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Effect of non-ionizing radiation on structure and medical content for plant



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Abstract

Plants are living chemical factories for enormous array of the secondary metabolites. Due to toxic and carcinogenic effects of the synthetic antioxidants, a great deal of attention has been focused in recent decades on natural antioxidants such as polyphenols derived from various natural sources. The aim of research is to study the effect of UVA on structure and medical content of yellow mustard. The results show non-enzymatic such as glutathione and phenolic contents in yellow mustard improved after exposure to UVA. Total proline, tocopherol total flavonoids in yellow mustard varied after exposure to UVA. Also internal structure as hydroxyl group of yellow mustard changed after exposure to UVA.

Key words: phenolic, glutathione, DPPH scavenging activity, proline, yellow mustard, UVA

2. Introduction

Solar radiation is a complex mixture of ultraviolet, visible light and infrared wavelengths. UVA radiation (315- 400 nm) is a component of solar radiation. Plants are living chemical factories for the biosynthesis of a huge array of the secondary metabolites. Plants are used medicinally in different countries and are a source of many powerful drugs. The World Health Organization stated that about 80% of the world's population depends mainly on traditional medicine that mainly includes the use of plant extracts. UV light is an important abiotic elicitor, and had use in phytochemical production in a variety of plant cultures in the past [1]. Exposure to UV light stress causes stimulation of defense mechanisms in plants, thus, producing commercially important secondary compounds [2]. Some reports discussed the effect of low levels of UV radiation on plant growth [3, 4]. There are many beneficial uses of radiation that offer few risks when properly employed. The exposure to radiations can have stimulatory effects on specific morphological parameters. The mustard plant belongs to the Cruciferae (Brassicaceae) family, used in medicine is external as a liniment and relieving pain from bruises or a stiff neck and relieving colic and respiratory problems. Growth behavior, secondary metabolites and vitamins of Nigella Sativa

and *garden cress* changed after exposure by UVC [5, 6]. UVs have adequate energy to break the chemical bonds causing photochemical reactions and inducing changes in plant metabolic enzyme, subsequently trigger the production of secondary metabolites [7-9]. Effect of UV is varied with duration and irradiation intensity. The objective of this work is to assess the effect of UVA radiation on structure, non-enzymatic and enzymatic antioxidants for yellow mustard.

3. Experimental methods

3.1 Structure measurements

Internal structure and molecular structure of yellow mustard are studied by Shimadzu X-ray diffractometer, (Dx-30, Japan), scanning electron microscope (JEOL JSM-6510LV, Japan) and NicoletTM iSTM 10 FT-IR Spectrometer from USA.

3.2 GSH determination: GSH is determined using UV/V spectrophotometer, Jenway, England.

3.3 DPPH determination: Concentration ranging from 0.4g/100g to 2g/100g are prepared with methanol from each sample (100 μ l) extract and DPPH radical (100 μ l, 2Mm) dissolved in methanol. The mixture is stirred and left to stand for 15 min in dark. Then the absorbance is measured at 517 nm against a blank. Percentage scavenging effect is calculated as [(A_o-

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 A_1 / A_0]×100 where A_0 is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample.

3.4 Total phenols determination: Total phenols are determined colorimetric by Folin-Ciocalteu reagent total phenolic content is calculated from the regression equation of the standard $plot(y=3.005\times-993.56, r^2=0.9974)$ and are expressed as mg Gallic acid equivalent/100g sample.

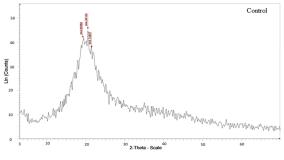
3.5 Flavonoid determination: Aluminum chloride colorimetric method is used to determine flavonoid content. 1 ml of sample extract is mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride. 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 minutes. The absorbance is measured at 420 nm. Rutin is used as standard (1mg/ml). Flavonoid content is calculated from the regression equation of the standard plot (y=16.122×-340.23, r²=0.9777) and are expressed as (mg Rutin equivalent/100g sample).

