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Effect of Pre-Sowing Treatments with Uv-Ray on the Chemical Components of Fennel (*Foeniculum Vulgare* Mill)

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Abstract. The objective of the present study was to evaluate the effect of pre-sowing treatments with UV-ray on the amount of essential oil and its components. The results showed that exposing seeds to UV-A $\times D_1 \times T_1$ significantly increased protein content comparing to control. Meanwhile it decreased the concentration of *trans*-anethole. However increasing time to 40 min. (UV-A $\times D_1 \times T_2$) decreased protein content. Concerning the studied chemical compounds, they were significantly affected by UV-ray, but each had an optimum dose for accumulating a high rate. When seeds were exposed to UV-C $\times D_2 \times T_2$ produced the higher concentrations of *trans*-anethole as the main compound of fennel essential oil and M-chavicol (estragol), compared to control. And (UVC $\times D_1 \times T_3$) also increased qualitative characters such as essential oil content, essential oil yield and fixed oil content compared to control. Protein profiles showed around 9 protein bands, clearly dominant. 1, 2 and 3 protein bands with down regulations were identified when seeds exposed to UV-A $\times D_2 \times T_2$ (mutant M_2) and UV-C $\times D_1 \times T_3$ (M_{12}). This was conformed the under production of cellular protein by UV mutation. SDS-polyacrylamide gel electrophoresis (PAGE) analysis did not show additional or deletion in number of protein bands in UV mutants.

Keywords: *Foeniculum vulgare* Mill; Protein content; Trans-anethole; UV-ray.

1. Introduction

Fennel (*Foeniculum vulgare* Mill.) which belongs to the family Apiaceae, was selected. Fennel is one of the aromatic and medicinal plants, small group of annual, biennial or perennial herbs [1]. Fennel is used as a spice and also as an important ingredient in various folklore medicines throughout the world [2].

Ultraviolet radiation is the part of the non-ionizing region of the electromagnetic spectrum that comprises approximately 8-9 % of total solar radiation [3]. UV-C (100-280 *nm*) is extremely active photo-chemically and biologically lethal, but it is completely excluded by the ozone layer. UV-B region (280- 320 *nm*) is the part of ultraviolet radiation which is biologically active and vulnerable to significant changes resulting from differences in the extent of blocking by the ozone layer. UV-A (320-400 *nm*) is present in significant amounts in natural daylight and is relatively little affected by changes in the ozone layer [4].

In recent years, there has been an increased interest in UV light techniques, because ultraviolet radiation is one important factor that in many cases has an effect on plant growth and stimulates the production of secondary metabolites. The degree of the effect of UV-ray on plant growth varies according to plant species, but their effecting on plant secondary metabolites may return to some specific reasons such as the secondary metabolites which have a sun screening effect and protect the cells from the radiation, this would be especially likely if the metabolites are concentrated in the epidermis or other superficial tissues, and the radiation aids in reaching a certain level of general differentiation necessary for production of secondary metabolites[5].

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2. Materials and Methods

This experiment was carried out at the research station field of the Faculty of Agricultural Sciences / Sulaymani Univesity in Bakrajo during the season (October 2011- July 2012). The seeds of *Foeniculum vulgare* Mill. were (local variety) standard seeds obtained from Pakan Bazaar product of Iran and before sowing they were treated with ultraviolet ray {Control: UV-C: 254 *nm* and UV-A: 366 *nm*)} at two different distances (D_1:6 cm and D_2: 12 cm) between seed lots and the source of UV-ray for different periods of time (T_1: 20, T_2: 40, and T_3: 60 min.). The field land was designed as a factorial experiment conducted in split-split plot design, with three replicates.

2.1. Studied Characters

2.1.1. Chemical components

2.1.1.1. Protein %

Protein content was determined in seeds by using Kjeldahl method [6] and for calculation, the following equations were used:

- T. N% = (V ×N ×E ×0.014 /S) ×100
- Protein%=T.N% ×6.25
- T.N= Total nitrogen
- V= Volume of 0.01 N HCL titrated for the sample (ml).
- N = Normality of HCL solution.
- E= Equivalent weight of nitrogen

2.1.1.2. Fixed oil %

The ratio (%) of fixed oil was determined by using the Soxhlet apparatus, the method used by [7], then fixed oil content calculated as follows [8]:

- Fixed oil% = (Weight of flask after extraction – weight of flask prior to extraction /Weight of sample) ×100

2.1.1.3. Essential oil %

Water steam distillation: Essential oil was quantified by using a Clevenger apparatus, according to [9]. Samples of 25 g from the dried and cleaned mixed seeds for each treatment were ground up; therefore, they were subjected to hydro-distillation for 120 min. [10] to obtain the yield of essential oil and the chemical component of the oil samples, using High Performance Liquid Chromatography (HPLC) technique. The following equation was used to determine the content of essential oil.

