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Research article

EFFECT OF PRE-SOWING TREATMENTS WITH UV-RAY ON AGRONOMIC CHARACTERS AND CHEMICAL COMPONENTS OF ANISE (*Pimpinella anisum*L.)

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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 UV-C, high energetic, had greater effect on studied characters, especially plant secondary metabolites than UV-A. And also D_2 distance between UV source and exposed seeds produced higher values of essential oil components of seeds compared to D_1. There were differences effect of exposed times on the studied characters, the highest fixed oil rate was found when the seeds exposed to UV for 40 min. while the optimum protein and essential oil contents were found at 20 min. radiation, whereas at 60 min. radiation, higher concentration of *trans*-anethole was accumulated. The results showed that exposing seeds to UV-C $\times D_2 \times T_2$ significantly increased essential oil yield (42.40 kg ha^{-1}) compared to control (10.63 kg ha^{-1}), while exposing seeds to UV-C at 6 cm distance for 20 min. significantly increased the rate of essential oil compared to control. While the higher rate of fixed oil was recorded after exposing the seeds to UV-A $\times D_2 \times T_2$ with values (12 %) control (5%). Concerning the studied chemical compounds, they were significantly affected by UV-ray, but each had an optimum dose for accumulating a high rate. The higher concentration of *trans*-anethole as the main compound of anise essential oil, was found to be (43.824 mg ml^{-1}) after exposing seeds to UV-C at 6 cm distance for 60 min. compared to control (7.955 mg ml^{-1}), while by decreasing exposure time to 40 min. for the same treatment, the concentration of estragole recorded the highest rate (1.87 mg ml^{-1}) compared to control (0.818 mg ml^{-1}). Moreover, anisaldehyde recorded high concentration with UV-C$\times D_1 \times T_1$ (2.131 mg ml^{-1}), dianethole with UV-A $\times D_2 \times T_2$ (15.03 mg ml^{-1}), anisic acid with UV-C$\times D_2 \times T_1$ (5.064 mg ml^{-1}), linalool with UV-A $\times D_1 \times T_1$ (4.044 mg ml^{-1}) and limonene in control treatment (3.689 mg ml^{-1}). The studied correlation coefficients verified that the essential oil was positively and significantly correlated with each of fixed oil and protein. *Trans*-anethole had negative and significant correlations with limonene and linalool (-0.668** and -0.468**), respectively, and also had positive and significant correlations with estragole with enisaldehyde (0.556**) and dianethole (0.716**).

Key words: *Pimpinella anisum* L; Protein content; Trans-anethole; UV-ray.

INTRODUCTION

The plant species Anise (*Pimpinella anisum* L.) which belongs to the family Apiaceae, was selected, because they are known to be rich in sources of essential oils mainly *trans*-anethole compounds. The genus *Pimpinella* contains 23 species, 3 of which are endemic in Iraq [1]. Anise is primarily grown for its fruit, commercially called “seed”, used as flavoring [2]. In general, the essential oil is extracted from its seed, but its roots may also be used. Essential oil of anise fruits contains from 75 to 95 % or more, Anethole (*trans*-anethole) as the main compound, followed by Estragole (Methyl chavicol), Anisaldehyde and some other compounds in a small amount [3]. Anise oil has been used in Iraqi folk medicine for the treatment of some diseases, including seizures and epilepsy [1, 4, 5]. Generally, the phytotherapeutic applications of the plant as well as the essential oil include carminative, mild expectorant, diuretic, antiseptic and antispasmodic effects [6, 7]. In addition to its medicinal value, its fruits and oil have been used in food industry, such as cookie, candy, toothpaste, liquor and in some alcoholic drinks for flavorings. Also it is added in American tobacco products because of its aromatic characteristics [8, 9].

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 [10].

Interest in the effects of UV on plants has increased considerably in recent years as evidence for ozone depletion continues to accumulate [11]. UV-A (320-400 *nm*) is present in significant amounts in natural daylight and is relatively little affected by changes in the ozone layer. 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 [12, 13]. The main objective of the present study was to apply the technique of UV-light at different wavelengths, distances and time of exposure as pre-sowing treatments of wild collected seeds and determination of the effects on chemical components of Anise (*Pimpinella anisum* L.).

MATERIALS AND METHODES

This experiment was carried out at the research station field of Faculty of Agricultural Sciences / Sulaimani University in Bakrajo during the season (October 2011- June 2012). The seeds of *Pimpinella anisum* L. were (local variety) standard seeds 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.

