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Effect of Ultraviolet on Morphological and Secondary Metabolites Content of Garden Cress

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ABSTRACT

Very little research covered the effect of UVC on morphological structure and properties of garden cress (Lepidium sativum Linn.). For this reason, the aim of this work is to study the effect of ultraviolet- C from UV lamp (UVC ~ 2000- 2800 Å) on growth stages (Leg and leaf growth), color, internal structure and secondary metabolites content of Lepidium sativum Linn. The results show that, garden cress plant may die and the color become yellow. Structure (future of formed peaks) and molecular structure (peak position and peak intensity) of garden grass changed after exposure to UVC for different time and distance. The values of total phenol**,** total Flavonoids, Vitamin E and Vitamin c in extracted garden cress decreased after exposure to UVC for different time and distance. Scavenging activity % (DPPH) and ABTS radical scavenging activity in extracted garden cress increased after exposure to UVC for different time and distance. The medicinal plant (Lepidium sativum Linn.) should protect from UVC radiation for best uses.

Keywords: Garden Cress, UVC, Internal Structure, Absorption, IR, X-ray

I. INTRODUCTION

The sun emits energy over a broad spectrum of wavelengths: visible light that you see, infrared radiation that you feel as heat, and ultraviolet (UV) radiation that you can't see or feel. UV radiation has a shorter wavelength and higher energy than visible light. UV radiation has a shorter wavelength and higher energy than visible light. Opportunely for life on earth, our atmosphere's stratospheric ozone layer shields us from most UV radiation. Garden cress has considered as an important medicinal plant since the Vedic era. It seeds are used as a medicine in Ayurvedic System of Medicine. Moreover, because the UV penetration through leaf tissues increases as wavelength increases, UV-A can reach much deeper target sites in the leaves than UV-B [1]. Indeed, although UV-A is less efficient than UV-B in mediating some biological responses such as DNA damage, the high UV-A levels reaching the deeper tissues can compensate for the lower reactivity [2]. When the germinating green bean seeds were exposed to UV C (254 nm) for different time intervals ranging from 15-60 min, and then subjected to salinity stress, the seedlings was less affected by salinity stress as compared to non-treated ones [3]. The groundnut seeds exposed to UVC for period varying from 5-60 min, the seedling vigour and flower production increased as compared to controls. Similarly, the pod biomass also increased in all UV C treatments [4]. Though changes in the UVA: photosynthetically active radiation (PAR; 400-700 nm) ratio are less marked than those in the UVB: PAR ratio, they are an important consideration in experimental design [5- 9]. The aim of this research is to study the effect of UVC on morphology, internal structure and antioxidant value of Lepidium sativum that affected in its medical applications.

II. METHODS AND MATERIAL

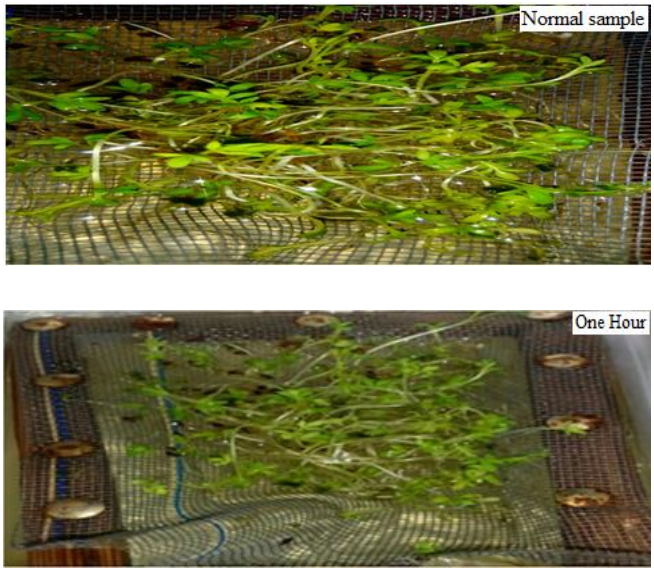
The experimental plant involved in the present investigation is garden cress (Lepidium sativum Linn.). Pure seeds received from Egyptian Ministry of Agriculture. The ultra-violet irradiation system used in this study consists of one fluorescent lamps (type-C ranged from 200-280 nm). The power of the lamp equal 15 watt, and the system covered totally with reflected surfaces (aluminium foil) to illuminate the sample from all sides. The aqueous extract was prepared from dried powder of garden cress seeds. Five grams of powder

