growth stimulants, mycorrhizal fungi and their combination treatments on N % and P % of Dutch fennel (*Foeniculum vulgare* Mill.), during 2018/2019 and 2019/2020 seasons.**

Growth stimulants	N %			P %		
	First season					
	Uninoculum AMF	Inoculum AMF	Mean (A)	Uninoculum AMF	Inoculum AMF	Mean (A)
Control (tap water)	1.68 k	1.78 j	1.73 f	0.214 j	0.220 i	0.217 f
Amino power at 2ml/l	2.71 e	2.75 d	2.73 c	0.271 e	0.276 d	0.273 c
Brassinolide at 50 mg/l	2.47 g	2.58 f	2.53 d	0.265 f	0.272 e	0.268 d
Humic acid at 5.0 ml/l	2.78 c	2.86 b	2.82 b	0.290 c	0.297 b	0.293 b
SA at 200 ppm	1.90 i	2.08 h	1.99 e	0.242 h	0.250 g	0.246 e
Seaweeds extract at 2 ml/l	2.79 c	2.88 a	2.84 a	0.296 b	0.301 a	0.299 a
Mean (B)	2.39 b	2.49 a		0.263 b	0.269 a	
	Second season					
Control (tap water)	1.72 l	1.80 k	1.76 f	0.216 i	0.220 h	0.218 e
Amino power at 2ml/l	2.68 f	2.76 e	2.72 c	0.271 e	0.275 d	0.273 c
Brassinolide at 50 mg/l	2.50 h	2.61 g	2.56 d	0.269 e	0.272 de	0.271 c
Humic acid at 5.0 ml/l	2.77 d	2.88 b	2.83 b	0.290 c	0.298 b	0.294 b
SA at 200 ppm	1.94 g	2.13 i	2.04 e	0.243 g	0.252 f	0.248 d
Seaweeds extract at 2 ml/l	2.79 c	2.91 a	2.85 a	0.297 b	0.306 a	0.302 a
Mean (B)	2.40 b	2.52 a		0.264 b	0.271 a	

AMF = Arbuscular mycorrhizal fungi; SA= Salicylic acid

**Table 10. Effect of some growth stimulants, mycorrhizal fungi and their combination treatments on K % and total carbohydrates % of Dutch fennel (*Foeniculum vulgare* Mill.), during 2018/2019 and 2019/2020 seasons.**

Growth stimulants	K %			Total carbohydrates %		
	First season					
	Uninoculum AMF	Inoculum AMF	Mean (A)	Uninoculum AMF	Inoculum AMF	Mean (A)
Control (tap water)	1.18 l	1.26 k	1.22 f	15.96 j	16.96 i	16.46 f
Amino power at 2ml/l	1.95f	2.01 e	1.98 c	18.77 fg	19.14 ef	18.96 d
Brassinolide at 50 mg/l	1.79 h	1.85 g	1.82 d	19.41 de	19.87 d	19.64 c
Humic acid at 5.0 ml/l	2.09 d	2.18 c	2.13 b	19.93 d	21.22 c	20.57 b
SA at 200 ppm	1.49 g	1.60 i	1.54 e	18.00 h	18.49 gh	18.24 e
Seaweeds extract at 2 ml/l	2.30 b	2.36 a	2.33 a	22.10 b	23.25 a	22.67 a
Mean (B)	1.80 b	1.88 a		19.03 b	19.82 a	
	Second season					
Control (tap water)	1.22 k	1.28 j	1.25 f	16.26 g	16.81 fg	16.53 e
Amino power at 2ml/l	1.92 f	2.01 e	1.96 c	17.81 e	19.07 d	18.44 d
Brassinolide at 50 mg/l	1.81 g	1.85 g	1.83 d	19.95 cd	20.83 bc	20.39 c
Humic acid at 5.0 ml/l	2.12 d	2.22 c	2.17 b	20.39 c	21.70 b	21.05 b
SA at 200 ppm	1.47 i	1.62 h	1.55 e	17.58 ef	19.11 d	18.34 d
Seaweeds extract at 2 ml/l	2.29 b	2.38 a	2.34 a	21.55 b	23.41 a	22.48 a
Mean (B)	1.80 b	1.90 a		18.92 b	21.15 a	

AMF = Arbuscular mycorrhizal fungi; SA= Salicylic acid

fungi (AMF). Also, the combined treatment between humic acid at 5.0 ml/l or amino acid at 2 ml/l and inoculum mycorrhizal fungi recorded highly increments of these parameters. In contrast, the minimum values of abovementioned parameters were obtained by control plants (tap water) or salicylic acid (SA) at 200 ppm especially uninoculum mycorrhizal fungi, in ascending order and in the both seasons.