- Essential oil% = Volume of essential oil / Weight of sample ×100

And essential oil yield was calculated from the following formula:

- Essential oil yield kg h^{-1} = Essential oil% w/w × seed yield kg h^{-1}

2.1.1.4. Essential oil analyzing

The extracts (essential oil) were qualitatively and quantitatively analyzed by High Performance Liquid Chromatography (HPLC) [Research Laboratory of the Green Field Company in Baghdad] using a model Shimadzu Corporation, Kyoto Japan, LC- 10 AV equipped with binary delivery pump model LC-10A Shimadzu. Column: Phenomenex C-18, 3µm particle size (50×4.6 mm I.D.)[11].

Calculation: Concentration of sample µm/ml = (Area of sample/ Area of standard) × Concentration of standard × dilution factor [12].

2.1.1.5. Protein extraction

Protein extracted in seeds by using gel electrophoresis [13].

2.2. Statistical Analysis

Statistical analysis of the data was carried out by using statistical program JMP Stat, for checking the significance of the different treatments, whereas LSD at 5% probability levels ($P< 0.05$) was used to compare the differences among the treatments.

3. Results and Discation

The results of (Table 1) indicated that irradiation of seeds to UV had significant effect on qualitative characters of fennel seeds. Exposing seeds to UV for T3 (60 min.) gave significantly the highest value of essential oil percent accumulated in seeds (3.9 %), followed by T2 (40 min.) with (3.6%). Similar results with positive effect of UV-ray on essential oil concentration in anise were found by [14], in Parsley by [15], and in lavender by [16].

Table 1. Effect of exposure time to UV ray on essential oil content (EO %), essential oil yield (EOY kg ha^{-1}, fixed oil content (FO %) and protein content (P %) in fennel seeds.

Exposure time / Character	T_1	T_2	T_3	LSD 0.05
EO%	3.4	3.6	3.9	0.11
EOY(Kg h^{-1})	51.7	62.6	55.9	2.8
FO%	6.6	19.5	19.7	0.8
P%	20.5	19.5	19.7	0.8

T_1:20 min., T_2:40 min., T_3:60 min.

Table 2 verifies the interaction between UV-C×D_1×T_3 produced higher values of essential oil content (4.5%) and essential oil yield (82.4 kg ha^{-1}) compared to control (3.6 % and 45.9 kg ha^{-1}), respectively, this interaction increased essential oil concentration by (25%), and essential oil yield by (79.5%). And the lowest value of essential oil yield was found by UV-C×D_1×T_1 (32.8 kg ha^{-1}). The results were in agreement with those by [17], who reported an increase (50%) of essential oil production when *Mentha spicata* irradiated by UV-ray.

Interaction between UV-C×D_1×T_3 significantly increased fixed oil (7.3%) compared to control (7.1%), but the lowest value with (5.6%) was found by the interaction UVC×D_2×T_2. Irradiation of seeds with UV-A×D_1×T_1 significantly increased protein content (22.3%), followed by UV-C×D_1×T_2 (21.6%) compared to control (19.8%) where their contents were increased by (12.6%) and (9.0%), respectively. These results were in agreement with those by [18] who reported an increase in protein content of *Brassica napus* plants when exposed to UV-ray. This increase was probably due to the synthesis of defense proteins and enzymes. The results of the same table showed that the minimum value of protein content (18.2%) was produced by the interaction UV-C×D_1×T_3.

The significant interaction between UV-C×D_1×T_3 increased fixed oil% ,essential oil% and essential oil yield by (2.8%, 25 % and 79.5%), respectively and protein content was increased by (9%) with the interaction UV-C×D_1×T_2 compared to control. The interaction between UV-A×D_1×T_1 increased protein concentrations by (12.6%) compared to control.

Table 2. Effect of UV_{WL}, distance and exposing time on fixed oil rate (FO %), protein (P %), essential oil (EO %) and essential oil yield (EOY kg ha^{-1}) in fennel seeds.

