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Effect of Nicotinamide on Growth and Volatile Oil Composition of Various Parts of Sweet Fennel (*Foeniculum vulgare* var. *dulce*) Plants

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Abstract: Pot experiment was conducted to study the response of *Foeniculum vulgare* plants to foliar application of nicotinamide. Data showed that, vegetative growth, essential oil percentage of different parts and yield significantly promoted by nicotinamide treatments up to 80 mg l^{-1}. Protein profile of the produced seeds and total carbohydrates were also influenced by the treatments. The most promising results with best yield and quality of fennel oil were obtained from plants treated with nicotinamide at 80 mg l^{-1} at both cuttings; essential oil yield was maximal at 1st cut (0.14 ml oil/plant); it was high in the fruiting umbels (0.93 %), low in the leaves (0.33 %), but only traces occurred in the stems (0.07 %). GC/MS analysis revealed that the major components of all oil samples was trans-anethole (17.32, 20.96, 59.31 % in leaf, stem, fruiting umel, respectively). The other main components namely, limonene, γ- terpinene, estagole, fenchone, α- pinene and β- pinene. The volatile oil of leaf and stem contained high amount of anis-aldehyde. These components showed some qualitative and quantitative differences under the effect of nicotinamide treatments in different parts of fennel plants.

Key words: *Foeniculum vulgare*, nicotinamide, vegetative growth, protein profile, total carbohydrates, essential oil components, yield

Introduction

Foeniculum vulgare (sweet fennel) is an *Umbelliferous* perennial plant and one of the most widespread spice in the world, the fresh leaves and dried fruits are used commonly as a flavouring in many food products and as a local mateia medica in Turkey (Akgül, 1986). The principle flavouring constituents of fennel fruits of sweet fennel, var. *dulce* (Ravid *et al.*, 1983), vegetable fennel, var. *azoricum* (Stahl, 1982) and bitter fennel var. *vulgare* (Kraus and Hammerschmidt, 1980) are trans-anethole and fenchone but the other parts of fennel plants have somewhat different chemical compositions (Akgül and Bayrak, 1988). Sweet fennel seed oil had a yellowish tint, characteristic odour and sweet taste. The main characteristic composition of the oil is the high content are trans-anethole which varied from 75.68 to 86.52 %, limonene (4.25-9.15%), estagole (3.25-5.21%), fenchone (1.05-2.80%), γ–terpinene (0.86-1.57%) and β- pinenc (0.47-1.14%) (Akgül, 1986). Nicotinamide is a nitrogen- containing aromatic compound known as growth regulating factor that influence many physiological processes such as the synthesis of enzymes, nucleic acids and protein, in addition they act as co-enzymes and changes the endogenous hormones (Deyab, 1989; Sheteawi, 1993; Hathout, 1995; Abdel-Halim, 1995). Since nicotinamide is recently isolated from some plants and little work including its effect on the physiology and yield of aromatic plants is known, also, no studies have been conducted so far on the oil composition of different parts of sweet fennel plants, therefore additional studies are required to elucidate the effects of treating fennel plants with nicotinamide. For this work, we studied some morphological criteria, some metabolic constituents, as well as essential oil production and composition of different parts of nicotinamide treated sweet fennel *(Foeniculum vulgare* L.) plants.