The abovementioned results of different treatments are nearly similar to those obtained by Maryam *et al.* (2014) on *Ocimum basilicum*, L., Mohamed *et al.* (2015) on basil plant, Mohamed *et al.* (2016) on *Ocimum basilicum*, L. cv. Genovese. El-Khateeb (2017) on *Majorana hortensis* plant, Ibrahim and Helaly (2017) on fenugreek plants, Mansori *et al.* (2019) demonstrated that, the application of seaweeds extracts enhances the chemical composition of *Salvia officinalis* plant., Moghith (2019) stated that, the maximum significant increases of chemical composition of chia (*Salvia hispanica* L.) plants was recorded by

inoculum mycorrhizal fungi as compared of uninoculated plants (control) in both seasons. Furthermore Ghatas (2020) on *Coriandrum sativum* L. plant.

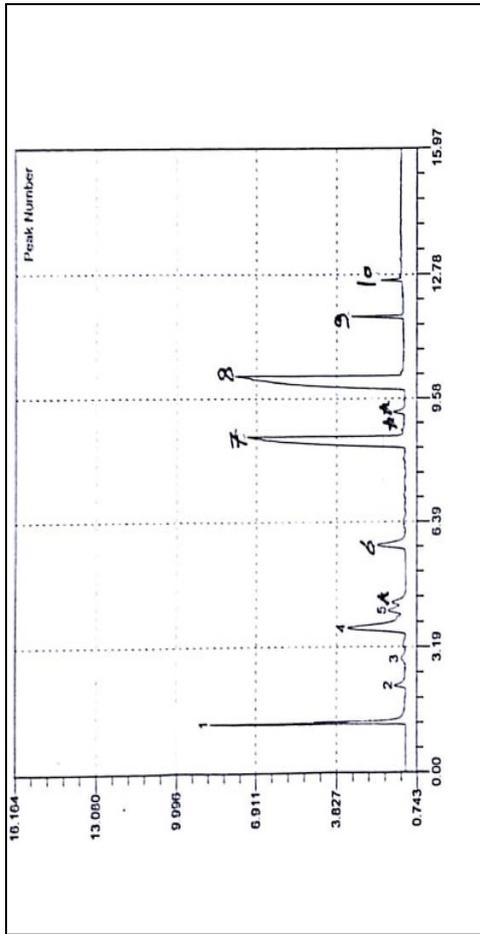
#### Essential oil constituents:

The GLC analysis of Dutch fennel essential oil as indicated in Table (11) and Figs. (1-4) suggested the presence of ten compounds with the presence of anethole as the main components, followed by estragole as well as  $\alpha$ -pinene, sabinene,  $\beta$ -pinene, myrcene, 1,8 cineole, fenchone, estragole, trans-anethole,  $\beta$ -caryophyllene and tetracosamethyl-cyclododecasiloxane. The master component of Dutch fennel seeds volatile oil was trans-anethole. However, the highest value of anethole (42.27%) was listed by seaweeds extract at 2 ml/l + AMF, followed by humic acid at 5.0 ml/l + AMF (40.21%). While, the maximum percentage of Estragole (32.20%) occurred with seaweeds extract at 2 ml/l + AMF in comparison of other treatments. In addition, the combined treatment of seaweeds extract at 2 ml/l + AMF gave the richest percentage

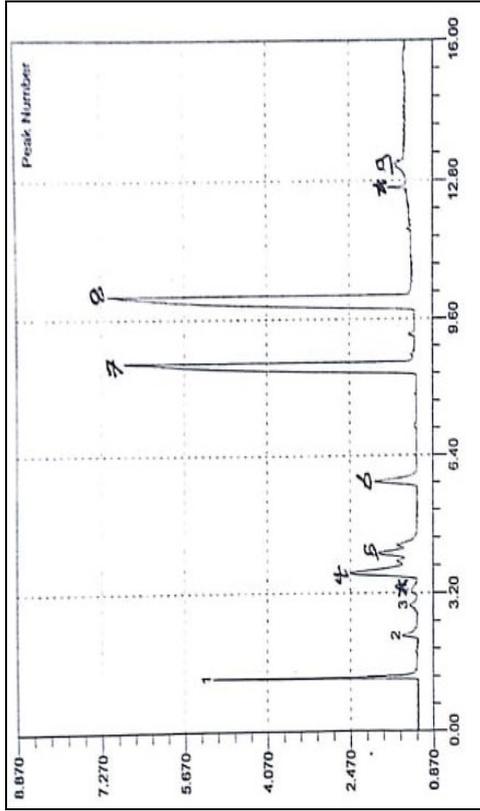
**Table 11. Influence of various treatments on essential oil composition of Dutch fennel (*Foeniculum vulgare* Mill.) plant, during the second season 2019/2020.**