Character / UV_{WL}×D×T	EO%	EOY(Kg h^{-1})	F0%	P%
Control	3.6	45.9	7.1	19.8
UV-C×D_1×T_1	2.9	32.8	6.1	19.9
UVC×D_1×T_2	4.0	64.0	6.0	21.6
UVC×D_1×T_3	4.5	82.4	7.3	18.2
UVC×D_2×T_1	4.0	62.8	6.3	20.5
UVC×D_2×T_2	3.2	67.7	5.6	18.2
UVC×D_2×T_3	4.0	50.8	6.5	19.9
UVA×D_1×T_1	3.3	72.3	6.0	22.3
UVA×D_1×T_2	4.0	69.9	6.3	17.9
UVA×D_1×T_3	4.2	47.6	6.8	20.9
UVA×D_2×T_1	3.3	50.7	6.8	20.6
UVA×D_2×T_2	3.6	81.9	6.1	19.6
UVA×D_2×T_3	3.7	63.0	6.8	19.5
LSD 0.05	0.2	6.9	0.8	2.0

Control, UV-C: 354 *nm*, UV-A: 365 *nm*, D_1:6cm, D_2:12 cm, T_1:20 min., T_2:40 min., T_3: 60 min.

Table 3 shows that the exposed seeds to UV-C and UV-A decreased concentrations of α-pienene, methyl chavicol, *trans*-anethole, fenchone and terpenene compared to control by (580.9%, 80%, 51.9%, 53.9% and 9.4%) compared to the lowest value for each treatment. But exposed seeds to UV-A significantly increased concentrations of eucalyptol, limonene, α- phellandrene and *p*-cymene to (1.50, 1.22, 0.93 and 0.82 mg ml^{-1}), respectively.

Exposed seeds to UV-C wavelength significantly increased concentration of estragole (methyl chavicol) and *trans*-anethole to (1.26 and 11.09 mg ml^{-1}), respectively compared to UV-A (1.06 and 8.76 mg ml^{-1}), while exposed seeds to UV-A wavelength significantly increased concentration of α-pinene, fenchone, eucalyptol, limonene, terpenene, α-phellandrene and p-cymene (1.05, 5.90, 1.50, 1.22, 0.86, 0.93 and 0.82 mg ml^{-1}), respectively compared to UV-C wavelength. These variations in the contents of chemical components of fennel seeds due to exposing seeds to different UV wavelengths could be explained by the different pathways through which each chemical is synthesized, such as the phenylpropanoid *trans*-anethole and estragole, found in the essential oil of fennel, are synthesized by shikimic acid pathway, while limonene, terpenene and α- pinene synthesized by acetate-mevalonic acid pathway [19].

Table 3. Effect of UV-ray (A and C) doses on chemical components of fennel essential oil as identified by (HPLC).

Chemical component / UV treatment	α –p mg ml^{-1}	m.ch mg ml^{-1}	*t*-a mg ml^{-1}	fe mg ml^{-1}	eu mg ml^{-1}	Li mg ml^{-1}	ter. mg ml^{-1}	α- ph mg ml^{-1}	p-cy mg ml^{-1}
Control	2.86	1.91	13.31	6.05	0.82	0.55	0.95	0.54	0.76
UV-C	0.42	1.26	11.09	3.93	0.94	0.57	0.56	0.44	0.11
UV-A	1.05	1.06	8.76	5.90	1.50	1.22	0.86	0.93	0.82
LSD0.05	0.012	0.002	0.014	0.011	0.019	0.015	0.023	0.11	0.04

Control, UV-C: 354 *nm*, UV-A: 365 *nm*.

The results of Table 4 showed that irradiation of fennel seeds to UV ray for 60 min. (T_3) significantly increased concentration of α-pienene, *trans*-anethole, terpenene and *p*-cymene (1.60, 12.03, 0.83 and 0.59 mg ml^{-1}), respectively. When exposed time was decreased the concentrations of these chemical components were decreased. Exposed seeds to UV-ray for 40 min. (T_2) significantly increased the concentrations of methyl chavicol, eucalyptol, limonene and α-phellandrene (1.92, 1.19, 0.84 and 0.73 mg ml^{-1}), respectively. While exposed seeds to UV-ray for 20 min. (T_1) significantly increased the concentration of fenchone (5.86 mg ml^{-1}) and with increasing exposed time, the concentration of fenchone was decreased.

Table 4. Effect of seed exposure to different times of UV-ray on chemical components of fennel essential oil as identified by HPLC.

