Physiological Responses of Fennel (*Foeniculum Vulgare* Mill) Plants to Some Growth Substances. The Effect of Certain Amino Acids and a Pyrimidine Derivative

M. E. El-Awadi* and Esmat A. Hassan
Botany Department, Division of Agricultural and Biological Research National Research Centre, Dokki, 12311, Cairo, Egypt.
*el_awadi@yahoo.com

Abstract: In the Green house of the Botany Department (winter season 2007/08-2008/09) fennel seeds (*Foeniculum vulgare* Mill), from Department of Medicinal and Aromatic Plants, were cultivated after 3 hours soaking in the amino acids methionine and tryptophan and in the pyrimidine derivative material (SG93) provided by the Department of The Pharmaceutical Industry, each at 100 and 500mg/l. Growth measurements and chemical analyses of the plant were carried out at juvenile and fruiting stages, i.e. age of 84 and 119 days respectively. The pre-sowing seed treatment with the growth substances; methionine, tryptophan and the pyrimidine derivative (SG93) resulted in significant increases in plant height, number of leaves, number of branches, fresh and dry weight of shoots, number of umbels per plant, weight of seeds per umbel and per plant , in comparison to control. The pre-sowing seed treatments led to an elevation of leaf photosynthetic pigments` content, total protein, total phenolic compounds in the shoots and in the yielded seeds as well as in the percentage of fixed and essential oils as compared to the control. The highest content of the essential oil percentage was obtained as a result of seed-soaking treatment in methionine at 100mg/l concentration. In this connection, anethol represented the major component of such a percentage. [Journal of American Science 2010; 6(7):120-125]. (ISSN: 1545-1003).

Key words: Essential oil, *Foeniculum vulgare,* growth, growth substances, productivity

1. Introduction

Fennel (*Foeniculum vulgare* Mill. *Apiaceae*) is a perennial hemicryptophyte, inhabits the Mediterranean basin is known as a medicinal aromatic herb. Its fruit is used in the remedy against digestive disorders while bitter fennel is used as food flavor, in liqueurs and in the perfumery industry (Tanira, *et al*. 1996). The major volatile (essential) oil of the plant is anethole and fenchone (Simandi *et al*. 1999). Fennel extracts proved to have anti-inflammatory, antispasmodic, carminative, diuretic, expectorant, laxative, analgesic, stimulant of gastrointestinal mobility and are used in treatment of nervous disturbances (Choi and Hwang 2004). Anand *et al* (2008) reported the anticancer activity of fennel seed anthenol.

Growth regulating substances were shown to enhance the biosynthesis of certain chemical constituents in plants. In this respect the amino acids which have a high integrity with different metabolic pools in plants were used to promote plant growth (Coruzzi and Last, 2000). However, S-adenosyl - methionine play a role via the methyl group as a donor to produce estragole and t-anethole in cell-free extracts of the bitter fennel plant (Gross *et al*., 2002). Later, Maxwell and Kieber (2004) indicated the link of methionine to the biosynthesis of growth regulating substances, e.g cytokinins, auxins and brassinosteroids in plants. Whereas the link of tryptophan to the biosynthesis of auxins, the phytoalexin camalexin, phenylpropanoids and other related natural products in plants was recently reported (Tao *et al*. 2008).

Pyrimidine derivatives, which are the building blocks for nucleic acid synthesis, energy sources, precursors for synthesis of sucrose, polysaccharides, and phospholipids, were found to have a role in cellular regulation and biosynthesis of some amino acids and secondary products in plants (Stasolla *et al*., 2003).

This implicated the enhancement of vegetative growth and chemical constituents caused by amino acid treatments in *Pelargonium graveolens* L. (Talaat 2005) and in *Philodendron erubescens* (Abou -Dahab and Abd El-Aziz 2006) and in fennel in our present results.

In our previous investigations, the pyrimidine derivative (SG93) was found to modulate plant growth response of different plant spieces under certain abiotic stresses (Hassasn *et al*., 2006 and El-Awadi, 2007).

In the present study, we aimed to investigate the physiological responses to the amino acids methionine, tryptophan and to the pyrimidine derivative material (SG93) at different concentrations

on growth, productivity and chemical constituents of fennel plants.

2. Material and Methods

The present experiments were carried out using two successive winter seasons (2007/08-2008/09) in the green house of Botany Department, National Research Centre, Egypt.

The effect of the amino acids mthionine, tryptophan and the pyrimidine derivative compound (SG93 – Fig. 1) were investigated on growth, productivity and biochemical constituents of fennel plant *(Foeniculum vulgare* Mill.).