was soaked in 100 ml of distilled H_2O for 24 hours at room temperature and filtered through filter paper to obtain 5% w/v. Then, the filtrate centrifuged at 3500 rpm for 25 min and the resultant supernatant used for chemical tests. Chemical investigations performed on the powdered materials of plants and on the aqueous extract using standard methods to know the various chemical components. Microstructure of garden cress is performed using Shimadzu X–ray Diffractometer, (Dx–30, Japan) Cu–Kα radiation with λ=1.54056 Å at 45 kV and 35 mA and Ni–filter, in the angular range 2θ ranging from 0 to 100° in continuous mode with a scan speed 5 deg/min. Molecular structure of garden cress is studied by Nicolet™ iS™ 10 FT-IR Spectrometer from USA. Absorption of extracted garden cress plant measured by UV- 2100 Spectrophotometer.

III. RESULTS AND DISCUSSION

A. Growth behavior

The growth behavior (Leg and leaf growth) of normal garden cress and after exposure to UVC radiation for 1, 2, 3 and 4 hours is shown in Figure 1. The garden cress plant behavior and color changed or died after irradiated by UVC radiation. That's meant the internal structure (microstructure and molecular structure) of the plant changed after exposure to UVC radiation for different time.



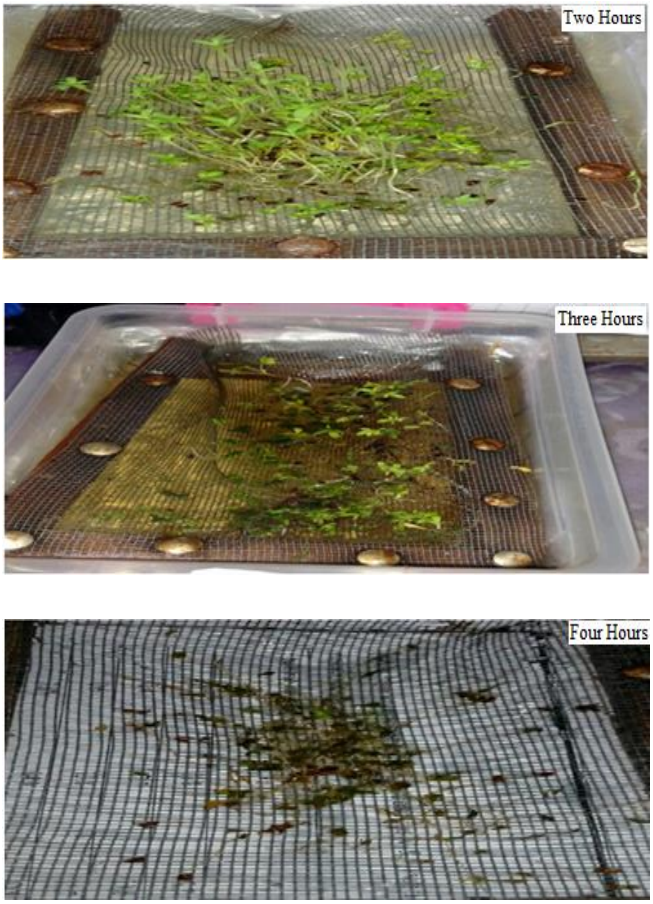
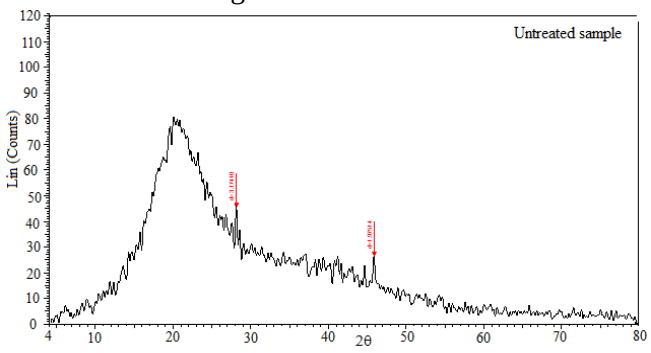


Figure 1. growth behavior of normal garden cress and after exposure to UVC for different time

B. Structure (X-ray analysis)

Figure 2 shows x-ray diffraction patterns of normal garden cress and after exposure to UVC for four hours at different distance (5, 10, 15, 20 and 25 cm). The analysis (formed peaks, peak position, area, full width half maximum and intensity) of x-ray whish listed in Table 1 show that, the internal structure (cluster formed from chains and chain size) of garden cress changed after exposure to UVC which is agreed with pervious results show in in Figure 1.