Chemical component / Time (min.)	α –p mg ml^{-1}	m.ch mg ml^{-1}	*t*-a mg ml^{-1}	fe mg ml^{-1}	eu mg ml^{-1}	li mg ml^{-1}	ter. mg ml^{-1}	α- ph mg ml^{-1}	p-cy mg ml^{-1}
T1 (20 min.)	1.41	1.09	10.32	5.86	1.06	0.77	0.74	0.57	0.54
T2 (40 min.)	1.32	1.92	10.81	4.32	1.19	0.84	0.81	0.73	0.55
T3 (60 min.)	1.60	1.22	12.03	5.69	1.02	0.72	0.83	0.62	0.59
LSD0.05	0.011	0.001	0.006	0.009	0.007	0.007	0.009	0.08	0.011

T_1:20 min., T_2:40 min., T_3: 60 min.

The results of Table 5 revealed that exposing seeds to UV-A×D_1×T_3 interaction significantly increased the concentrations of α-pinene (3.03 mg ml^{-1}) and terpenene (1.46 mg ml^{-1}) compared to control (2.86 and 0.95 mg ml^{-1}), respectively. The interaction between UV-C×D_2×T_2 significantly increased the concentrations of methyl chavicol and *trans*-anethole (4.33 and 14.83 mg ml^{-1}), respectively compared to control (1.91 and 13.31 mg ml^{-1}). The results showed that after exposure of seeds to UV-C treatment increased the concentrations of compounds synthesized by shikimic acid pathway, while the concentrations of limonene, synthesized by acetate-mevalonic acid Pathway were decreased [19], this explained that UV-C had positive effect on shikimic acid pathway and negative effect on acetate-mevalonic acid pathway. The interaction between UVA×D_2×T_1 significantly increased concentration of fenchone (10.67 mg ml^{-1}) compared to control and the rest of other treatments for the same compounds, while interaction between UVA×D_1×T_2 gave the lowest value for the concentration of fenchone (0.78 mg ml^{-1}). The interaction between UV-A×D_2×T_2 significantly increased the concentration of eucalyptol (2.25 mg ml^{-1}) compared to control and the rest of

other treatments for the same compounds, while interaction between UVA×D_2×T_3 gave the lowest concentration value of eucalyptol (0.35 mg ml^{-1}) .

The interaction between UV-A×D_1×T_2 significantly increased the concentration of limonene, α-phellandrene and *p*-cymene (2.07, 2.15 and 1.45 mg ml^{-1}), respectively compared to control treatments (0.55, 0.54 and 0.76 mg ml^{-1}), respectively. The interaction between UV-C×D_2×T_1 gave the lowest concentration values of terpenene and α-phellanderene (0.18 and 0.14 mg ml^{-1}), respectively. The interaction between UV-A×D_2× T_3 gave the lowest concentration values of eucalyptol and limonene (0.35 and 0.150 mg ml^{-1}), respectively.

Table 5. Interaction effect of UV $_{WL}$, distance and exposure time on chemical components of fennel essential oil as identified by HPLC.

Chemical component / UV_{WL}×D×T	α –p mg ml^{-1}	m.ch mg ml^{-1}	*t*-a mg ml^{-1}	fe mg ml^{-1}	eu mg ml^{-1}	li mg ml^{-1}	ter. mg ml^{-1}	α- ph mg ml^{-1}	p-cy mg ml^{-1}
Control	2.86	1.91	13.31	6.05	0.82	0.55	0.95	0.54	0.76
UV-C×D1×T1	0	0.32	5.90	6.74	1.14	1.06	0.91	0.66	0
UV-C×D1×T2	1.18	0	8.51	5.15	1.44	0.35	0.65	0.54	0.15
UV-C×D1×T3	0	0.81	14.54	3.39	1.11	0.86	0.74	0.64	0
UV-C×D2×T1	1.008	1.63	14.49	2.72	0.76	0.35	0.18	0.14	0.14
UV-C×D2×T2	0	4.33	14.83	1.25	0.44	0.45	0.34	0.27	0
UV-C×D2×T3	0.37	0.45	8.26	4.34	0.76	0.35	0.57	0.38	0.36
UV-A×D1×T1	1.44	0.58	4.49	2.93	1.55	1.27	0.52	0.85	0.75
UV-A×D1×T2	0	2.86	5.86	0.78	1.37	2.07	1.37	2.15	1.45
UV-A×D1×T3	3.03	1.85	14.04	4.52	2.23	1.85	1.46	1.16	0.94
UV-A×D2×T1	0.33	0.16	10.42	10.67	1.25	0.86	0.93	0.67	0.85
UV-A×D2×T2	1.01	0.51	9.02	6.65	2.25	1.09	0.57	0.32	0.16
UV-A×D2×T3	0.505	0.38	8.72	9.83	0.35	0.15	0.33	0.45	0.75
LSD0.05	0.02	0.003	0.016	0.022	0.018	0.018	0.023	0.21	0.028

Control; UV-C: 254 *nm*, UV-A: 365 *nm* D_1: 6 cm, D_2: 12 cm, T_1: 20 min., T_2: 40 min., T_3: 60 min.