Studied characters

Chemical components

Protein %: Protein content was determined in seeds by using Kjeldahl method [14], and for calculation, the following equations were used:

T. N% = (V×N×E×0.014 /S) ×100

Protein % = T.N % × 6.2

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

S= weight of sample (g)

Fixed oil %: The ratio (%) of fixed oil was determined by using the Soxhlet apparatus, the method used by [14], then fixed oil content calculated as follows [15]:

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

Essential oil %

Water steam distillation

Essential oil was quantified by using a Clevenger apparatus, according to European Pharmacopoeia (2000). 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. [16] 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}

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.) [17].

Calculation

Concentration of sample µm/ml = (Area of sample/ Area of standard) × Concentration of standard × dilution factor [18].

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.

RESULTS AND DISCUSSION

Effect of exposure time duration to UV light

Table (1) revealed that exposing seeds to UV for 40 min. T_2 gave highest value of fixed oil content with (8.95 %) and essential oil yield (24.77 kg ha^{-1}) followed by T_3 (7.75 % and 17.64 kg ha^{-1}) respectively. These results were in agreement with those of [19] who reported that irradiated seeds with UV for 45 min. enhanced accumulation of oil in *Helianthus annuus* L. comparing to other exposure times (0, 15, 30 min.). By increasing exposing time to UV- ray, protein and essential oil were decreasing but meanwhile all treatments showed superiority on control treatment. $T_1$was the best treatment to produce high content of protein and essential oil (25.1 % and 3.16 %) compared to control (20.43 % and 1.6 %) respectively, where their contents were increased by (22.86 % and 97.5 %) compared to control, this increment of the amount of the essential oil in seeds may be due to the UV-rays which may cause genetic variations that reflected on the metabolism of plants [20], similar results of positive effect of UV- ray on essential oil concentration in anise were found by [21], in Parsley [22] and in Lavender [23].

However comparing to UV-treated seeds, protein content was significantly increased by first dose (20 min.) of UV-ray, while essential oil was significantly increased by both T_1 and T_2 and decreased by irradiating seeds for 60 min., and fixed oil significantly increased by all doses of exposure time. Concerning protein content, our results were close to those of [24] who reported that increasing exposure times to UV- ray decreased protein content in both white beet and red cabbage plants, while it had contrary effects on kidney bean.

Table 1: Effect of anise seed exposure to different times of UV- ray on fixed oil content (FO %), protein content (P %), essential oil content (EO %) and essential oil yield (EOY).

Exposure time / Character	T 1 (20 min.)	T 2 (40 min.)	T 3 (60 min.)	LSD		Control
				0.05	0.01	
FO (%)	7.25	8.95	7.75	0.69	0.96	5
P (%)	25.1	22.5	22.42	2.15	2.97	20.43
EO (%)	3.16	2.73	2.04	0.061	0.084	1.6
EOY(kg ha^{-1})	13.04	24.77	17.64	2.79	3.85	10.63

Interaction of UV $_{WL}$, distance and exposure time

Table (2) verified a highly significant effect of the interaction UV-A×D_2×T_2 (12%) where their content was increased by (140 %) compared to control, followed by UV-A ×D_1×T_2 (9.3 %), while the lowest fixed oil content(6 %) found in treatment UV-C×D_1×T_1, but generally the results showed that treating seeds with UV at both distances and for all three time exposures increased the content of fixed oil comparing to control. Data in table (2) also showed that the irradiated seeds with UV-C×D_2×T_1 treatment produced plant seeds with the highest content of essential oil (3.69 %) more than control (1.6 %) by (130.62 %), followed by UV-A ×D_2×T_2 with (3.66 %), while the content of essential oil was negatively affected by treatment UV-C×D_1×T_3 where their concentration was decreased by (20.3 %) compared to control. The maximum yield of essential oil was found when seeds exposed to UV-C×D_2×T_2, four times higher than control treatment (42.40 kg ha^{-1} and 10.63 kg ha^{-1}) respectively, followed by UV-A×D_1×T_3 with (31.8 kg ha^{-1}) but the lowest yield of essential oil was found when seeds exposed to UV-A×D_2×T_3 (6.09 kg ha^{-1}). Generally yields of essential oil from anise have been reported in the range of (10 to 24 kg ha^{-1}) [25, 26], while [27] reported the range between (1.6 to 41 kg ha^{-1}), while essential oil yield in the present study ranged from (6.09 to 42.40 kg ha^{-1}). In fact, the essential oil yield was directly associated with seed yield and essential oil content. Variation in these components directly affected essential oil yield [25, 28].

