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Research article

Gamma radiation effects on vitamins, antioxidant, internal and molecular structure of Purslane seeds

Soad Hasanin[1], A. G. ELshahawy[1], Hamed M El-Shora[2] and Abu Bakr El-Bediwi[1]

[1] Physics Department, Faculty of Science, Mansoura University, 35516 Mansoura, Egypt
[2] Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt

* **Correspondence:** Email: baker_elbediwi@yahoo.cm.

Abstract: Purslane contains the highest amount of omega-3 fatty acids, antioxidants, and has better nutritional quality. Gamma irradiation is harmless and dependable method for refining the shelf life and nutritional quality of the stored seeds. The aim of this research is to study the influence of irradiation by gamma on structure, ascorbic acid and phenolic content of Purslane seeds. The results show that, ascorbic acid, phenolic content, carbohydrate and protein content in Purslane seeds decreased after exposed by gamma rays. Super oxidase dismutase, fat and fiber content in Purslane seeds increased after exposed by gamma radiation. The shape, size, interconnection and arrangement of molecules in/or around benzene ring in Purslane cells changed after exposure to gamma radiation.

Keywords: vitamins; gamma radiation; Purslane seeds; phenolic compounds; super oxidase dismutase

1. Introduction

Purslane is a member of the Portulacaceae Juss has nutritional, medicinal and therapeutic properties, and use as folk medicine and traditional food since ancient times. Recent years the use of radiation technology in different areas agriculture increased where the stimulating effect of gamma rays improve the adaptive response of living organisms. Gamma radiation is a form of ionizing radiation and due to their easy availability and penetration power, proved that more economical and effective compared to other ionizing radiations, contribute to the service of human society through applications in medicine, agriculture, pharmaceutical and other technological processes [1]. The irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein, hormone balance

and enzyme activity [2,3]. The morphological structural and functional changes dependent on the strength and exposure time of gamma radiation doses, where caused some changes physiological and biochemical of seed [4]. Gamma irradiation at a dose of 500 Gy caused a significantly decreased seed viability compared with dose of 250 Gy, also irradiation dose of 500 Gy enhanced negative effect on rice seed germination capacity [5,6]. Plant height decreased as the radiation dose increased and is directly proportional with increasing dose [7], also the number of leaves produced by irradiated plants at 250 and 500 Gy are more than the untreated. Low doses of gamma radiation improved vigor and yield of wheat [8,9]. The percentage of survival in the field, production and the seedling height was decreased after exposure to gamma radiation dose above 300 Gy [10]. Also the percentage of germination decreased at irradiated at 300 Gy dose [11]. The enhanced germination and vigor in seeds were noticed after exposed by 20 Gy gamma radiation compared with non-irradiated. Also a significant increase in the hydrogen peroxide, phenol and lignin deposition was noticed in 20 Gy-irradiated rice plants [12]. This work aimed study internal structure, ascorbic acid and phenolic compounds of irradiated Purslane seeds.

2. Materials and experimental methods

The seeds of Purslane were irradiated at doses (50, 100, 150, 200, 250 and 500 Gy) using cobalt-60 gamma source, (with dose rate 0.863 kGy/h), at the Egyptian atomic energy authority. Internal structural studied by scanning electron microscope (JEOL JSM-6510LV, Japan), while molecular structure by Nicolet™ iS™ 10 FT-IR Spectrometer from USA.

2.1. Phenolic compounds

The test was run for the extracted seeds to quantify the phenolic contents. Folin-Ciocalteu (F-C) assay was used following the procedure reported by Wolfe et al [13] and Issa et al. [14] in which the standard curve of Gallic acid was used to calculate the characteristic values as milligram Gallic acid equivalents/grams of the dried plant. The process involved the use of a Gallic acid standard curve ($y = 0.0062\ x$, $r^2 = 0.987$).

The test was run for the extracted seeds using aluminum chloride colorimetric assay following the procedure reported by Zhishen et al. [15] using the standard curve of Catechin "secondary metabolite". The total flavonoids were estimated from the following standard curve ($y = 0.0028\ x$, $r^2 = 0.988$).