Peak No.	Component name	Area %			
		Control (tap water)	Amino acid at 2 ml/l + AMF	Humic acid at 5.0 ml/l + AMF	Seaweeds extract at 2 ml/l + AMF
1	$\alpha$ -Pinene	9.34	5.25	4.41	0.58
2	Sabinene	1.29	1.44	1.42	1.47
3	$\beta$ -Pinene	1.40	0.95	0.99	0.80
4	Myrcene	10.51	8.70	8.82	12.14
5	1,8 cineole	2.16	6.27	6.34	1.89
6	Fenchone	3.69	4.02	3.80	3.57
7	Estragole	27.69	29.11	30.16	32.20
8	Trans-anethole	36.50	39.15	40.21	42.27
9	$\beta$ -caryophyllene	2.57	2.11	1.92	2.29
10	Tetracosamethyl-cyclododecasiloxane	0.76	-	-	0.51
-	Total identified	95.91	96.90	98.97	97.72
*	Unknown	4.09	3.10	1.03	2.28
	<b>Total</b>	100.00	100.00	100.00	100.00

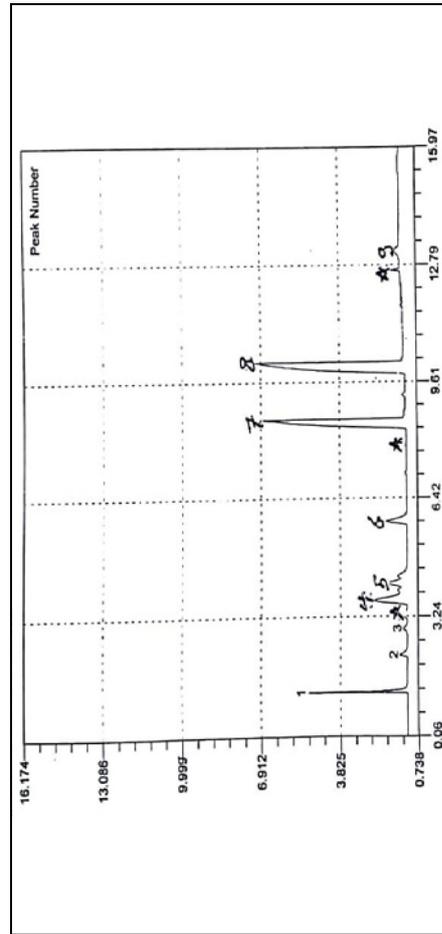
AMF = Arbuscular mycorrhizal fungi



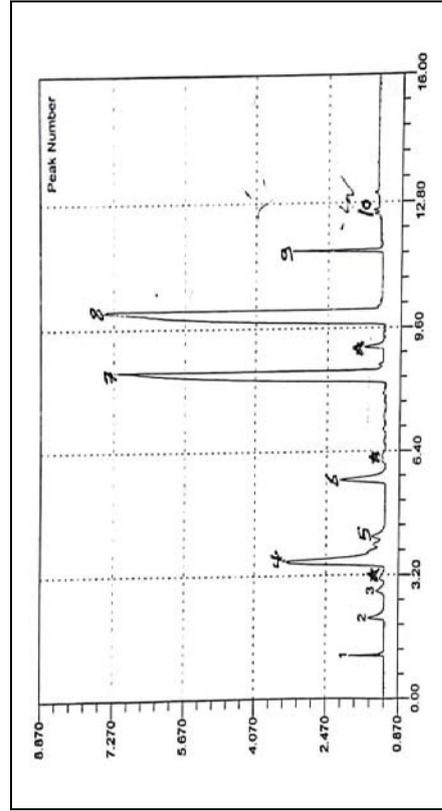
**Fig. 1. Influence of control (tap water) treatment on essential oil constituents of Dutch fennel.**



