EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

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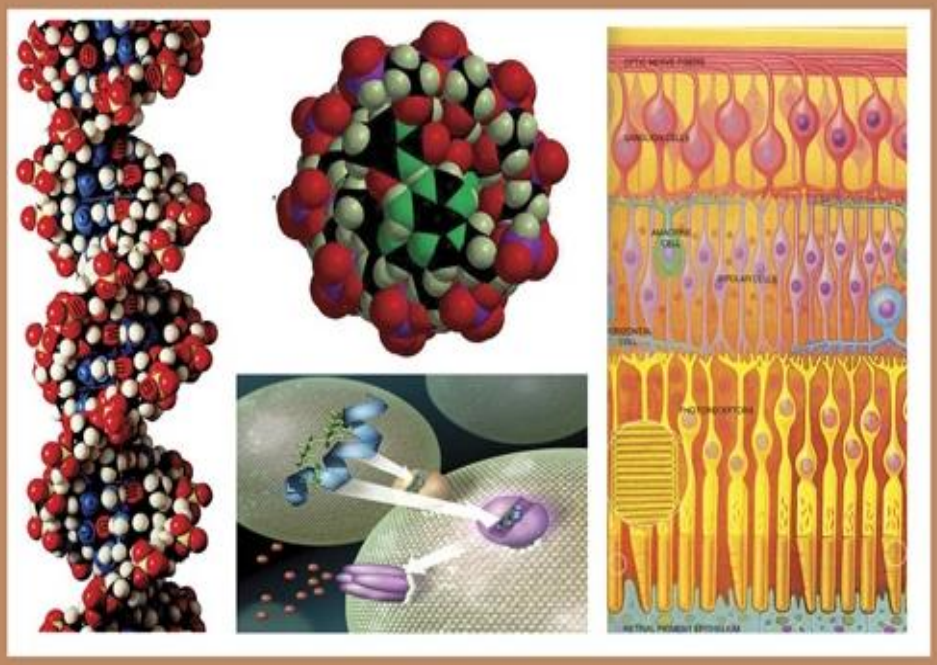

ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 14 No. 1 (2022)

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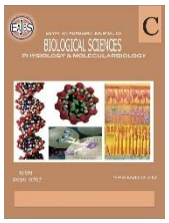
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 14(1) pp337-344 (2022)*
DOI: 10.21608/EAJBSC.2022.231287

Egypt. Acad. J. Biolog. Sci., 14(1):337-344 (2022)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Ultraviolet Effect on Internal Chemical Composition, Phenolic and Glutathione of Yellow Mustard

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ARTICLE INFO
Article History
Received:2/3/2022
Accepted:15/4/2022
Available:20/4/2022

Keywords:
Glutathione, phenolic, DPPH scavenging activity, chemical composition, yellow mustard.

ABSTRACT
Background: The production by plants of compounds useful as medicines or raw materials for the manufacture of medicines is influenced by ultraviolet radiation. Ultraviolet is, UV, very important when used to improve medicine products in plants. No available studies covered the effect of UVC for a long period of time and its high power on the chemical composition and antioxidants of medical plants. For this reason, the aim of this research is to study the effect of UVC on internal structure, enzymatic and non-enzymatic antioxidants for *yellow mustard*. **Methods:** The irradiated system consists of a fluorescent lamp is used. Pure *Yellow mustard* seeds (Sinapis alba) are used as research samples, received from the Egyptian Ministry of Agriculture. Total phenols were determined calorimetrically by the Folin-Ciocalteu reagent. UV/Vis spectrophotometer, Jenway, England was used to determine GSH. **Results:** The chemical composition of *yellow mustard* is highly affected after exposure to UVC. Glutathione in *yellow mustard* varied after being exposed to UVC. DPPH scavenging activity for *yellow mustard* decreased but total phenolic content increased after exposure to UVC. The results from scanning electron microscope and IR spectrum analysis showed the internal structures such as molecule arrangement, orientation and size or molecular bonds (as strength and position) for *yellow mustard* changed after exposure to UVC.