2.2. Ascorbic acid content

Pipette out 5 mL of the working standard solution into a 100 mL of conical flask. Add 10 mL of 4% oxalic acid and titrate against the dye (V_1 mL). End point is the appearance of pink colour which persists for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid. Extract the sample (0.5–5 g depending on the sample) in 4% oxalic acid and make up to a known volume (100 mL) and centrifuge. Pipette out 5 mL of this supernatant, add 10 mL of 4% oxalic acid and titrate against the dye (V_2 mL) [16]. Amount of ascorbic acid mg/100 mL sample = (0.5 mg × V_2 mL × 100 mL × 100) / (V_1 mL × 5 mL × Wt.) of the sample.

2.3. Superoxide dismutase (SOD) determination

1 g seed material (whole seeds, endosperms or embryos) was homogenized in 10 cm^3 of 25 mol/m^3 EPPS (yV-2-hydroxyethylpiperazine propane sulphonic acid) buffer (pH 7–8) containing 0–2 mol/m^3 EDTA and 2% polyvinyl pyrrolidone (insoluble). The homogenate was filtered through a nylon mesh and then centrifuged at 18000 g for 15 min. The supernatant obtained was used for enzyme assays. All operations were carried out at 4 ℃. With the exception of SOD, all enzyme activities were measured in a final volume of 1 cm^3 at 25 ℃.

Superoxide dismutase (SOD) was assayed by a photochemical method as described by Giannopolitis and Ries (1977) [17]. One unit of SOD activity was defined as the amount of enzyme resulting in 50% inhibition of the rate of p-nitro blue tetrazolium chloride reduction at 560 nm.

2.4. Chemical composition determination

Moisture content determined according to the method described by AOAC (2019) [18]. A known weight of air dried seeds (2 g) was dried at 105 ℃ in an air drying oven to a constant weight. Percentage of moisture content was calculated.

Ash content determined according to AOAC (2019), as follow: Exactly 2 g of air dried seeds were place in a silica crucible and ignited at 600 ℃ in a muffle furnace till a constant weight; the percentage of ash content is calculated.

Crude fiber was estimated according to the method described by AOAC (2019). A known weight of the air dried seeds (2 g) is mixed with 0.5 g asbestos, then 200 mL of sulphuric acid (1.25% v/v), are added. The mixture is boiled under reflex for 30 minutes, followed by filtration through Gooch crucible. The residue is boiled again with aqueous sodium hydroxide solution (200 mL, 1.25% w/v) for 30 minutes, and then filtration was repeated in the same manner. Finally, the residue is washed with hot water followed by diethyl ether and dried at 110 ℃ to constant weight. The content of Gooch crucible was then ignited in the muffle furnace at 600 ℃ to a constant weight. Fiber content is calculated by subtraction of ash content from the weight of digested sample. Percentage of crude fiber content was then calculated.

Crude protein content determined by the official Kjeldahl method described in AOAC (2019), as follow: A known weight of air dried seeds (0.5 g) was digested with 8 mL of concentrated sulphuric acid in Kjeldahl flask in the presence of (2.14 g) digestion mixture [1 kg potassium sulphate and 60 g of mercuric oxide (red)]. After digestion the solution was treated with 10 mL of 40% sodium hydroxide solution. The liberated NH_3 was received into 10 mL of 1% boric acid in the presence of 2 drops of Tachero indicator (1.25 g methyl red + 0.32 g methylene blue in one liter of 90% ethanol). The received ammonia was titrated with 0.01 N sulphuric acid. The percentage of total nitrogen was estimated and the crude protein content was calculated by using 6.25 as a factor of protein.

2.5. Statistical analysis

Data were analyzed by SPSS software using analysis of variance (ANOVA) and differences among means were determined for significance at $P < 0.05$ using Tukey's test.

3. Results

3.1. Phenolic compounds

Total phenolic compound content in Purslane seeds decreased after irradiated by gamma doses from 50 to 500 Gy as shown in Table 1, where decreased gradually up to 199.55 mg/g. Table 2 showed that total flavonoids compound content in Purslane seeds gradually decreased after exposure to 50, 100, 150, 200, 250 and 500 Gy, where decreased up to 87.9 mg/g at 500 Gy. These results show that, there is a significant, $P < 0.001$, reduction of total phenolic compounds content with increased gamma doses.