Materials and Methods

A pot experiment had been conducted at the Experimental Farm of National Research Centre, Dokki, Cairo, Egypt. Seeds of sweet fennel (*Foeniculum vulgare* var. *dulce*) plants were secured from the medicinal and aromatic section, Ministry of Agriculture, A. R. E. The seeds were sown on 10 th of November during 1998-1999 in pots (30 cm in diameter) filled with loamy clay soil. Water requirements were regularly fulfilled every 3 to 4 days according to weather conditions. Fertilization was carried out for each pot at proportion of 1 g calcium nitrate (33.5 % N), 2 g calcium superphosphate (15.5 % P_2O_5) and 1 g potassium sulphate (48 % K_2O). These fertilizers were applied in two doses at 30 and 60 days from planting and repeated after the first and second cuts. Plants were foliarly sprayed with nicotinamide at zero, 20, 40, 80 and 100 mg/l three times. The first was two months from transplanting, the second was two month from the first cut and the third was two month after the second cut. Three cuts were taken by about 6, 9 and 12 months from sowing. Control plants were sprayed with distilled water and the volume of the spraying solution was maintained just to cover completely the plant foliage till drip, Tepol was added @ 0.1 % of the spraying solution as wetting agent. The plant herbage was harvested at fruiting stage by cutting above 10 cm over the soil surface and plant growth parameter for the 2 cuts were recorded in terms of plant height, number of (branches, leaves and umbels) per plant, also fresh and dry weights of the (stem, leaves and umbels) were recorded. Plant samples were dried in an electric oven with drift fan at 60 °C for 48 hr till constant dry weight. Representative fresh samples were taken from each treatment for determination of essential oil content and oil constituents. Air dried seeds of fennel plants developed from third cut were used for determination of protein pattern using sodium dodecyl sulphate polyacrylamide gel electrophoresis in the central laboratory of Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Total carbohydrate in the dried samples of first cut were determined by using phenol sulphuric method (Dubois *et al.*, 1956). Qualitative determination of fennel oil obtained from the fresh umbels, leaves and stems of different treatments was achieved by hydro-distillation according to Egyptian Pharmacopoeia (1984) during the first and second cuts. Essential oils from the first cut were separated and analyzed qualitatively by GC/MS. Conditions for the GC analysis were as follows: Varian 3400 GC equilibrating chromatograph equipped with a DB-5 fused silica capillary column (30 m X 0.25 mm i.d., 0.25 μm film thickness). The multi step temperature program was increased from 60 °C held for 3 min., to 260 °C held for 10 min. with rate of 5°C/min. The carrier gas was helium at a flow rate of 1 ml/ min and the sample size was 1 μl (Injector temperature was 250 °C). Mass spectrometer was a Varian-finnigan SSQ 7000 operating in ionizing potential 70 ev, spectra were scanned in the range of 35-400 amu analysis. Experiment were distributed in complete randomized blocks, each represented by 2 plants. Data obtained were subjected to statistical analysis of variance according to (Snedecor and Cochran, 1980) and the values of least significant differences (L. S. D. at 0.05% level) were calculated to compare the means of different treatments.

Results and Discussion

Data (Table 1) shows that foliar application of nicotinamide at 0.0, 20, 40, 80 and 100 mg/l promoted the growth eriteria 20, 40, 80 and 100 mg l^{-1} promoted growth criteria (plant height, number of branches, leaves and umbels as well as fresh and dry

Table 1: Effect of nicotinamide on vegetative growth of *Foeniculum vulgare* L. plants. Mean data of the first and second cuts

	Plant height (cm)		Number of						Fresh weight (gm/plant)						Dry weight (gm/plant)					
	1st cut	2st cut	branches/ plant		Leaves/ plant		Umbles/ plant		Stem/ plant		Leaves/ plant		Umbels/ plant		Stem/ plant		Leaves/ plant		Umbels/ plant	
Treatments			1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Control	87.67	66.00	6.00	6.00	13.67	8.33	7.00	5.67	37.34	10.91	17.45	4.57	12.38	4.32	8.06	2.65	3.57	1.17	2.58	1.35
Nicotinamide 20 mg l^{-1}	105.33	75.67	7.33	7.33	16.67	9.33	8.00	6.67	50.07	12.42	21.87	4.78	13.84	4.45	10.80	2.91	4.67	1.28	2.72	1.78
Nicotinamide 40 mg l^{-1}	108.00	76.67	8.33	7.67	22.00	11.33	11.33	9.67	52.14	13.47	22.88	5.69	17.36	5.82	12.50	3.11	4.93	1.40	3.63	2.40
Nicotinamide 80 mg l^{-1}	113.33	78.33	8.67	8.00	23.00	12.33	14.33	10.67	53.83	17.24	24.74	7.59	19.58	6.71	13.14	4.67	5.83	2.27	3.76	2.57
Nicotinamide 100 mg l^{-1}	103.33	64.33	7.00	6.33	12.33	5.67	5.67	4.33	33.85	10.16	8.77	4.07	5.57	3.47	6.22	2.44	2.27	1.09	1.09	1.11
LSD at 0.05 level	3.33	5.38	0.81	1.24	2.30	1.48	1.38	1.22	2.07	1.96	2.65	0.75	1.66	0.80	1.44	0.88	0.64	0.21	0.60	0.31