INTRODUCTION

The mustard plant belongs to the Cruciferae family. *Brown mustard* is used as a medicine to treat simple toothaches and severe convulsions in small children. The most common use of mustard as a medicine is a liniment, poultice, or plaster, relieving bruises pain, or a stiff neck and relieving colic and respiratory problems. There are many beneficial uses of radiation that offer few risks when properly employed. Exposure to radiations has stimulatory effects on specific morphological parameters. The irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein, hormone balance and enzyme activity (Hameed *et al.*, 2008). Growth behavior, internal structure, enzymes and free radicals of *Nigella Sativa* and *garden cress* changed after being exposed UVC (El-Bediwi *et al.*, 2018; El-Bediwi *et al.*, 2018). Ultraviolet radiation has wavelengths between 200 and 400 nm and accordance with International Commission on Illumination, was categorized into UVC (200-280 nm), UVB (280-320 nm) and UVA (320-400 nm). UVs possess sufficient energy to break the chemical bonds causing photochemical reactions and inducing changes in plant metabolic enzymes, subsequently triggering the production of secondary metabolites (Zhang and Björn, 2009; Hectors *et al.*, 2014; Ghasemi *et al.*, 2019).

The objective of this research is to study the effect of UVC on internal chemical composition, and non-enzymatic and enzymatic antioxidants for *yellow mustard*.

MATERIALS AND METHODS

Radiation Source:

The irradiated system consists of a fluorescent lamp (Type-C with $\lambda \cong 2540$ Å). The system is covered totally with aluminium foil from all sides.

Internal Structure:

The structure of *yellow mustard* is studied by scanning electron microscope (JEOL JSM-6510LV, Japan). Also, molecular structure of yellow mustard is studied by Nicolet™ iS™ 10 FT-IR Spectrometer from the USA.

Determination of GSH:

UV/Vis spectrophotometer, Jenway, England was used to determine GSH.

Method Description for Total Phenols:

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

Method Description for DPPH Radical-Scavenging Activity:

Concentrations ranging from 0.4g/100g to 2g/100g were prepared with methanol from each sample (100 µl) extract and DPPH radical (100 µl, 2Mm) dissolved in methanol. The mixture was stirred and left to stand for 15 min in dark. Then the absorbance was measured at 517 nm against a blank. The percentage scavenging effect was calculated as $[(A_o- A_1)/A_0]\times100$ where A_o is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample.

RESULTS AND DISCUSSION

Structure:

Figure 1 shows scanning electron micrographs, SEM, of *yellow mustard* after being exposed to UVC for different periods of time and dissimilar distances. The micrographs show a change in internal structure, such as molecules' shape and size or orientation, for *yellow mustard* after being exposed to UVC. That is because, the interaction of UVC ray with it, break or modify bonds or form free radical and this agrees with other studies (Kovács and Keresztes, 2002; Ashraf *et al.*, 2003).

Molecular Structure (IR analysis):

IR spectrum of *yellow mustard* is a plot of wavenumber (X-axis) vs. present transmittance (Y-axis) as shown in Figure 2. IR analysis of *yellow mustard* shows the transmittance intensity is increased after exposure to UVC for 1 and 4 hours at 5 cm and 1 hour at 20 cm from the source. Also, there is a significant change in the main peak position, O-H, after exposure to UVC for 4 hours at 20 cm and little variation for other exposure times at 5 cm and 20 cm distances. That is because UVC breaks or modifies the position of molecular bonds. However, the change of some bands after exposure to UVC caused either degradation or switching off of the transcription-translation machinery during radiation exposure (Sen Raychaudhuri and Deng, 2000).

Glutathione Content:

Glutathione is one of the most important non-enzymatic antioxidants. **Figure 3** shows glutathione content, GSH, in *yellow mustard* is varied, where it decreased by 42.7% and 23.9% after one and four exposed hours then increased by 22.47% and 32.6% after two and three hours exposed to UVC at 5 cm distance. It decreased by 21.1% after one-hour exposure at a distance of 20 cm from UV source then increased by 21.02%, 14.22%, and 15.14% after being exposed for two, three and four hours. That is due to the hermetic type of response under applied doses of radiation (Štajne *et al.*, 2009; Chakravarty and Sen, 2001).