Table 1. Phenolic of Purslane seeds after exposed by gamma radiation.

Sample dose/Gy	Phenolic content (mg/g)
Non-irradiated	321.24 ± 24.07
50	310.45 ± 27.9
100	285.73 ± 17.1
150	262.02 ± 20.2
200	234.13 ± 16.38
250	203.54 ± 12.24
500	199.55 ± 7.96

Table 2. Flavonoids of Purslane seeds after exposure to gamma radiation.

Sample dose/Gy	Flavonoids content (mg/g)
Non-irradiated	189.26 ± 14.175
50	175.26 ± 11.37
100	152.93 ± 12.24
150	141.59 ± 11.28
200	113.74 ± 10.17
250	98.42 ± 6.86
500	87.91 ± 7.04

3.2. Vitamin C (ascorbic acid)

Table 3. Ascorbic acid in Purslane seeds after exposure to gamma radiation.

Samples doses/Gy	Ascorbic acid (mg/100 g)
Non-irradiated	17.143 ± 1.53
50	14.636 ± 1.098
100	15.452 ± 1.082
150	14.732 ± 1.325
200	13.842 ± 1.176
250	11.942 ± 0.96
500	10.528 ± 0.895

Table 3 showed that ascorbic acid in Purslane seeds decreased, (a significant decreased $P < 0.001$), after exposure to gamma radiation, where decreased from 17.17 to 14.63 and 10.53 mg/100 g at 50 and 500 Gy. That is meant, ascorbic acid (Vitamin C) is the most sensitive soluble vitamins to radiation.

3.3. Superoxide dismutase activity (SOD)

Superoxide dismutase (SOD) activity, as antioxidant enzyme, enhances with increasing gamma radiation doses, due to increase the free radicals. Table 4 showed that, super oxidase dismutase in Purslane seeds increased gradually with increasing gamma radiation doses, where a significant change happened, $P < 0.001$, in SOD at 500 Gy.

Table 4. SOD in Purslane seeds after exposure to gamma radiation.

Samples doses/Gy	Super oxidase dismutase (μg^{-1})
Non-irradiated	228.39 ± 16.03
50	284.43 ± 19.88
100	321.63 ± 28.89
150	384.92 ± 33.49
200	405.21 ± 28.35
250	428.00 ± 29.96
500	498.05 ± 29.88

3.4. Chemical composition

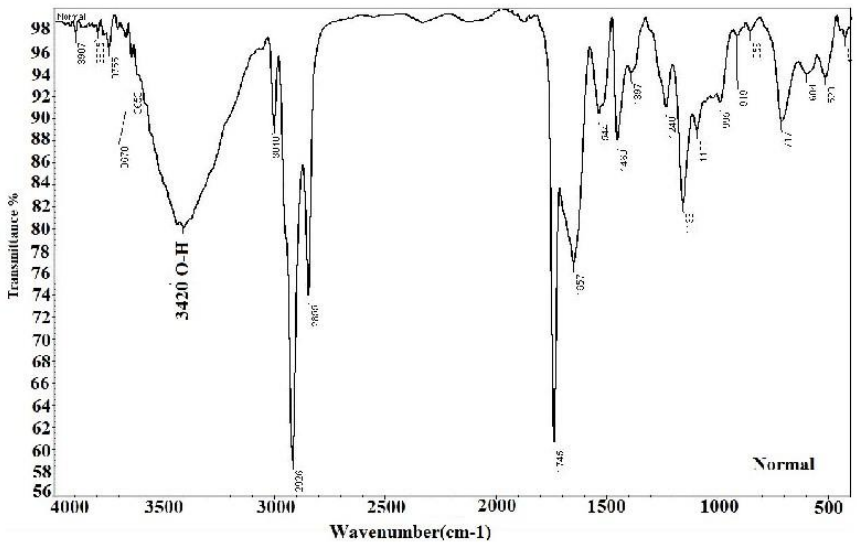
Carbohydrate and protein content in Purslane seeds decreased but fat and fiber content increased after exposure to gamma radiation at 250 and 500 Gy as listed in Table 5, where carbohydrate and protein content decreased by 19.52% and 27.04% but fiber and fat content increased by 22.9% and 23.8%.