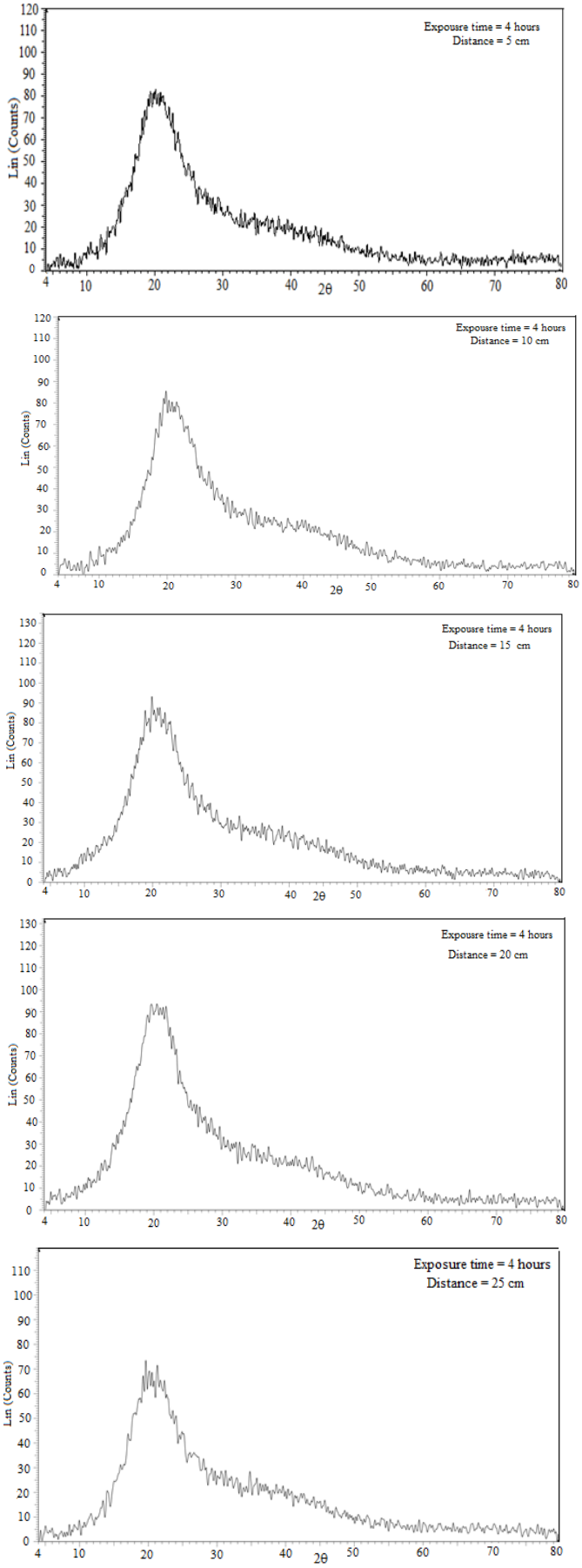


Figure 2. x- ray diffraction patterns of normal garden cress and after exposure to UVC

Table 1. x- ray diffraction analysis of normal garden cress and after exposure to UVC

Normal sample (Irradiated sample)				
2θ	d Å	Int. Count	Area	FWHM
20.459	4.33758	80.9	1054.1	9.141
28.09	3.1741	45.4		
45.909	1.97514	26.4		

Distance = 5 cm		**Exposure time = 4 hours**		
2θ	d Å	Int. Count	Area	FWHM
20.459	4.33758	83.1	827.417	8.25172

Distance = 10 cm		**Exposure time = 4 hours**		
2θ	d Å	Int. Count	Area	FWHM
20.77	4.27329	83.1	789.72	8.66

Distance = 15 cm		**Exposure time = 4 hours**		
2θ	d Å	Int. Count	Area	FWHM
20.459	4.33758	94.2	1262	8.81

Distance = 20 cm		**Exposure time = 4 hours**		
2θ	d Å	Int. Count	Area	FWHM
20.614	4.30519	96	1108	8.49