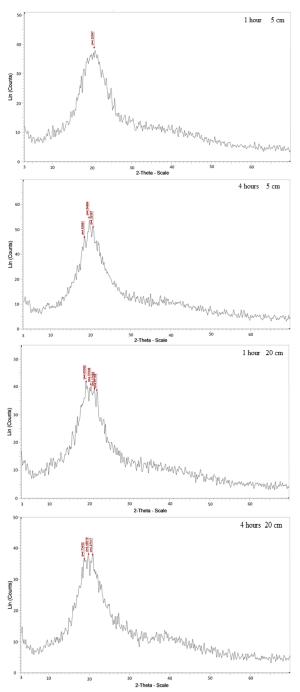
3.6 Proline determination: The proline concentration is determined after extraction with 3% (W/V) aqueous sulfosalicylic acid from a standard curve using D-Proline (y=36.738x+1.2739, r2=0.9777) which give by used UV/V spectrophotometer, Jenway, England.

4. Results and discussions

4.1 Internal structure

Figure 1 shows x-ray diffraction patterns of normal yellow mustard after exposure to UVA for different period times at dissimilar distances. There is a change in the main peak of yellow mustard as listed in Table 1 after exposed to UVA at 5 and 20 cm far the source for 1 and 4 hours. That is because the interaction of UVA with atoms or molecules in the cell, break or modify bonds or rearrangement it or produce free radicals [10]. These change can damage or modify important components for plant cells effected differentially the morphology, anatomy, biochemistry and physiology of plants [11]. Scanning electron micrographs show different features and qualities of the main cell and molecules a round it as show in Figure 2. It agreed and confirmed the x-ray and IR results.





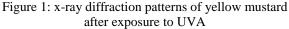


Table 1: x-ray analysis of yellow mustar	rd before and after exposed
to 1137 A	

	u.	5011		
Exposure time (hour)	Area under	the peak	Width of	the peak
Zero (Control)	4.6	1		
	5 cm	20 cm	5 cm	20 cm
1	6.14	4.524	7.3240309	6.758
4	4.363	5.21	6.2214994	6.423
	(hour)	Exposure time (hour)Area under (hour)Zero (Control)4.65 cm5 cm16.14	(hour) 4.61 Zero (Control) 5 cm 20 cm 1 6.14 4.524	Exposure time (hour)Area under the peakWidth of Width of 4.61Zero (Control)4.615 cm20 cm16.144.5247.3240309

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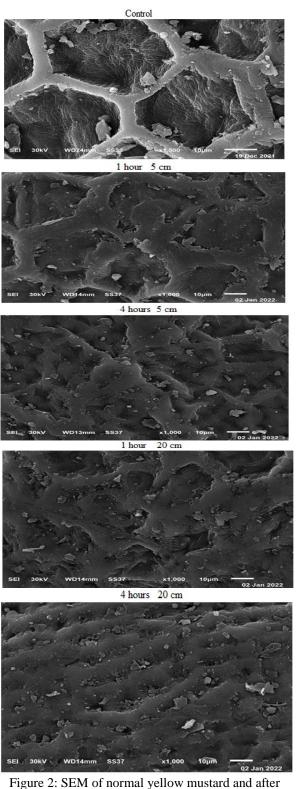


Figure 2: SEM of normal yellow mustard and after exposed to UVA

4.2 Molecular structure

Figure 3 shows IR spectrum of yellow mustard, a plot of wave number (X- axis) vs. present transmittance (Y- axis). IR analysis of yellow mustard listed in Table 2 show transmittance the intensity increased after exposure to UVA at 5 cm for 1 and 4 hours but decreased at 20 cm for 1 and 4 hours. A significant change occurred in the main peak position, O-H, after exposure to UVA for 1 hour at 5 cm, but a little variation is occurred during other exposure times at 5 and 20 cm distance. That is because the absorption of UVA in cell, break or modify position or degrade some molecular bond or switching off of the transcription– translation machinery during radiation exposure [12].