I-Cultivation .and treatments:

Fennel seeds were selected, sterilized in sodium hypochlorite solution (1%) for 15 minutes, washed thoroughly with distilled water, and then soaked in the following solutions for 3 hours:

1- Distilled water (control).
2- In methionine
3- In tryptophan and
4- In the pyrimidine derivative (SG93- Fig.1), provided by the Department of Pharmaceutical Industry of the National Research Centre.

All at 100 and 500mg/l. At 2cm depth, each 10 seeds were sown in pots (30-cm diameter filled with clay and sand; 2: 1 v/v) at the 17th of Nov. Standard agricultural practices were carried out as recommended. Each treatment included 5 replicates = 50 plant. The pots / treatments were distributed following a complete randomized design of distribution.

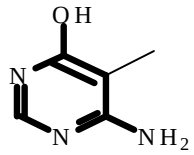

Figure 1: Chemical formula of SG93

II- Growth and yield measurements:

Plant samples were taken at the juvenile stage (age of 84 days) and at fruiting one (age of 119 days).Plant height, number of leaves, number of branches, fresh and dry weight of the shoots were recorded at both stages.

Yield of fennel plant was recorded as the number of umbels per plant, the number of seeds per umbel and per plant and weight of seeds per umbel and per plant.

III- Biochemical analyses:

Photosynthetic pigments were estimated in fresh tissues of fennel leaves according to **(**Wettstein 1957**)**. Protein percentage was determined according to A.O.A.C. (1990). Total Free amino acids were determined using the ninhydrin colorimetric method defined by Plummer (1978). Following the method reported by Snell and Snell (1952), total phenolic compounds were estimated.

Seed fixed oil content, was determined as reported in the (A.O.A.C., 1990) with Soxhelt apparatus using petroleum ether (40-60^{o}C).

The essential oil was extracted from the yielded dry seeds and estimated referring to the British Pharmacopoeia (1980), dehydrated over anhydrous sodium sulfate and then kept at refrigerators (-4^{o}c) till GLC analysis.

Essential oil of fennel seeds was analyzed by GC using a Agilent Technologies, (6890N Network GC system, U.S.A.) using capillary column HP 5% (30 m x 320 Um), 0.25um film thickness. Oven temperature was programmed at 70°C for 2 min. from 700°.190°C at rate of 4 ml/min. and finally 250°C (15 min) with N2: H2: Air at 30:30:300 ml/min. The temperature of the detector (FID) was maintained at 280°C. Identification of the oil components was based on the comparison of the Rts of the separated compounds with those of standard compounds that injected under the same conditions and confirmed for the major compounds by their relative retention indices.

IV- Statistical analysis:

A complete experimental randomized block design with 5 replicates was adopted. Combined results` analysis of the average values of the two seasons was carried out and the values of LSD were calculated as described by Snedecor and Cochran (1980).

3. Results and Discussion

1- Effect on growth and productivity

Data presented in Tables (1and 2) indicated that the pre-sowing seed soaking treatment in the amino acids methionine and tryptophan and in the pyrimidine derivative material (SG93) resulted in the promotion of growth and productivity of fennel plants. Both amino acids each at 100mg/l and the pyrimidine derivative at its high (500mg/l) concentrations caused significant increases in plant height, number of leaves, number of branches, fresh and dry weight of shoots as compared to the control (Table -1). The increases were remarkable at both the juvenile and fruiting growth stages. While observed at lower values, significant enhancement in growth was also obtained with other treatments. Therefore, the maximum effect was gained at the low concentration of each of the amino acids methionine and tryptophan followed by the high concentration

treatment of the pyrimidine derivative material (SG93).

From Table (2) significant increases were obtained in the number of umbels per plant, seed number per umbel and per plant and weight (gm) of seeds per umbel and per plant with methionine seed treatment at 100mg/l in comparison to the control. Similar results were obtained with the tryptophan at its low concentration (100 mg/l) and with the pyrimidine derivative material at its high concentration (500 mg/l).

These results are supported by those previously reported by of Talaat (2005) on *Pelargonium graveolens* L., and Abu-Dahab and Abd El-Aziz (2006) on *Philodendron erubescens.* In addition the pyrimidine derivative under test was found to enhance the growth and productivity of some monocots and dicot plants under certain abiotic stress conditions (Hassan *et al*., 2006) and El-Awadi, 2007).