Distance = 25 cm		**Exposure time = 4 hours**		
2θ	d Å	Int. Count	Area	FWHM
20.77	4.27329	73.1	623	8.36

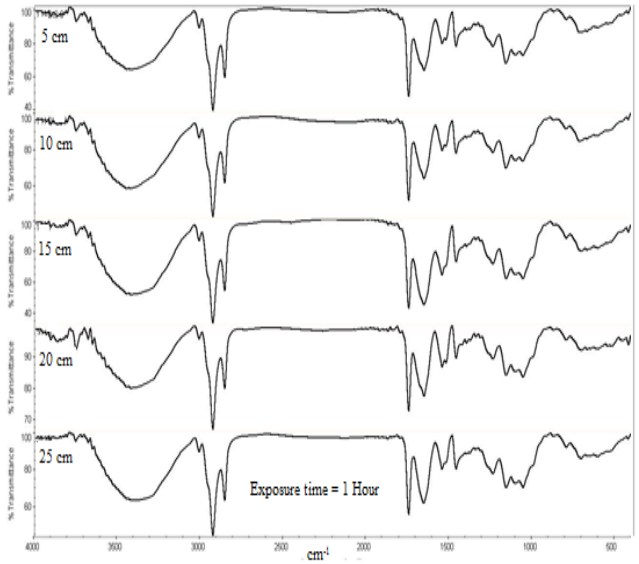
C. Molecular structure (IR analysis)

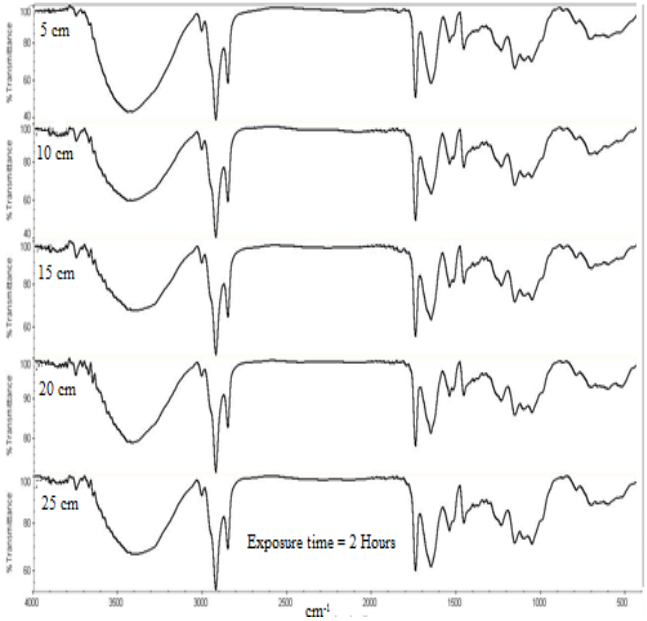
Infrared spectroscopy is widely used in research and industry as simple and reliable technique for measurement, quality control and dynamic measurement. Infrared radiations are a part of electromagnetic spectrum between the visible and microwave regions. They absorbed by organic molecules and converted into energy of molecular vibration. Different types of bonds and thus different functional groups absorb infrared radiation of different wavelengths. Radiation in this region (4000- 400 cm^{-1}) can be utilized in organic structure determination by making use of the fact that it is absorbed by interatomic bond in organic compounds. Chemical bonds in different environments will absorb varying intensities and at varying frequencies. The frequencies at which their absorptions of IR radiation (peaks or signals) can correlated to bonds within compound.

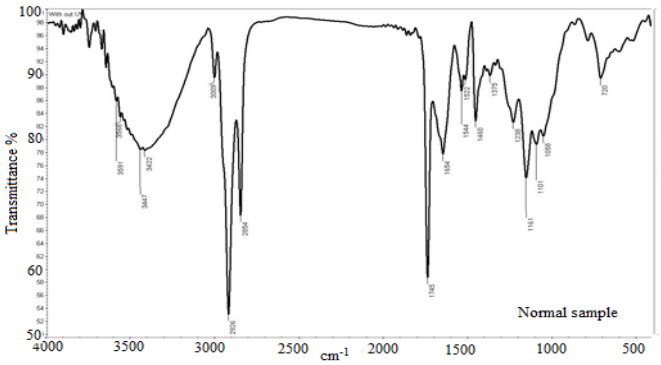
IR spectrum of garden cress plant is shown in Figure 3 which a plot of wave number (X- axis) vs. present