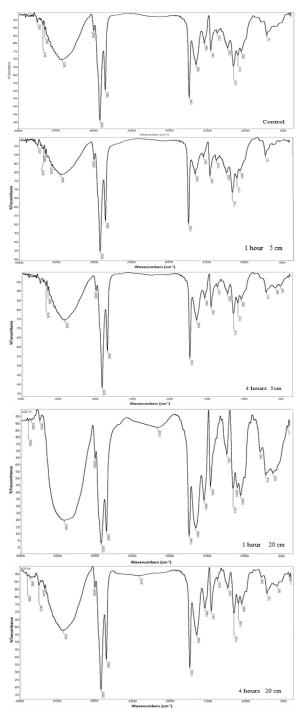


Figure 3: IR spectrum of yellow mustard after exposure to UVA

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Control (untreated sample)						
I	Position	Int	Intensity %		Band	
	1746		45	.6	C- O	
	2925		31.	04	C-H	
	3422		69.	.4	O-H	
1 ł	nour at 5 cm	1 4 hour at 5 cm				
Positio	Intensit	Ban		Positio	Intensit	Ban
n	у %	d		n	у %	d
1746	50.4	C- O		1746	54.12	C- O
2925	34.36	C-H		2925	38.11	C-H
3450	78.4	O-H		3423	74.26	O-H

Table 2: IR spectrum analysis of yellow mustard after exposure to UVA

1 he	our at 20 cr	n	4 h	our at 20 cr	n
Positio	Intensit	Ban	Positio	Intensit	Ban
n	у %	d	n	у %	d
1746	8.32	C- 0	1746	32.9	C- 0
2925	2.75	C-H	2925	18.21	C-H
3421	19.22	O-H	3421	57.37	O-H

4.3 Glutathione content

The glutathione which one of the most important nonenzymatic antioxidants. Glutathione content for yellow mustard is increased after exposure to UVA at 5 and 20 cm for different period of times as seen in Table 3 and Figure 4. The results show it is increased by 17.44%, 19.3%, 7.8% and 4.58% and, by 14.68%, 19.3%, 0.45% and 5.5% at 5 and 20 cm distance far from UVA source for different period times. That is because is positively affected by radiation as a hermetic type of response under applied of radiation [13, 14].

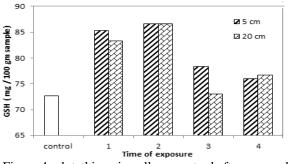


Figure 4: glutathione in yellow mustard after exposed to UVA

Table 3: glutathione content of yellow mustard after exposed to UVA

Exposure time (hour) Zero (Control)	GSH (mg/ 100g) 72.66	
	5 cm	20 cm
1	85.33	83.33
2	86.66	86.66
3	78.33	72.99
4	75.99	76.66

4.4 Phenolic content

Phenolic compounds are plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl groups. Total phenolic content for yellow

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mustard increased by 32.57%, 43.5%, 19.5% and 48.35% and by18.17%, 8.31%, 59.71% to 1.82.5% after exposure to UVA at distances 5 and 20 cm for 1, 2, 3 and 4 hours as shown in Table 4 and Figure 5. That is because UV radiation increases the accumulation of phenolic compounds along with antioxidant properties. Also a change in the intensity and position of O-H band caused increased in phenolic. The other results also shown phenolic compounds is increased or induced after UV radiation exposure during different period times [15-19].

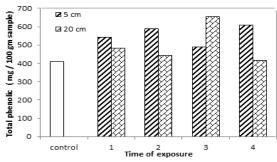


Figure 5: total phenolic content in yellow mustard exposed to UVA

Table 4: total phenolic content of yellow mustard exposed to UVA

Exposure time (hour) Zero (Control)	Total phenolic (mg/ 100g) 409.48	
	5 cm	20 cm
1	542.86	483.89
2	587.57	443.50
3	489.33	654.21
4	607.44	416.92

4.5 Flavonoids content

Flavonoid is a term that is a bit ambiguous literally it means flavone-like compound. Table 5 and Figure 6 show total flavonoids in yellow mustard decrease after exposure for 1, 3 and 4 hours and 1 and 3 hours at 5 and 20 cm from UVA source but it increased after exposure for 2 hours at 5 cm distance and 2 and 4 hours at 20 cm. That is because flavonoids is plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl groups which changed as shown in IR analysis.