Table 2: Protein profile of the produced seeds by fennel plants in response to nicotinamide treatments

Treatments mg l^{-1}	Control	Nico. 20 (mg l^{-1})	Nico. 40 (mg l^{-1})	Nico. 80 (mg l^{-1})
Mol. Wt. Kda	Mol. Wt. Kda	Mol. Wt. Kda	Mol. Wt. Kda	Relative front
0.051	-	330.09x	327.62x	-
0.065	-	-	-	269.32x
0.086	198.96	-	-	201.85x
0.108	144.64	152.79	-	-
0.117	-	-	-	126.34
0.134	110.07	106.22	109.34	106.34
0.159	89.00	-	-	87.06
0.174	77.47	-	-	77.14
0.180	-	73.70	74.13	-
0.194	65.67	-	65.92	-
0.210	-	62.80	-	-
0.216	-	-	61.41	61.60
0.230	59.11	-	-	-
0.244	-	56.76	-	56.63
0.251	-	-	55.5	-
0.259	54.01	-	-	-
0.294	-	48.57	-	49.34
0.304	47.18	-	47.01	-
0.327	-	43.90	-	44.33
0.342	42.14	41.90	41.45	-
0.425	-	-	-	32.40
0.438	31.20	-	-	-
0.448	-	3.21	-	-
0.458	-	-	29.33	-
0.490	-	-	-	26.56
0.511	25.28	24.87	-	-
0.524	-	-	23.90	-
0.634	17.10	17.25	17.05	-
0.711	13.34	-	-	13.65
0.732	-	12.63	-	-
0.744	12.28	-	12.15	-
0.796	-	10.56	-	10.37
0.814	9.80	-	9.83	-
0.830	-	8.33x	-	-
0.841	-	-	9.02x	-

Table 3: Effect of nicotinamide on carbohydrates and oil content of different parts of *Foeniculum vulgare* L. plants

	Total carbohydrates (%)			Oil (%)						Oil yield ml/plant	
	Seeds 1st cut	Leaves 1st cut	Stem 1st cut	Fruiting umbel		Leaves		Stem		1st cut	2nd cut
Treatments				1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut		
Control	25.710	14.800	30.920	0.711	0.948	0.221	0.328	0.030	0.273	0.138	0.086
Nicotinamide 20mgl^{-1}	27.120	17.930	33.260	0.842	1.004	0.275	0.432	0.053	0.329	0.203	0.106
Nicotinamide 40mgl^{-1}	27.230	18.290	34.530	0.896	1.020	0.301	0.447	0.062	0.332	0.256	0.130
Nicotinamide 80mgl^{-1}	28.070	19.480	35.010	0.933	1.159	0.329	0.478	0.070	0.348	0.302	0.174
Nicotinamide 100mgl^{-1}	26.160	17.170	31.710	0.758	0.994	0.241	0.428	0.046	0.317	0.079	0.084
L.S.D. at 0.05 level	0.530	0.460	1.810	0.008	0.005	0.006	0.004	0.003	0.006	0.015	0.010

Table 4: Effect of nicotinamide on the essential oil components of different parts of *Foeniculum vulgare* L. plants