**Fig. 2. Influence of amino acid at 2ml/l+ AMF treatment on essential oil constituents of Dutch fennel.**



**Fig. 3. Influence of humic acid at 5.0 ml/l + AMF treatment on essential oil constituents of Dutch fennel.**



**Fig. 4. Influence of seaweeds extract at 2 ml/l + AMF treatment on essential oil constituents of Dutch fennel.**

of myrcene (12.14%). In contrast, different treatments caused decreases in the percentage of  $\alpha$ -pinene from (9.34%) in control reaches to the minimum values (0.58%) of seaweeds extract at 2 ml/l + AMF. Finally, humic acid at 5.0 ml/l + AMF score the large values of 1,8 cineole (6.34%). In this regard, El Laban *et al.* (2017) on Dutch fennel, the results showed that, the main chemical constituents i.e. trans-anethole, estragole, fenchone, limonene, alpha-pinene. Mady (2020) on Dutch fennel (*Foeniculum vulgare* Mill.) stated that, the presence of 16 compounds with the presence of anethole as the main components, followed by estragole.

### CONCLUSION

Generally, it is preferable from the results mentioned above that, the tallest plant, the highest values of branches number and the heaviest fresh and dry weights, were scored by spraying Dutch fennel plant with seaweed extract combined with superior for the inoculum mycorrhizal fungi in the two seasons (Tables, 2 and 3). Using growth stimulant treatments progressively increased the seeds yield parameters i.e. number of umbels/plant, weight of 1000 seed (g), seeds yield/plant (g) and seeds yield/feddan (ton) especially the inoculum mycorrhizal fungi. In this respect, the richest leaf chlorophyll a, b and carotenoids, N, P, K and total carbohydrate were resuled by seaweed extract combined with the inoculum mycorrhizal fungi as well as their combinations in most cases (Tables, 7, 8, 9 and 10). Hence, the afore enhancement of different parameters of Dutch fennel plant growth, greatly affected were reflexed the superior of essential oil (%) and their constituents, as well (Tables 7, 11 and Figs. 1-4). Finally, it could be recommended that the combinations of seaweed extract at 2 ml/l and inoculum arbuscular mycorrhizal fungi (AMF) could achieved the largest growth, seeds yield, chemical constituents and oil productivity of Dutch fennel (*Foeniculum vulgare* Mill. spp. *vulgare*).

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## تأثير بعض منشطات النمو بالتعاون مع فطريات الميكوريزا على النمو، الانتاجية، المكونات الكيميائية لنبات الشمرو الهولندي

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الشمرو نبات طبي وعطري مهم يحتل المرتبة الأولى في قائمة الصادرات المصرية من الأعشاب والتوابل. يتميز الشمرو الهولندي بنسبة أعلى من الأنيثول (anethole) ونسبة أقل من الاستراجول (estragole) من الشمرو المصري، لذلك يتم استخدام الشمرو كمضاد لبعض الأمراض الفيروسية. يهدف البحث الحالي إلى دراسة تأثير بعض منشطات النمو (الأحماض الأمينية، البراسينوليد، حمض الهيوميك، حمض الساليسيليك، مستخلص الطحالب البحرية و ماء الصنبور للمقارنة) وفطريات الميكوريزا (arbuscular mycorrhiza) بالإضافة إلى معاملات التفاعل بينهم على النمو الخضري وإنتاجية الزيت العطري وبعض المكونات الكيميائية لنبات الشمرو الهولندي، وقد أجريت هذه الدراسة في مزرعة التجارب وفي معمل قسم البساتين بكلية الزراعة بمشتهر، جامعه بنها، مصر خلال مواسم ٢٠١٨/٢٠١٩ و ٢٠١٩/٢٠٢٠. تم الحصول على أعلى زيادة معنوية من القراءات المدروسة من خلال معاملة التفاعل لمستخلص الطحالب البحرية عند ٢ مل/لتر مع تفوق التلقيح بالميكوريزا في الموسمين الأول والثاني. وسجلت أكبر نسبة زيت بذور (٢,٢٢ و ٢,٢٤) بمعاملة التفاعل لمستخلص الطحالب البحرية عند ٢ مل/لتر مع التلقيح بالميكوريزا. المكونات الكيميائية لزيت الشمرو الهولندي الأساسي حوالي عشرة مكونات، والمكونات الرئيسية لتكوين الزيت العطري trans-anethole و estragole و myrcene. بشكل عام يمكن التوصية بأن معاملة التفاعل لمستخلص الطحالب البحرية عند ٢ مل/لتر مع التلقيح بالميكوريزا يمكن أن تحقق أكبر نمو، وإنتاجية، ومكونات كيميائية للشمرو الهولندي.