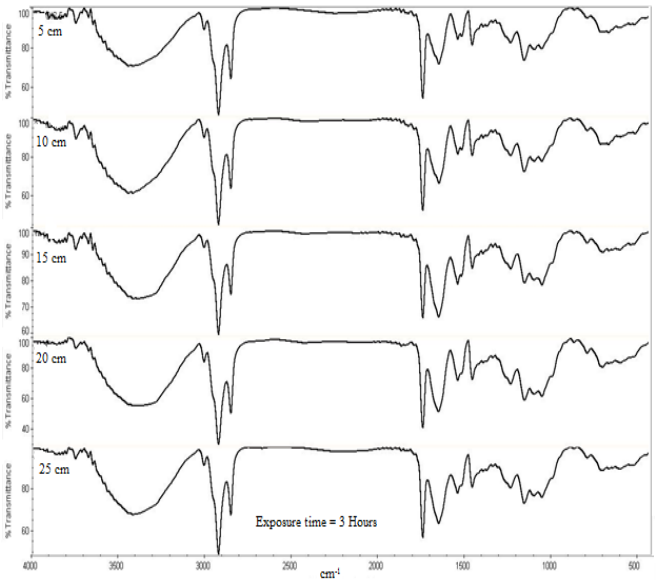
transmittance (Y- axis). The analysis of IR spectrum listed in Table 2 shows that, transmittance Int. % at position 2926 -2925 cm^{-1} and the main peak at position ~ 3421 decreased (varied) after exposure to UVC radiation for one, two, three and four hours at distance 5, 10and 25 cm from UV source. Transmittance Int. % at position 2926 cm^{-1} decreased (varied) after exposure for one and two hours then increased after exposure for three and four hours to UVC at 15 cm from the source. But the main peak decreased (varied) and transmittance Int. % decreased (varied) after exposure for one, two and three hours then increased after exposure for four hours to UV. Transmittance Int. % at position 2926 cm^{-1} increased after exposure for one and two hours then decreased after exposure for three and four hours to UVC at 20 cm from source. But the main peak varied and transmittance Int. % increased (varied) after exposure for one and two hours then decreased (varied) after exposure for three and four hours to UVC.

From IR results (graphs and analysis) molecular structure (C-H and O- H bonded usually broad and strong) changed after exposure to UVC for different time (1, 2, 3 and 4 hours) and dissimilar distance (5, 10, 15, 20 and 25 cm) from the source. Because each interatomic bond may vibrate in several different motions (stretching or bending), individual bonds may absorb at more than one IR frequency. Stretching absorptions usually produce stronger peaks than bending.









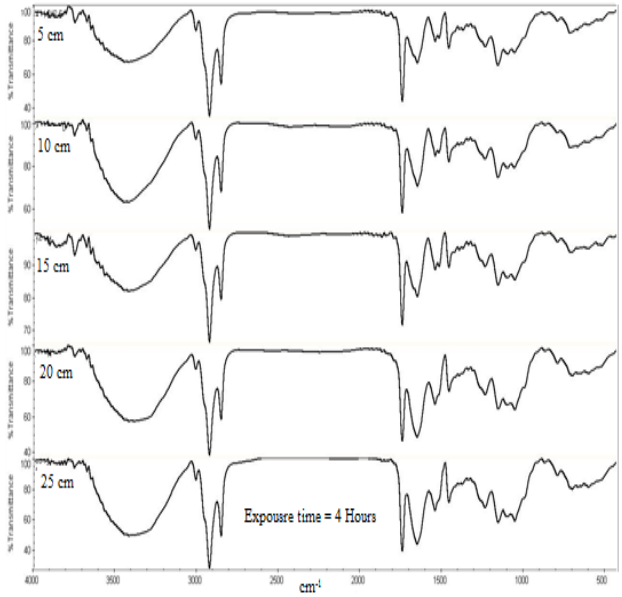


Figure 3. IR spectrum of normal garden cress and after exposure to UVC

Table 2. IR analysis of garden cress before and after exposure to UVC

Distance from UV source = 5 cm				
Exposure Time	Position cm^{-1}	Trans. Int. %	Position cm^{-1}	Trans. Int. %
Normal	2926	52.96	3422	78.24
1	2925	38.93	3421	63.27
2	2926	38.29	3446	42.38
3	2926	45.34	3421	69.93
4	2926	34.51	3421	66.4