Treatments components (%)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Fruiting umbel oil components (%)																	
Control	0.957	0.037	0.129	0.163	0.007	21.519	2.281	1.351	2.065	0.694	59.312	-	-	0.087	-	1.087	10.220
Nicotinamide 20 mg l^{-1}	0.068	0.368	0.283	0.415	0.194	13.327	-	0.219	5.751	0.275	57.243	-	-	-	-	-	21.857
Nicotinamide 40 mg l^{-1}	2.972	0.074	0.509	0.331	0.440	28.110	-	4.394	1.658	0.639	35.556	-	-	0.184	-	1.163	23.970
Nicotinamide 80 mg l^{-1}	0.056	0.502	0.249	0.385	0.027	14.999	-	0.209	5.652	0.277	66.476	-	-	0.024	-	0.131	11.140
Nicotinamide 100 mg l^{-1}	0.021	2.688	0.166	-	0.595	19.881	0.008	0.245	4.661	0.030	66.578	-	-	-	-	0.018	5.109
Leaf oil components (%)																	
Control	0.296	-	-	0.056	-	18.250	0.017	0.714	0.874	-	17.323	2.096	1.946	15.638	1.398	1.323	40.096
Nicotinamide 20 mg l^{-1}	1.188	0.031	-	0.205	-	16.531	0.082	1.671	4.078	-	27.598	2.143	0.026	0.110	0.285	0.278	45.774
Nicotinamide 40 mg l^{-1}	-	0.749	-	0.021	-	29.688	0.861	0.131	0.813	-	48.055	1.908	0.036	0.267	0.310	0.087	17.074
Nicotinamide 80 mg l^{-1}	0.394	0.010	-	0.043	-	80.912	0.009	0.389	0.239	-	15.240	0.025	0.029	0.023	-	0.014	2.687
Nicotinamide 100 mg l^{-1}	-	0.532	-	0.037	0.017	91.481	0.026	-	0.275	-	5.446	-	0.270	-	0.056	0.026	1.834
Stem oil components (%)																	
Control	1.257	0.032	0.068	0.065	31.480	0.028	0.100	0.135	0.277	20.955	1.960	-	11.460	-		0.666	31.517
Nicotinamide 20 mg l^{-1}	0.013	2.793	0.060	0.016	96.976	-	-	0.063	0.026	14.579	0.040	-	0.046	-		0.056	12.332
Nicotinamide 40 mg l^{-1}	0.766	0.015	0.031	-	54.704	0.023	-	-	0.009	40.085	0.159	-	0.068	-		0.016	4.124
Nicotinamide 80 mg l^{-1}	0.020	0.095	0.100	-	65.188	-	-	0.094	0.045	27.354	1.766	-	0.164	-		0.024	50.179
Nicotinamide 100 mg l^{-1}	-	0.263	-	-	37.291	-	0.004	0.045	-	59.913	0.715	-	0.015	-		0.177	1.577

1 = α -Pinene, 2 = Camphene, 3 = α -Thujone, 4 = β -Pinene, 5 = α -Phellandene, 6 = Limonene, 7 = γ -Terpinene, 8 = Fenchone, 9 = Estragole, 10 = cis-Anethole, 11 = trans-Anethole, 12 = Fenchol acetate, 13 = Carvone, 14 = Anis-aldhyde, 15 = Carveol acetae, 16 = Anis-ketone, 17 = Not-idenfified comp.