Essential oil composition

Effect of UV wavelength

Table (3) indicated that UV-C increased concentrations of each anisaldehyde total, estragole, *trans*-anethole and anisic acid (1.177, 1.148, 31.897 and 1.728 mg ml^{-1}) respectively, while UV-A significantly increased the concentration of limonene and linalool (1.863 and 2.124mg ml^{-1}) respectively. Dianethole was not significantly affected by changing in UV wavelength. UV-C wavelength increased the concentrations of both compound s*trans*-anethole and estragole compared to UV-A wave length by (95.53 % and 11.62 %) respectively. The results showed that, UV-C increased the concentrations of compounds synthesized by shikimic acid pathway, while the concentrations of both limonene and linalool synthesized by acetate-mevalonic acid pathway [29], were decreased after explosion of seeds to UV-C, this explained that UV-C has positive effect on shikimic acid pathway and negative effect on acetate-mevalonic acid pathway.

Table 2: Interaction effect of UV $_{WL}$, distance and exposure time on fixed oil content (FO %), protein content (P %), essential oil content (EO %) and essential oil yield (EOY) in anise seed.

Characters / UV_{WL}×D×T		FO (%)	P (%)	EO (%)	EOY(kgha^{-1})
UV-A×D_1×T_1		9	25.4	3.33	18.14
UV-A×D_1×T_2		9.3	19.68	1.66	11.77
UV-A× D_1×T_3		7	22.68	2.23	31.8
UV-A×D_2 ×T_1		7	26.62	2.4	8.38
UV-A×D_2×T_2		12	21.16	3.66	19.77
UV-A×D_2×T_3		7	25.1	1.6	6.09
UV-C×D_1×T_1		6	21.8	3.25	16.52
UV-C×D_1×T_2		6.5	25.18	3.63	25.14
UV-C×D_1×T_3		8.5	18.4	1.33	9.18
UV-C×D_2×T_1		7	26.54	3.69	9.14
UV-C×D_2×T_2		8	23.9	2	42.4
UV-C×D_2×T_3		8.5	25.5	3	23.47
LSD	**0.01**	1.92	NS	1.168	7.7
	0.05	1.39	NS	0.1122	5.59
Y UV×D ×T		7.98	23.49	2.64	18.48
Y Control		5	20.43	1.6	10.63
Tcal		2.972	1.806	2.006	2.61
$t_{0.05}$(13)		2.16			
$t_{0.01}$(13)		3.01			

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

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

Characters / UV Treatment		**A Ald** mg ml^{-1}	**ES** mg ml^{-1}	**TA**mg ml^{-1}	**DA**mg ml^{-1}	**A Acid** mg ml^{-1}	**Lim** mg ml^{-1}	**Lin** mg ml^{-1}
UV-A (365 nm)		0.968	1.001	15.017	6.609	1.192	1.863	2.124
UV-C (254 nm)		1.177	1.148	31.897	6.851	1.728	1.132	0.957
LSD	0.05	0.041	0.088	1.127	N.S	0.436	0.071	0.093
	0.01	0.095	N.S	2.599	N.S	N.S	0.165	0.215

AAld: Anis aldehyde total, **ES**: Estragole (Methylchavicol),**TA.** anethole, **DA**: Di-anethole, **AAcid**:Anisic acid, **Lim**:Limonene and **Lin**: Linalool.

Effect of exposing time duration to UV-ray

Table (4) showed that irradiated seeds with UVxT_1 gave the highest concentration of anisaldehyde total (1.245 mg ml^{-1}) where its concentration was increased by (67.34 %) compared to control, while UVxT_2 radiation had the lowest value with (0.879 mg ml^{-1}), but generally exposing seeds to UV for all exposure times significantly increased the concentration of anisaldehyde total compared to control treatment. The high concentration of estragole (1.155 mg ml^{-1}) found in 60 min. radiation which was higher by (41.2%) than control, followed by T_1 treatment. The major essential oil constituent in the seeds of anise is*trans*-anethole, it can be observed from results of (Table 4) that it was the most affected compound by UV-ray.

According to the present results, *trans*-anethole had a direct relationship with exposure times, i.e. by increasing exposing time to UV-ray, the concentration of this compound was increased such as in case of irradiating seeds for 60 min. gave the highest concentration with (25.766 mg ml^{-1}) compared to other treatments where *trans*-anethole concentration was three times higher than control (7.955 mg ml^{-1}). These findings were in line with [21] who demonstrated that irradiated seeds for 60 min. increased the concentration of *trans*-anetholeby three times more than non-irradiated seeds. The reason for this was that the radiation affects the components of the essential oil [30].