Distance from UV source = 10 cm				
Exposure Time	Position cm^{-1}	Trans. Int. %	Position cm^{-1}	Trans. Int. %
Normal	2926	52.96	3422	78.24
1	2926	42.2	3422	57.58
2	2926	39.63	3421	58.58
3	2926	44.31	3421	60.52
4	2926	50.18	3423	62.6

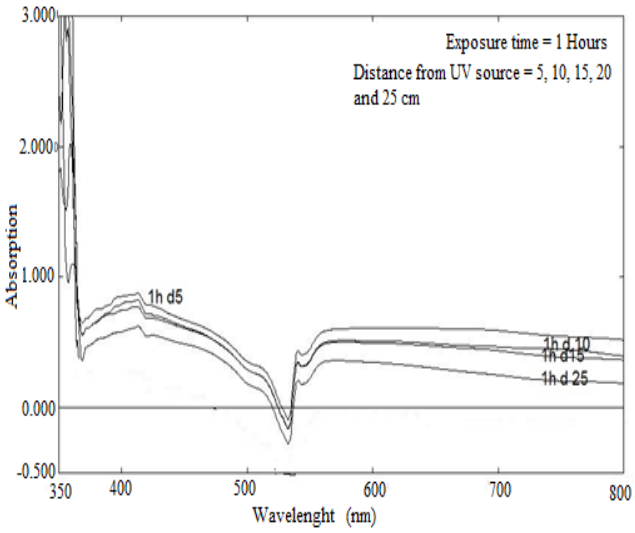
Distance from UV source = 15 cm				
Exposure Time	Position cm^{-1}	Trans. Int. %	Position cm^{-1}	Trans. Int. %
Normal	2926	52.96	3422	78.24
1	2926	33.08	3418	50.996
2	2926	46.52	3420	66.89
3	2926	59.03	3420	72.59
4	2926	66.11	3421	81.8

Distance from UV source = 20 cm				
Exposure Time	Position cm^{-1}	Trans. Int. %	Position cm^{-1}	Trans. Int. %
Normal	2926	52.96	3422	78.24
1	2926	66.5	3420	79.53
2	2926	71.25	3422	78.5
3	2926	29.1	3419	54.32
4	2926	37.85	3419	56.83

Distance from UV source = 25 cm				
Exposure Time	Position cm^{-1}	Trans. Int. %	Position cm^{-1}	Trans. Int. %
Normal	2926	52.96	3422	78.24
1	2926	43.7	3384	62.57
2	2926	51.11	3420	66.17
3	2926	48.36	3420	67.07
4	2926	27.32	3419	48.58

D. UV analysis

UV graphs, which is, draw between absorption and wavelength of garden cress plant shown in Figure 4. The results show that, absorption quantity increased with decreased the distance from UV source and increased exposure time.



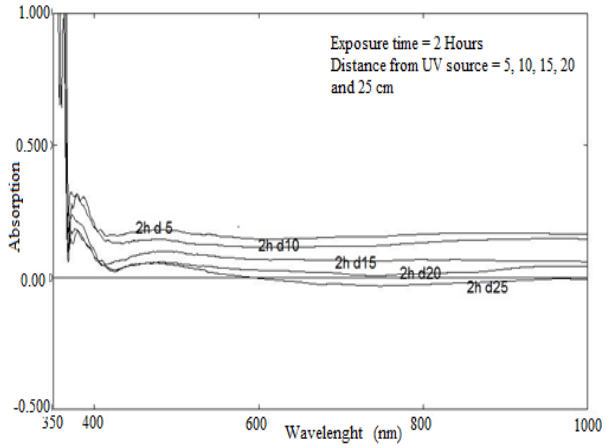


Figure 4. absorption versus wavelength at different exposure time and dissimilar distance from UV source

E. Preparation of Aqueous seed Extract from lepidium sativum.

Sample (10) g of the air–dried powder was mixed with 100 ml distilled water followed by shaking overnight at 4 ^{0}C. The mixture filtered through cheesecloth and then centrifuged for 30 min at 3000 g. The resulting supernatant considered as a stock solution of concentration as 10% (w\v). This concentration used in preparing the various tested concentration (2, 4, 6, 8 and 10 %) by subsequent dilutions with distilled water.