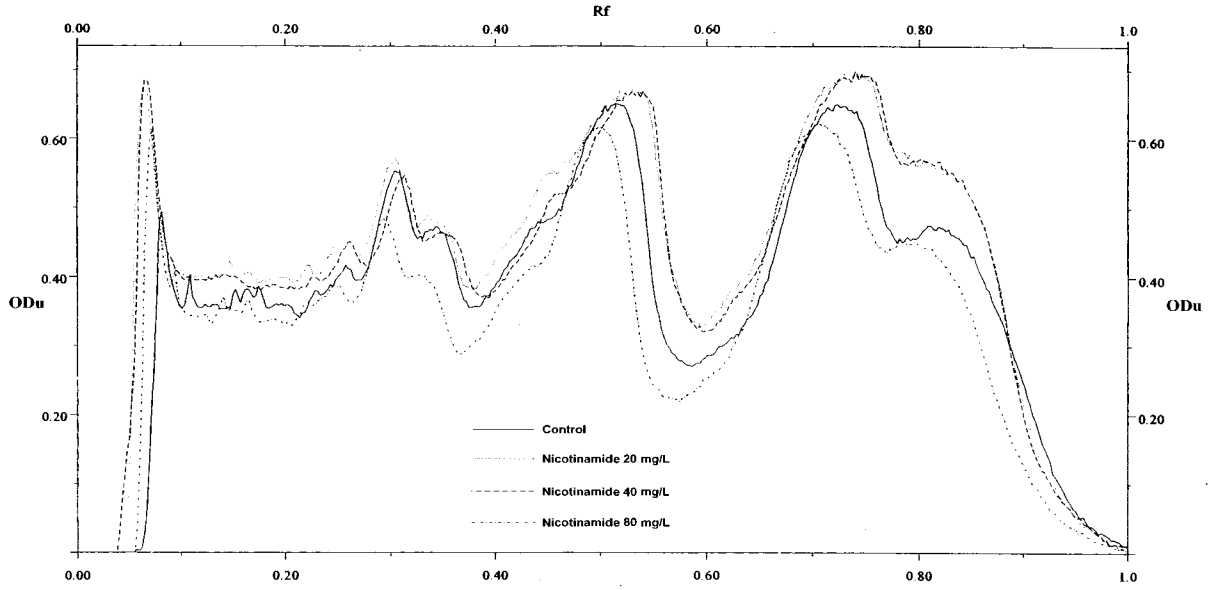

Fig.1: Protein profile of the produced seeds by fennel plant in response to nicotinamide treatments

weights of stem, leaves and umbels at fruiting stage). In all cases, the increments in growth parameters accompanied with parallel increase in nicotinamide concentration up to 80 mg l^{-1}, the increase were often highly significant in comparison with untreated ones. On the other hand, it could be observed that the higher concentration seemed to exhibit the opposite trend during the first and second cuts. The most effective treatments on growth parameters was nicotinamide at 80 mg l^{-1}. This favorable increase in growth of fennel plants as a result of nicotinamide treatments might be due to the enhancement of plant growth, formation of secondary branches and stimulation of metabolic processes. These results are in full agreement with those obtained by Khotyleva (1992) and Hathout *et al.* (1993) who reported that foliar application of nicotinamide to tomato plants increased stem length, number of branches, leaves, flowers, fruit set as well as fresh and dry weights of the plant organs consequent to spraying mainly with 80 ppm concentration. Moreover, Abdel-Halim (1995) on tomato plants, revealed that the regular increase in contents and levels of promoters (IAA, IAN and GA-like substances) due to foliar spray with nicotinamide at 50 ppm accompanied by decrease in content and activity of inhibitors (ABA) leading to hormonal balance. The qualitative and electrophoretic studies of protein profiles in control fennel seeds as well as in the nicotinamide produced seeds was presented in Table 2 and Fig. 1. The protein bands showed a wide variation in nicotinamide produced seeds as compared to control seeds. The control seeds showed 16 bands with mobility from 0.086 to 0.814 with molecular masses ranged approximately from 198.96 to 9.80 KDa. In the 80 mg l^{-1} nicotinamide produced seeds, some bands were missing, like those with molecular masses of 198.96, 144.64, 110.07, 65.67, 54.01, 17.10, 12.28 and 9.80; meanwhile some new bands appeared with molecular masses of 269.32, 201.85, 126.34, 106.09, 61.60, 56.63, 26.56 and 10.37. The appearance of proteins having high molecular weight was the marked feature in the produced seeds after nicotinamide treatments especially at 80 mg l^{-1}. This changes in protein patterns may reflect that nicotinamide treatments retard partial proteins degradation, as well as activate the enzymes involved in the metabolism of essential oil formation concerning the total carbohydrates of sweet fennel plants, it is clear that foliar application of nicotinamide had the beneficial effect upon the total carbohydrates content of different plant organs in the first cut (Table 3). There was a gradual increase in carbohydrates content of seeds, leaves and stem above their corresponding controls by increasing the applied concentration of nicotinamide reaching its