Table 1: Effect of methionine, tryptophan and the pyrimidine derivative seed soaking on growth parameters of fennel plant at the age of 84 (A) and 119 (B) days.

	mg/l	Shoot length (cm)		No. of leaves		No. of branches		Shoot FW (g)		Shoot DW (g)	
		A	B	A	B	A	B	A	B	A	B
Control	**0**	52.48	67.80	7.56	20.33	4.19	7.84	35.36	16.90	1.30	3.20
Methionine	**100**	58.27	81.97	10.50	24.61	6.54	13.25	39.52	20.61	1.43	3.97
	500	57.04	79.93	10.18	21.73	5.03	11.49	39.41	20.32	1.37	3.85
Tryptophan	**100**	57.78	75.31	10.59	25.93	6.47	10.38	39.34	19.47	1.47	3.50
	500	54.79	75.10	9.28	21.42	5.23	11.83	32.41	18.12	1.30	3.59
Pyrimidine derivative	**100**	54.84	69.92	9.24	22.73	4.37	11.23	43.66	19.71	1.22	3.57
	500	54.99	75.31	10.87	23.41	5.20	12.40	39.36	17.74	1.35	3.24
L.S.D 5%		1.07	1.57	0.58	1.68	0.25	0.43	0.40	1.19	0.10	0.32

Table 2: Effect of methionine, tryptophan and the pyrimidine derivative (SG93) seed soaking on yield characteristics of fennel plant.

	mg/l	No. of umbels/ plant	No. of seeds/ umbel	Wt. of seeds/umbel	Wt. of seeds /plant
Cont	**0**	9.82	108.24	1.43	13.89
Methionine	**100**	10.80	134.21	1.85	19.28
	500	10.09	122.49	1.59	15.88
Tryptophan	**100**	10.61	128.53	1.68	17.05
	500	9.99	115.49	1.52	14.80
Pyrimidine derivative	**100**	10.17	113.88	1.56	15.47
	500	10.78	115.92	1.78	18.41
L.S.D 5%		0.92	5.39	0.15	1.16

In an implication, Tao *et al* (2008) pointed to the enhancement in plant growth as a result of amino acids` conversion to the growth promoter IAA.

2- Effect on chemical constituents:

Results in Table (3) indicated that the pre-sowing seed treatment of fennel in methionine and tryptophan both at 100 mg/l and in the SG93 at 500 mg/l caused significant increases in chlorophyll a, b and cartenoids contents as compared to the control. This was true at both the juvenile and fruiting growth stages. The influence of other treatments appeared to be insignificant.

At the juvenile stage, significant increases in total phenolic contents were recorded with the methionine and tryptophan treatments both at 100mg/l and with the pyrimidine material at 500mg/l. While at the fruiting stage the tryptophan at 100mg/l-seed treatment showed a sole effect (Table-3).

The amount of total protein at the juvenile stage was elevated as a result of all the treatments (Table 3). The significant promotion of protein biosynthesis was obtained at the fruiting stage with the pyrimidine material SG93 and with trypyophan both at100mg/l concentration-seed treatment.

In seeds produced by fennel plant, except with the pyrimidine material at 100mg/l, the total phenolic compounds increased in response to the pre-sowing seed treatments with all the growth factors under test in comparison to control (Table 4).

Table 3: Effect of the amino acids methionine and tryptophan and the pyrimidine derivative material (SG93) on leaf photosynthetic pigments, total phenols and protein content at the age of 84 (A) and 119 (B) days-old fennel pants.

		Ch. A (mg /g f. wt.)		Ch. B (mg /g f. wt.)		Cart. (mg /g f. wt.)		Total phenolic compounds (mg/g d. wt.)		Total protein (mg/g d. wt. equiv.)	
	mg/l	A	B	A	B	A	B	A	B	A	B
Cont	0	0.66	0.79	0.20	0.23	0.30	0.42	5.40	5.44	10.61	16.43
Methionine	100	0.71	0.79	0.24	0.26	0.34	0.45	5.90	4.71	14.56	16.64
	500	0.68	0.82	0.21	0.24	0.32	0.43	5.44	5.21	13.52	16.64
Tryptophan	100	0.70	0.84	0.23	0.25	0.33	0.44	5.48	5.65	12.90	18.72
	500	0.66	0.79	0.21	0.24	0.32	0.41	5.72	5.48	13.10	16.02
Pyrimidine derivative	100	0.67	0.82	0.20	0.24	0.32	0.42	4.61	5.01	15.60	18.10
	500	0.69	0.82	0.23	0.25	0.33	0.44	5.57	5.13	13.52	18.10
L.S.D 5%		0.02	0.04	0.01	0.02	0.02	0.02	0.14	0.09	1.40	1.00

Table 4: Effect of methionine, tryptophan and the pyrimidine derivative (SG93) material on some biochemical constituents of the yielded fennel seeds.