Table 4: Effect of seed exposure to different timesof UV-ray on chemical components of anise essential oil as identified by (HPLC).

Characters / Time (min.)		A Aldmg ml^{-1}	ES mg ml^{-1}	TAmg ml^{-1}	DAmg ml^{-1}	A Acid mg ml^{-1}	Lim mg ml^{-1}	Lin mg ml^{-1}
T 1 (20 min.)		1.245	1.069	21.358	7.924	2.615	1.905	2.652
T 2 (40 min.)		0.879	1	23.247	7.463	0.968	1.008	0.874
T 3 (60 min.)		1.093	1.155	25.766	4.803	0.798	1.58	1.097
LSD	**0.05**	0.024	0.089	0.916	0.786	0.266	0.096	0.094
	0.01	0.034	0.123	1.262	1.084	0.367	0.133	0.13
Y Control		0.744	0.818	7.955	4.38	2.769	3.689	2.11

AAld: Anis aldehyde total, **ES**: Estragole (Methylchavicol),**TA**: *Trans*-anethole, **DA**: Di-anethole, **AAcid**: Anisic acid, **Lim**: Limonene and **Lin**: Linalool.

Table 5: Interaction effect of UV $_{WL}$, distance and exposure timeon chemical components of anise essential oil as identified by (HPLC).

Characters / UV_{WL}×D×T		A Aldmg ml^{-1}	ES mg ml^{-1}	TAmg ml^{-1}	DAmg ml^{-1}	A Acid mg ml^{-1}	Lim mg ml^{-1}	Lin mg ml^{-1}
UV-A × D_1×T_1		1.115	1.121	8.668	4.121	1.754	1.813	4.044
UV-A ×D_1×T_2		0.802	0.487	13.205	3.1	0.599	0.908	0.554
UV-A ×D_1×T_3		1.56	1.069	16.288	2.583	0.471	1.896	1.295
UV-A ×D_2 ×T_1		0.785	1.218	18.719	12.924	1.747	3.182	4.314
UV-A ×D_2×T_2		0.886	1.644	16.419	15.03	1.214	1.334	1.305
UV-A ×D_2×T_3		0.657	0.467	16.803	1.895	1.369	2.046	1.233
UV-C ×D_1×T_1		2.131	1.03	24.436	7.252	1.897	1.04	1.627
UV-C ×D_1×T_2		0	0	40.076	2.141	0.84	0.795	0.883
UV-C ×D_1×T_3		1.826	1.604	26.151	5.808	0.744	1.273	0.777
UV-C ×D_2×T_1		0.948	0.908	33.61	7.4	5.064	1.583	0.621
UV-C ×D_2×T_2		1.83	1.87	23.287	9.58	1.217	0.993	0.754
UV-C ×D_2×T_3		0.327	1.478	43.824	8.927	0.607	1.106	1.081
LSD	**0.01**	0.068	0.246	2.525	2.168	0.735	0.266	0.26
	0.05	0.049	0.179	1.833	1.573	0.533	0.193	0.189
Y UV×D ×T		1.072	1.074	23.457	6.73	1.46	1.497	1.54
Y Control		0.744	0.818	7.955	4.38	2.769	3.689	2.11
t cal		0.853	0.781	2.405	0.915	-1.685	-5.496	-0.752
$t_{0.05}$(13)		**2.16**						
$t_{0.01}$(13)		**3.01**						

UV-A: 365 nm, **UV-C**: 254 nm, **D_1**: 12 cm, **D_2**: 6 cm, **T_1**: 20 min, **T_2**: 40 min, **T_3**: 60 min.
AAld: Anis aldehyde total, **ES**: Estragole (Methylchavicol),**TA**: *Trans*-anethole, **DA**: Di-anethole, **AAcid**:Anisic acid, **Lim**: Limonene and **Lin**: Linalool

Interaction of UV_{WL}, distance and exposure time

Table (5) revealed that anisaldehyde total was highly significantly affected by interaction between UV-C×D_1×T_1 (2.131 mg ml^{-1}), significantly superior over control treatment by (186.43 %) but the lowest concentration of this compound was found in interaction between UV-C ×D_2×T_3 with (0.327 mg ml^{-1}). The Interaction between UV-C×D_2×T_2 significantly increased the concentration of estragole (1.87 mg ml^{-1}) compared to control (0.818 mg ml^{-1}) by (128.6 %), followed by UV-A×D_2×T_2 with (1.644 mg ml^{-1}). Moreover, compounds, anisaldehyde total and estragole were not identified by HPLC after exposing seeds to UV-C ×D_1×T_2 treatment.