F. Estimation of total phenols

Total phenolic content of the seed extract was estimated using Folin-Ciocalteau assay procedure with some modifications according to Slinkard and Singleton [10]. Plant extract (100 µl) added to 300 µl Folin-Ciocalteau reagent and the mixture incubated for 10 min at room temperature. Sodium carbonate solution (800 µl) added, mixed and incubated for 3 hours at room temperature. The absorbance was measured spectrophotomtrically at 765 nm. The standard curve was prepared using gallic acid. The above steps repeated by using gallic acid instead of leaf extracts. The content of phenols in the seed extract measured based on the derived equation from the standard curve and expressed as gallic acid equivalent (GAE g/100 g dry weight). Total phenol value in extracted garden cress decreased after exposure to UVC for different time and distance as shown in Table 3.

Table 3. total phenol in garden cress before and after exposure to UVC at different distance and time

Time (h)	5 cm	10 cm	15 cm	20 cm	25 cm
0	18.2±0.4	18.2±0.4	18.2±0.4	18.2±0.4	18.2±0.4
1	17.6±0.5	16.3±0.4	15.5±0.5	11.4±0.4	9.5±0.4
2	16.3±0.4	14±0.5	11±0.3	8.8±0.2	6.3±0.3
3	15.4±0.3	11.7±0.3	8.4±0.4	5.2±0.3	4±0.2
4	14.3±0.3	10±0.2	6.9±0.2	3.4±0.2	2.2±0.1

G. Estimation of flavonoids

$Alcl_3$ colorimetric method adopted for flavonoid determination [11]. One mL of sample solution mixed with 4 mL of distilled water. Sodium nitrite300 µl added, and after 5 min, 300 µL aluminium chlorides added and allowed to stand for 5 min. Then, 2 mL of sodium hydroxide added and the mixture shaken for mixing well. The absorbance measured at 510 nm spectrophotometrically.

Flavonoids value of extracted garden cress decreased after exposure to UVC for different time and distance as presented in Table 4.

Vitamin E value in extracted garden cress decreased after exposure to UVC for different time and distance as listed Table 5.

Table 4. Flavonoids in garden cress before and after exposure to UVC at different distance and time

Time (h)	5 cm	10 cm	15 cm	20 cm	25 cm
0	14.3±0.5	14.3±0.5	14.3±0.5	14.3±0.5	14.3±0.5
1	11±0.3	9.4±0.2	8±0.3	6.9±0.3	5±0.2
2	9.6±0.3	7.2±0.3	6.3±0.3	4±0.1	2.8±0.08
3	7±0.2	5±0.3	3.4±0.2	2±0.05	1.9±0.02
4	6±0.2	4.2±0.2	2.8±0.1	1.3±0.05	1.1±0.05

Table 5. Vitamin E in garden cress before and after exposure to UVC at different distance and time

Time (h)	5 cm	10 cm	15 cm	20 cm	25 cm
0	82.3±1.3	82.3±1.3	82.3±1.3	82.3±1.3	82.3±1.3
1	79.6±1.5	78.4±1.2	70±1	67.4±0.8	60.8±0.5
2	75.4±1.6	70.3±1.2	64.3±0.6	55.3±0.7	41±0.5
3	70.3±1	63.2±0.8	47.7±0.4	30.3±0.6	26.4±0.6
4	59.4±1	45.4±0.4	30.2±0.6	20±0.5	12±0.4

H. Non-enzymic antioxidants assay Ascorbate content

Total ascorbate (vitamin c) estimated according to Cakmak and Marschner with some modifications. A sample of 0.5 g of leaves extracted with 5 ml of 5% meta-phosphoric acid and centrifuged at 4000 rpm for 20 min. Total ascorbate (AsA + DHAsA) measured after reduction of DHAsA to AsA with DTT (1.4 dithiothreitol). The reaction mixture contained 0.2 ml of the supernatant, 0.5 ml of 150 mM phosphate buffer (PH 7.4) containing 5 mM EDTA, 0.1 ml of 10 mM DTT and 0.1 ml of 0.5% (w/v) N-ethylmaleimide (NEM) to remove excess DTT.

In the reaction mixture, the color was developed after addition of the following reagents: 0.4 ml of 10% trichloro acetic acid (TCA), 0.4 ml of 44% ortho-phosphoric acid, 0.4 ml of 4% (2, 2-bipyridine) in 70% ethyl alcohol, and 0.2 ml of 3% Fecl3. The mixture incubated at 40 °C for 30 min and the color produced was recorded at 525 nm.