		Essential oil %	Fixed oil %	Total phenolic compounds (mg/g d. wt.)	Major essential oil constituents (%)							
	mg/l				αPinene	D Limonene	1,8 Cineol	Fenchone	Anethol	Estragol(Methyl chavicol)	Known	Unknown
Cont	0	0.79	5.82	1.17	0.37	0.07	5.09	4.13	86.11	0.05	4.18	95.82
Methionine	100	0.78	6.05	1.22	0.36	0.02	4.93	4.13	87.58	0.53	2.45	97.55
	500	1.01	6.49	1.24	0.39	0.11	4.50	4.12	85.61	0.84	4.43	95.57
Tryptophan	100	1.03	5.28	1.21	0.23	0.11	5.72	4.10	87.53	0.18	2.13	97.87
	500	1.06	6.68	1.31	0.33	0.13	6.22	3.70	87.14	0.23	2.25	97.75
Pyrimidine derivative	100	0.89	4.80	1.13	0.34	0.09	3.97	4.47	86.22	0.73	3.37	96.63
	500	0.95	5.46	1.20	0.48	0.17	4.38	3.80	86.77	0.67	3.73	96.27
L.S.D 5%		0.04	0.31	0.03	-	-	-	-	-	-	-	-

In this respect, the tryptophan and methionine were previously found to promote the biosynthesis of photosynthetic pigments in *Foeniculum vulgare* L, (Hassanein, 2003). This however, explained the present results (Table-3). El-Awadi (2007) reported similar observations in other plant spieces under abiotic stress condition, i.e. the increase in the photosynthetic pigments, total phenolic compounds and total amount of protein in other plants.

In addition Abu-Dahab and Abd El-Aziz (2006), proved the effect of tryptophan in increasing the total free amino acids and total phenolic compound in *Philodendron erubescens.*

The importance of the estimated phenolic contents of the fennel plant is referred to the observations mentioned by Oktay *et al* (2003) on their active role as strong antioxidant .This is coincided by the findings of Parejo *et al* (2004) who identified 42 phenolic compounds, 27 of which were confined to the fennel plant for the first time.

3- Effect on oil percentage and composition

The essential oil of the produced fennel seeds in all treatments were subjected to fractionation using gas liquid chromatography (GLC) and data are represented in Table (4).

The essential and fixed oil percentage in the produced fennel seeds were significantly influenced

in response to the pre-sowing seed soaking treatments in the methionine , tryptophan as well as in the pyrimidine material (Table 4). Methionine at its low and high concentrations had favored other treatments followed by tryptophan and the pyrimidine derivative material (SG93).

In comparison to the control, methionine seed treatment resulted in a significant increase in the fixed oil percentage at both its concentrations followed by tryptophan at its high concentration, whereas the effect of the pyrimidine derivative compound on the fixed oil percentage resulted in slightly values (Table 4).

From the same table, as compared to the control, anethol, 1,8 cineol and fenchone are recorded as the main components of the essential oil of the produced fennel seeds. Anethol percentage ranged from 85.61 to 87.58, while 1,8cineol and fenchone ranged from 3.70 to 5.72%. The highest percentage of anethol was obtained in the 100mg/l methionine-seed –treated plants. Whereas, a considerable increase in the percentage of 1.8cineol was obtained within response to the pyrimidine derivative material seed treatment (Table 4). On the contrary, the application of tryptophan and the pyrimidine compound (SG93) both at 500mg/l resulted in markable decreases in the percentage of fenchone (Table 4).

The findings of Tallat (2005) on *Pelargonium graveolens* L are in support to our present results on fennel.

According to Anand *et al* (2008) anethole, which is the principal active constituent of fennel seeds, showed an anticancer activity.

In earlier studies, El-keltawi and Croteau (1986) explained the influence of growth regulators on essential oil production to the alternation in levels and /or relevant enzymes. However, Gross *et al* (2002) referred the role of the S-adenosyl-L-methionine to the methyl group as a donor to produce estragole and t -anethole in cell-free extracts from bitter fennel.

From the present results, it is concluded that the treatments had affeced the metabolism of the important constituents of fennel plant. Therefore, the treatments could be concerned in elevating the productive value of the plant as a source for the use in medical research against certain diseases. Further experiments are currently taking place to learn more on such a subject.

Corresponding Author:

Dr. Mohamed. E. El-Awadi

Botany Department, National Research Centre, Dokki, 12311, Cairo, Egypt.

el_awadi@yahoo.com

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