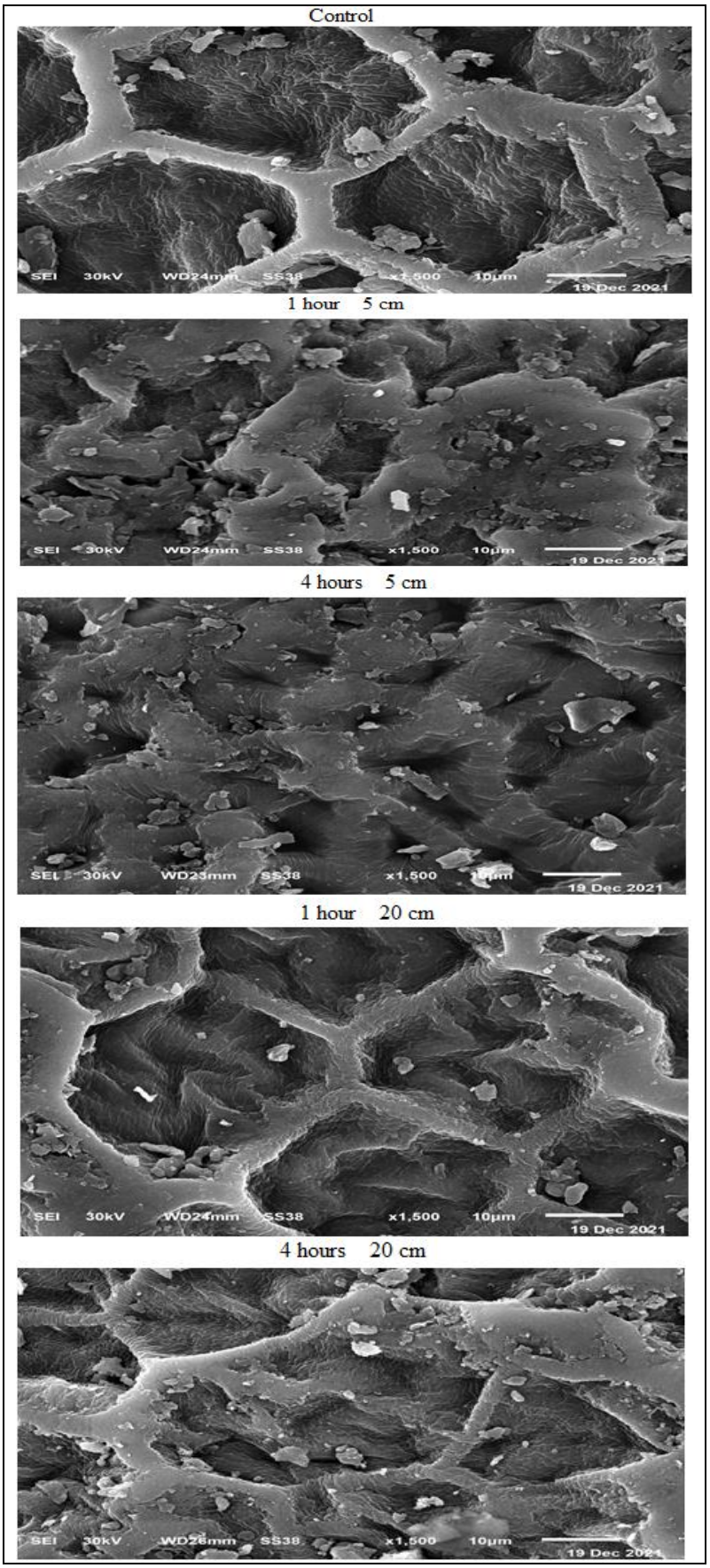

Fig. 1. SEM of *yellow mustard* after exposed to UVC

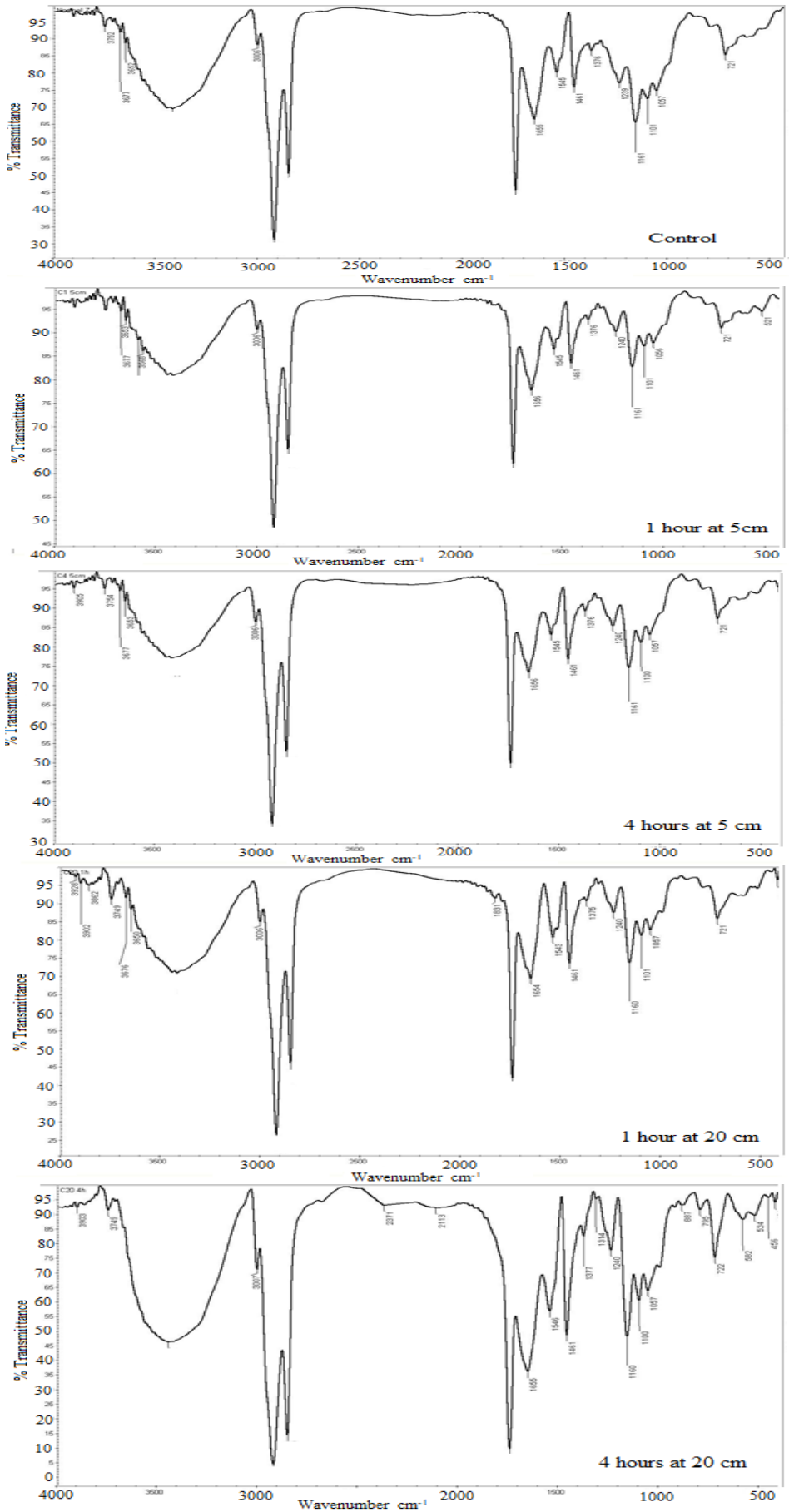

Fig. 2. IR spectrum of *yellow mustard* after exposed to UVC

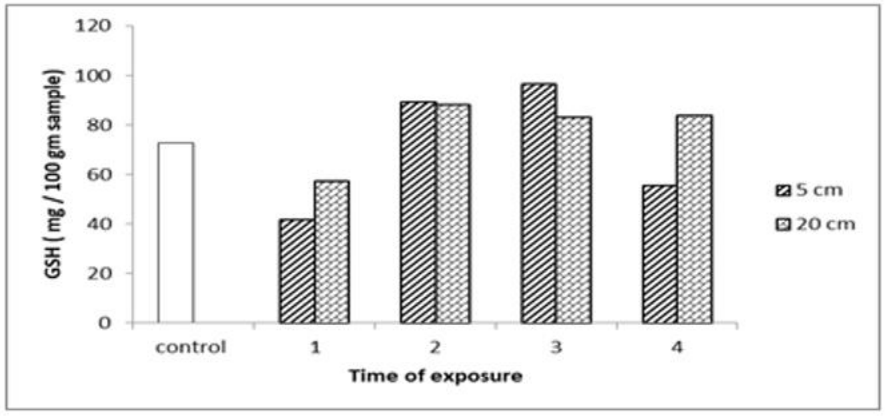

Fig. 3. Glutathione content in *yellow mustard* after exposure to UVC

Phenolic Content:

Phenolic compounds represent a huge class of plant secondary metabolites, have diverse structures containing one or more hydroxyl groups and are produced by plants mainly for protection against stress. Total phenolic