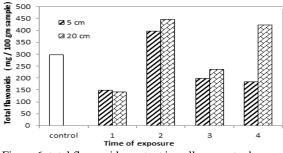


Figure 6: total flavonoids content in yellow mustard exposed to UVA

Exposure time (hour) Zero (Control)		ids (mg/ 100g) 97.6
	5 cm	20 cm
1	148.45	141.95
2	395.71	446.75
3	198.54	236.8
4	183.07	422.64

Table 5: total flavonoids content of yellow mustard exposed to UVA

4.6 Total proline content

Proline plays important roles in protein synthesis and structure, metabolism and nutrition. Total proline content in yellow mustard decreased after exposure to UVA for different period times and distances as shown in Table 6 and Figure 7. The chemical composition of yellow mustard such as protein changed after exposure to UVA. That is because proline is a substrate for protein synthesis. Also proline accumulation is response to biotic and abiotic stresses caused to the effect of UVA.

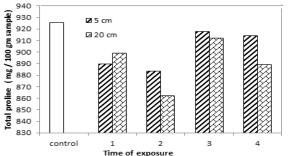


Figure 7: total proline content in yellow mustard exposed to UVA

Table 6: total proline content of yellow mustard exposed to UVA at

5 and 20 cm for different period times

Exposure time (hour) Zero (Control)	Total proline (mg/ 100g) 925.84		
	5 cm	20 cm	
1	889.96	899.24	
2	883.56	862.40	
3	917.54	911.96	
4	914.41	888.98	

4.7 DPPH scavenging activity

UVR carries higher energy than visible light and its effects on tissues include gene mutations, DNA damage, oxidative stress immunosuppression, and inflammatory responses. Therefore, some compounds increase and others may decrease due to abiotic stress as pathways for secondary metabolite production are interrelated. DPPH scavenging activity (%) for yellow mustard decreased after exposure to UVA for 1, 2, 3 and 4 hours at 5 and 20 cm as shown in Table 7 and Figure 8.

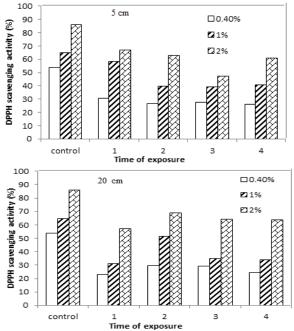


Figure 8: DPPH scavenging activity of yellow mustard exposed to UVA

Table 7: DPPH scavenging activity (%) of yellow mustard exposed to UVA

Exposure time (hour)	DPPH scavenging activity (%) 5 cm		
	0.4%	1%	2%
Zero (Control)	53.90	64.89	85.82
1	30.56	58.33	67.01
2	26.67	39.56	62.85
3	27.8	39.03	47.43
4	26.04	40.60	60.76
Exposure time (hour)	DPPH scav	enging activity	y (%) 20 cm
	0.4%	1%	2%
Zero (Control)	53.90	64.89	85.82
1	23.14	31.25	57.29
2	29.51	51.39	68.75
3	29.17	35.07	64.24
4	24.31	34.03	63.54

4.8 Tocopherol content

Table 8 and Figure 9 show, tocopherol content in yellow mustard decreased by 10.1%, 6.01%, 47.5% and 42.6% after exposure to UVA at 5 cm for 1, 2, 3 and 4 hours. Also it decreased gradually after exposure for 1, 2, 3 and 4 at 20 cm.

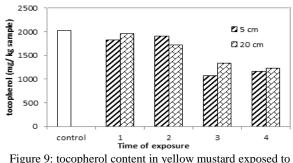


Figure 9: tocopherol content in yellow mustard exposed to UVA

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Table 8: tocopherol of yellow mustard exposed to UVA

Exposure time (hour) Zero (Control)	Tocopherol (mg/ kg) 2029.19		
	5 cm	20 cm	
1	1824.13	1965.43	
2	1907.79	1726.63	
3	1065.43	1335.00	
4	1165.43	1234.16	

Conclusion

Variation in radiation resulted in, significant differences in biomass accumulation, protein and total non-structural carbohydrates contents in plants. Exposure to UVA improve/or disprove some antioxidants for yellow mustard. Also UVA caused a change in yellow mustard internal structure.

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