Fig. 1 shows the effect of UV $_{WL}$ (A and C) on gene expression in fennel seeds. The results revealed that the profiles of the extracted proteins from different genotypes, visualized on 15% SDS-PAGE, displayed few differences indicating the effect of the mutagen effect. Protein profiles showed that around 9 protein bands were clearly dominant. 1, 2 and 3 protein bands with down regulation were identified in the UV mutants M_2 and M_{12}, respectively. This was confirmed the under production of cellular protein by UV mutation. SDS-poly acrylamide gel electrophoresis (PAGE) analysis did not show additional or deletion in number of protein bands in UV mutants.

Earlier studies by [20] showed that the UV radiation damages ribosomes by cross linking cytosolic ribosomal proteins S14, L23a, and L32, and chloroplast ribosomal protein L29 to RNA as [21] reported the synthesis of new protein bands under UV-C stress. UV damages to specific binding proteins are considered to play an important role in early responses of cells irradiated with UV, including damage of recognition in the DNA repair process. Exposure to UV-B light produced effects which were very similar to those obtained from UV-C exposure [22]. UV-C and UV-B rays were found to cause a decline in total RNA content, enzyme activity and protein levels of several key photosynthetic proteins including RUBISCO [23] and [24] and also decline in D1 polypeptide of photosystem II, chlorophyll a/6-binding protein and the ATPase complex [25].

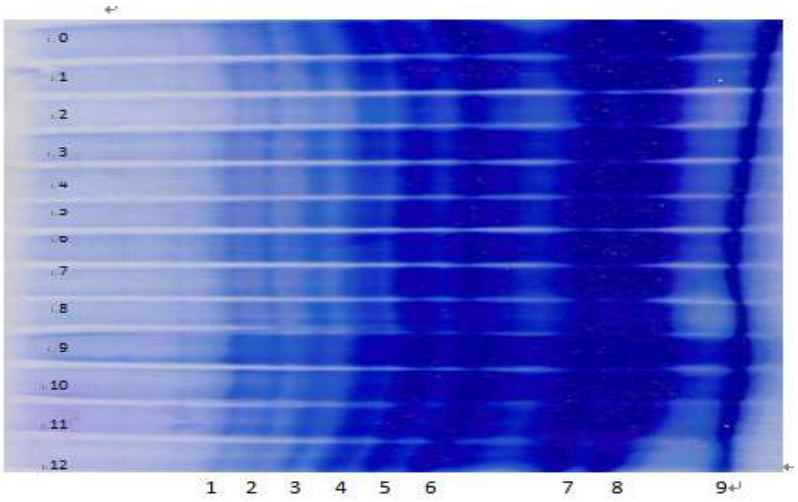

Fig. 1: Effect of UV_{WL} (A and C) on gene expression in fennel seed:

UV- 0 (Control);

M_1: UV-A $\times D_2 \times T_1$; (UV-A at distance 12 cm for 20 min.)

M_2: UV-A $\times D_2 \times T_2$; (UV-A at distance 12 cm for 40 min.)

M_3: UV-A $\times D_2 \times T_3$; (UV-A at distance 12 cm for 60 min.)

M_4: UV-A $\times D_1 \times T_1$; (UV-A at distance 6 cm for 20 min.)

M_5: UV-A $\times D_1 \times T_2$; (UV-A at distance 6 cm for 40 min.)

M_6: UV-A $\times D_1 \times T_3$; (UV-A at distance 6 cm for 60 min.)

M_7: UV-C $\times D_2 \times T_1$; (UV-C at distance 12 cm for 20 min.)

M_8: UV-C $\times D_2 \times T_2$; (UV-C at distance 12 cm for 40 min.)

M_9: UV-C $\times D_2 \times T_3$; (UV-C at distance 12 cm for 60 min.)

M_{10}: UV-C $\times D_1 \times T_1$; (UV-C at distance 6 cm for 20 min.)

M_{11}: UV-C $\times D_1 \times T_2$; (UV-C at distance 6 cm for 40 min.)

M_{12}: UV-C $\times D_1 \times T_3$. (UV-C at distance 6 cm for 60 min.)

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