content in *yellow mustard* presented in Figure 4 shows a variable increase after being exposed to UVC at different times at dissimilar distances. It's increased by 66.7% and 6.06%, 3.46% and 5.46%, 32.16% and 19.5%, 0.5% and 4.67% after being exposed to UVC for 1, 2, 3 and 4 hours at 5 and 20 cm distances and this agreement with the other study (Polovka *et al.*, 2006). UVC effected on the accumulation of phenolic compounds and is capable of breaking the chemical bonds of polyphenols thereby releasing soluble phenols of low molecular weight (Kim *et al.*, 2009; Wi *et al.*, 2007; Štajner *et al.*, 2007, Ashraf, 2009; Younis *et al.*, 2010).

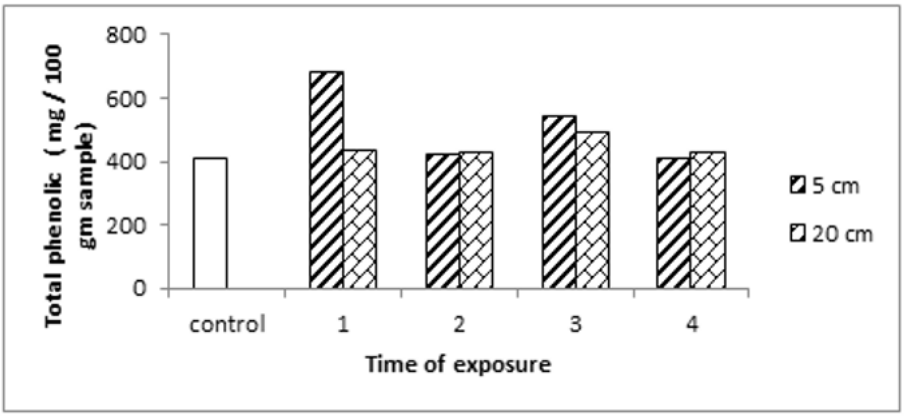

Fig. 4. Total phenolic content of *yellow mustard* after exposed to UVC

DPPH Scavenging Activity:

Some compounds increase and others decrease in plants due to abiotic stress as pathways for secondary metabolite production are interrelated. Figure 5 shows there is a variable decrease in DPPH scavenging activity (%) for *yellow mustard* after exposure to UVC at different times at dissimilar distances.