Exposed seeds to UV-C×D2×T3 stimulated plants to produce seeds with high concentration of *trans*-anethole (43.824 mg ml^{-1}) where its concentration was higher than control (7.955 mg ml^{-1}) by (450.9 %), while irradiated seeds by UV-A× D_1×T_1 treatment gave the minimum concentration of this compound (8.668 mg ml^{-1}) compared to other interaction treatments. Interaction between UV-A×D_2×T_2 positively increased the concentration of dianethole (15.03 mg ml^{-1}) compared to control (4.38 mg ml^{-1}) followed by UV-A×D_2 ×T_1 with (12.924 mg/ml). Anisic acid was only significantly affected by treatment UV-C×D_2×T_1 (5.064 mg ml^{-1}) compared to control (2.769 mg ml^{-1}), while the effects of other treatments were not fallen in significant range. The concentration of limonene was not significantly changed after irradiation of seeds with different treatments compared to control, while UV-A ×D_2 ×T_1 is the best treatment for this compound comparing to other treatments with (3.182 mg ml^{-1}). The high concentration of linalool was found in interaction between UV-A ×D_2 ×T_1 with (4.314 mg ml^{-1}) followed by UV-A× D_1×T_1 (4.044 mg ml^{-1}), while other treatments negatively decreased the concentration of Linalool.

Correlation coefficients among characters

Table (6) showed the presence of positive significant correlations of essential oil with fixed oil, protein, *trans*-anethole and dianethole content; these results were in agreement with [16]. While essential oil was negatively correlated with limonene but it had positive and significant correlation with estragole and *trans*-anethole contents. Positive correlation was found between essential oil yield and *trans*- anethole in anise plant found by [16]. However essential oil yield had negative significant correlations with anisic acid and Limonene.

According to the present results, no significant correlation was existed between protein and fixed oil. These results were in agreement with [31], but were contrary to the work reported by [32]. *Trans*-anetholeas a main component of essential oil was negatively and significantly correlated with each of limonene and linalool (-0.668** and -0.468**) respectively, this explained that the*trans*-anetholein high concentration caused a decreases in concentrations of limonene and linalool, while in low concentration the reverse was true. Also positive and significant correlation was found between estragole with anisaldehyde total (0.556**) and with dianethole (0.716**), and also limonene had positive and significant correlation with anisic acid.

Table 6: Correlation coefficients among almost all studied characteristics.

Traits	FO	P	EO	EOY	A Ald.	ES.	TA	DA	A. Acid	Lim
FO										
P	-0.026									
EO	**0.361***	**0.477****								
EOY	0.267	0.14	0.267							
A Ald.	0.108	-0.243	-0.107	0.268						
ES.	**0.428****	-0.03	0.037	**0.366***	**0.55****					
TA	0.206	**0.407****	**0.540****	**0.310***	-0.11	0.08				
DA	**0.453****	0.155	**0.370***	0.147	0.16	**0.71****	0.206			
A Acid	**-0.375***	0.038	0.065	**-0.405****	-0.05	-0.104	-0.163	0.06		
Lim	**-0.610****	-0.197	**-0.515****	**-0.469****	-0.24	-0.15	**-0.66****	-0.11	**0.488****	
Lin	-0.169	0.196	0.01	-0.259	-0.08	0.05	**-0.46****	0.17	0.164	**0.56****

Tab (p) % 1 = 0.402 ** Tab (p) % 5 = 0.312 *

FO %:Fixed oil content, **P %**: protein content , **EO %**: essential oilcontent,**EOY**: essential oil yield, **A Ald**: Anis aldehyde total, **ES**: Estragole (Methylchavicol),**TA**:*Trans*-anethole, **DA**: Di-anethole, **AAcid**:Anisic acid, **Lim**:Limonene and **Lin**: Linalool.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. Pre-Sowing seed treatments with different UV wavelength especially UV-C had a positive effect on the chemical components and the diversity of essential oil of (*Pimpinella anisum* L.).

2. The short distance treatment between UV-ray sources and exposed seeds had greater effect plant chemical components.
3. Plant seeds required optimal dose of exposure time to UV-ray, below this limit, it may not be sufficient to affect the plant and above this limit may have inhibiting effect on plant growth and development.
4. The present findings indicated that if the purpose was essential oil, irradiating seeds with UV-C×D_2×T_1 was more effective while for high concentration of *trans*-anethole, seeds were radiated with **UV-C×D_2×T_3** was more effective.

Recommendations

Investigating the effect of different UV- ray treatments on other medicinal plants.

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