maximum value at 80 mg l^{-1}. In support, Hathout *et al.* (1993) found that carbohydrates, mainly polysaccarides, were increased in tomato plants by the increase of nicotinamide applied up to 80 ppm concentration. Data (Table 3) shows, that fruiting umbels of fennel plants contain the highest content of volatile oil than that of leaves and stem. Moreover, foliar spray of nicotinamide increased oil % and oil yield of different parts during the first and second cuts. Nicotinamide at 80 mg l^{-1} recorded the maximum volatile oil percent % and oil yield in stem, leaves and fruiting umbels as compared with their corresponding controls. These results hold true during the two successive cuts. Nevertheless, the higher mean values of oil yield/plant for the two cuts when pooled together are gained by plants treated with nicotinamide at 80 mgl^{-1}. However, the higher concentration of 100 mg l^{-1} exhibited the opposite trend.

This increment of volatile oil might be due to the increase in vegetative growth as the plant advanced towards maturity as well the increase in carbohydrates synthesis and also the effect of nicotinamide on metabolism and enzymes level responsible for mono or sesqueterpene biosynthesis. This conclusion is in accordance with the findings of Gamal El-Din *et al.* (1997) on Lemon—grass and Naguib *et al.* (1998) on dill. The monoterpenes hydrocarbon fraction of the essential oils of different parts (fruiting umbels, leaves and stems) resulted from spraying the foliage parts of fennel plants with different concentrations of nicotinamide identified 16 compounds. There was a remarkable qualitative and quantitative difference among the hydrocarbon of the 15 samples (Table 4). The principle flavouring constituents of control sweet fennel fruit oils are trans-anethole (59.31%), limonene (21.52 %), estragole (2.16 %), fenchone (1.35 %), γ- terpinene (2.28 %) and α- pinene (0.96 %). On the other hand, anis aldehyde (0.087 %) and anis keone (1.087 %) which were the autoxidation products of trans-anethole and cis- anethole were generally found in small amounts in fruit oils, as a result the fruit oil are rich in oxygenated compounds. In this connection, Lawrence (1979) reported that, the volatile oil of sweet fennel fruit is characterized by relatively high concentrations of trans-anethole, limonene, estragole, γ- terpinene and low concentrations of α- pinene and fenchone. In the fruit oils of sweet fennel plants, nicotinamide treatments increased the percentage of trans-anethole and estragole but decreased limonene, especially at 80 and 100 mg l^{-1} nicotinamide. However, the opposite trend was obtained at 40 mg l^{-1} nicotinamide, where α- pinene, limonene and fenchone were increased but trans- anethole and estragole markedly decreased in comparsion with the fruit oil of control plants. Control fennel oils contained high percentage of limonene (18.25 and 31.48 %), anis aldehyde (15.64 and 11.46 %) and low percentage of trans-anethole (17.32 and 20.96 %) for leaves and stems oils, respectively. Moreover, spraying the foliage parts of fennel plants with different concentrations of nicotinamide gradually increases the percentage of limonene in the leaves up to 91.48 % at 100 mg l^{-1} nicotinamide treatment accompanied by parallel decrease in carvone (0.28 %), anis aldehyde (0.0 %) and anis keone (0.03 %) at the same concentration. In accordance, De Vottero *et al.* (1980) and Ravid *et al.* (1983) reported that sweet fennel herb oils had high concentration of limonene. With regard to seed oil, our findings suggest that stems and leaves oils of control sweet fennel plants have little value because of their low yield of oil, low percentage of trans-anethole and large amounts of hydrocarhones. However, nicotinamide treatments markedly improved the oil quantity and quality of stem and leaves and consequently increased its commercial value. In addition, nicotinamide at 40 mg l^{-1} increased d- limonene and trans-anethole and decrease anis aldehyde and anis keone to trace amounts in leaves and stem oils. On the other hand, the fruit oils with a high percentage of trans-anethole and low amounts of cis- anethole due to nicotinamide treatments seems to be a valuable flavouring agent for various food products. Similar results were found on bitter fennel by (Akgül and Bayrak, 1988).

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