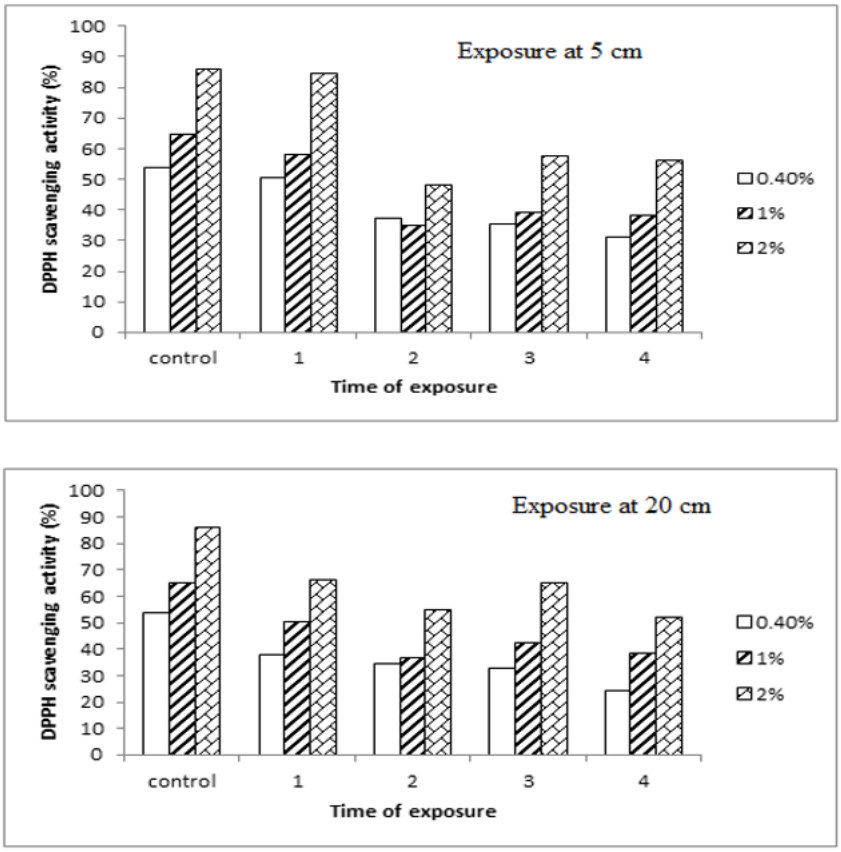

Fig. 5. DPPH scavenging activity of *yellow mustard* after exposed to UVC

Chemical Composition

Internal chemical composition for plants is changed due to environmental conditions and is not always regarded as a stress phenomenon as in many cases increase in the secondary metabolites can be achieved by radiation. The results presented in Table 1, show the carbohydrates in *yellow mustard* increased by 92.6% with decreasing protein, fats, moisture, total fibres and ASH by 15.2%, 32.7%, 13.7%, 6.1 and 0.2% respectively after exposure to UVC. That is because UVC react rapidly with almost all structural and functional organic molecules, including proteins, lipids and nucleic acids caused a change in their quantities.

Table 1. Chemical composition of *yellow mustard* after exposed to UVC at 5 and 20 cm for different period times

Exposure Time (h)	Chemical composition of yellow mustard (Exposure at 5 cm)					
	Carbohydrates	Protein	Fats	Moisture	Total fibres	ASH
control	16.54%	14.50%	32.70%	5.76%	26.52%	3.98%
1	31.86%	12.30%	22.00%	4.97%	24.90%	3.97%
4	15.50%	25.70%	27.10%	5.11%	23.30%	3.29%

Exposure Time (h)	Chemical composition of yellow mustard (Exposure at 20 cm)					
	Carbohydrates	Protein	Fats	Moisture	Total fibres	ASH
control	16.54%	14.50%	32.70%	5.76%	26.52%	3.98%
1	17.40%	11.28%	35.00%	5.73%	26.80%	3.79%
4	15.03%	19.70%	37.00%	4.81%	19.60%	3.86%

Conclusion

SEM and IR analysis show that accumulation or arrangement or orientation of molecules or chemical composition of *yellow mustard* changed after exposure to UVC. Also, the change in bio contents of *yellow mustard* (GSH, total phenolic and DPPH scavenging activity) is dependent on UVC power.

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