The effect of UVC on Vitamin c in extracted garden cress for different time at dissimilar distance shown in Table 6. Vitamin c in extracted garden cress value decreased after exposure to UVC for different time and distance.

Table 6: Vitamin c in garden cress before and after exposure to UVC at different distance and time

Time (h)	5 cm	10 cm	15 cm	20 cm	25 cm
0	79.4±0.9	79.4±0.9	79.4±0.9	79.4±0.9	79.4±0.9
1	77.3±1.8	76.2±1.1	64.7±0.9	55.4±0.8	42±0.6
2	72±1.2	62±0.5	54±0.6	48.2±0.5	31.3±0.6
3	51±0.8	45.6±0.7	39.9±0.5	25±0.5	16.4±0.4
4	40.3±0.5	37±0.5	28.2±0.4	18.3±0.4	9.7±0.3

I. Determination of the antioxidant activity by DPPH

The diluted working solution of the test extracts were prepared in methanol. Ascorbic acid used as standard in 1-100 µg/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution mixed with 1 ml of sample solution and standard solution separately. These solution mixtures kept in dark for 30 min and optical density measured at 517 nm using Cecil-Elect spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density recorded and % inhibition calculated using the formula given below:

(%) inhibition of DPPH activity = $(A_{blank} - B_{sample} \backslash A_{blank}) \times 100$

Where A =optical density of the blank and B =optical density of the sample.

The effect of UVC on scavenging activity % in extracted garden cress for different time at dissimilar distance is listed Table 7. Scavenging activity % (DPPH) in extracted garden cress value increased after exposure to UVC for different time and distance.

Table 7: scavenging activity (DPPH) in garden cress before and after exposure to UVC at different distance and time

Exposure time (h)	5 cm	10 cm	15 cm	20 cm	25 cm
0	21.3±0.4	21.3±0.4	21.3±0.4	21.3±0.4	21.3±0.4
1	28±0.5	44±0.6	49.2±0.8	58.4±0.7	64.5±1.1
2	31.2±0.6	48.2±0.5	54±0.6	62±0.5	72±1.2
3	34.6±0.7	53.6±0.7	59.3±0.5	69±0.8	79.4±1.4
4	42±0.6	55.4±0.8	61±0.8	72.2±1.1	84.3±1.8

J. Determination of ABTS radical scavenging activity

The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution prepared by mixing the two stock solutions in equal quantities and allowing them to react for 14 h at room temperature in the dark. Mix 1ml working solution to one ml of fucoidan solution (10% w\v) and the absorbance tacked at 734 nm after 7 min using a spectrophotometer.

ABTS radical scavenging activity (%) =

$Abs_{control} - Abs_{sample} \backslash Abs_{control}$

Where: $Abs_{control}$ is the absorbance of ATBS solution radical in methanol; Abs_{sample} is the absorbance of ABTS solution mixed with the sample.

ABTS radical scavenging activity in garden cress increased after exposure to UVC at different distance and time as shown in Table 8.

Table 8. ABTS radical scavenging activity in garden cress before and after exposure to UVC at different distance and time

Exposure time (h)	5 cm	10 cm	15 cm	20 cm	25 cm
0	15.2 ± 0.3	15.2 ± 0.3	15.2 ± 0.3	15.2 ± 0.3	15.2 ± 0.3
1	18.4 ± 0.4	25.3 ± 0.5	44 ± 0.7	54.4 ± 0.9	68.3 ± 0.9
2	21.2 ± 0.5	28.4 ± 0.5	49.5± 0.5	60 ± 1	77.2 ± 1.2
3	28,3 ± 0.5	33.4 ± 0.6	53.2 ± 0.6	66 ± 0.6	82.3 ± 1.3
4	36.4 ± 0.7	39.2 ± 0.7	58.4 ± 0.8	75.2 ± 0.8	89.4 ± 1.6

IV. CONCLUSIONS

The behavior growth and internal structural (Structure and molecular structure) of garden cress plant changed after exposure to UVC for different time. Total phenol**,** total Flavonoids, Vitamin E and Vitamin c in extracted garden cress decreased after exposure to UVC for different time and distance. Scavenging activity percentage (DPPH) and ABTS radical scavenging activity in extracted garden cress increased after exposure to UVC for different time and distance.

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