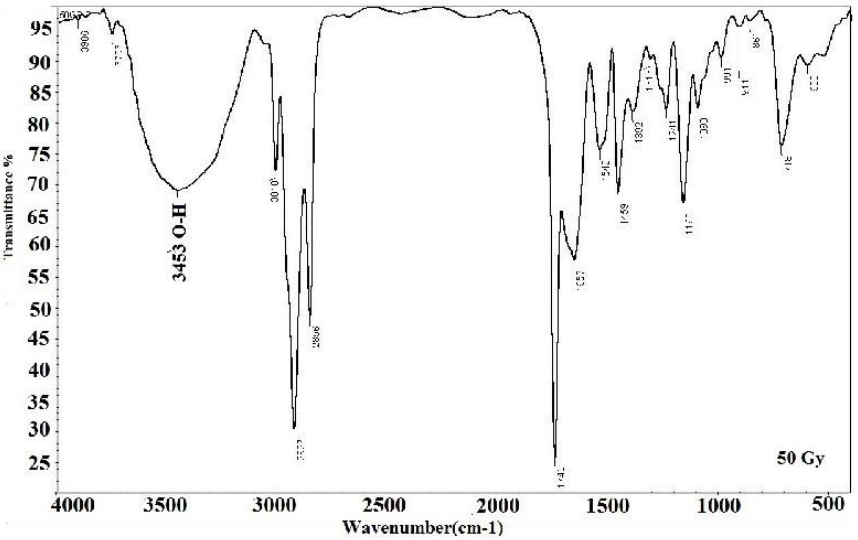
Table 5. Chemical composition of Purslane seeds after exposure to 250 and 500 Gy doses.

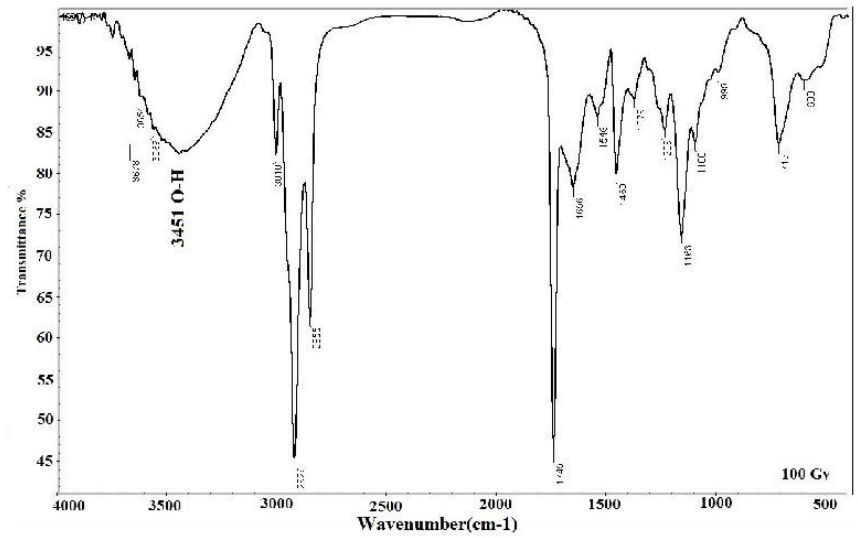
Composition %	Non-irradiated	250 Gy	500 Gy
Ash	7.68	6.538	5.026
Fat	6.138	6.9372	7.577
Fiber	44.586	49.456	54.768
N	1.568	1.298	1.144
Protein	9.798	8.536	7.151
Carbohydrates	30.229	26.52	24.334

3.5. Molecular structure

Infrared (IR) spectrum for Purslane seeds after exposure to gamma radiation doses, ranging from 50 to 500 Gy was shown in (Figure 1). There is a change in the peak position and transmittance intensity of hydroxyl band as listed in Table 6, where each molecular band in the cell absorbed enough energy during gamma radiation penetrating to vibrate at different positions.


Normal
3420 O-H
Transmittance %
Wavenumber(cm-1)


50 Gy
3453 O-H
Transmittance %
Wavenumber(cm-1)


100 Gy
3451 O-H
Transmittance %
Wavenumber(cm-1)

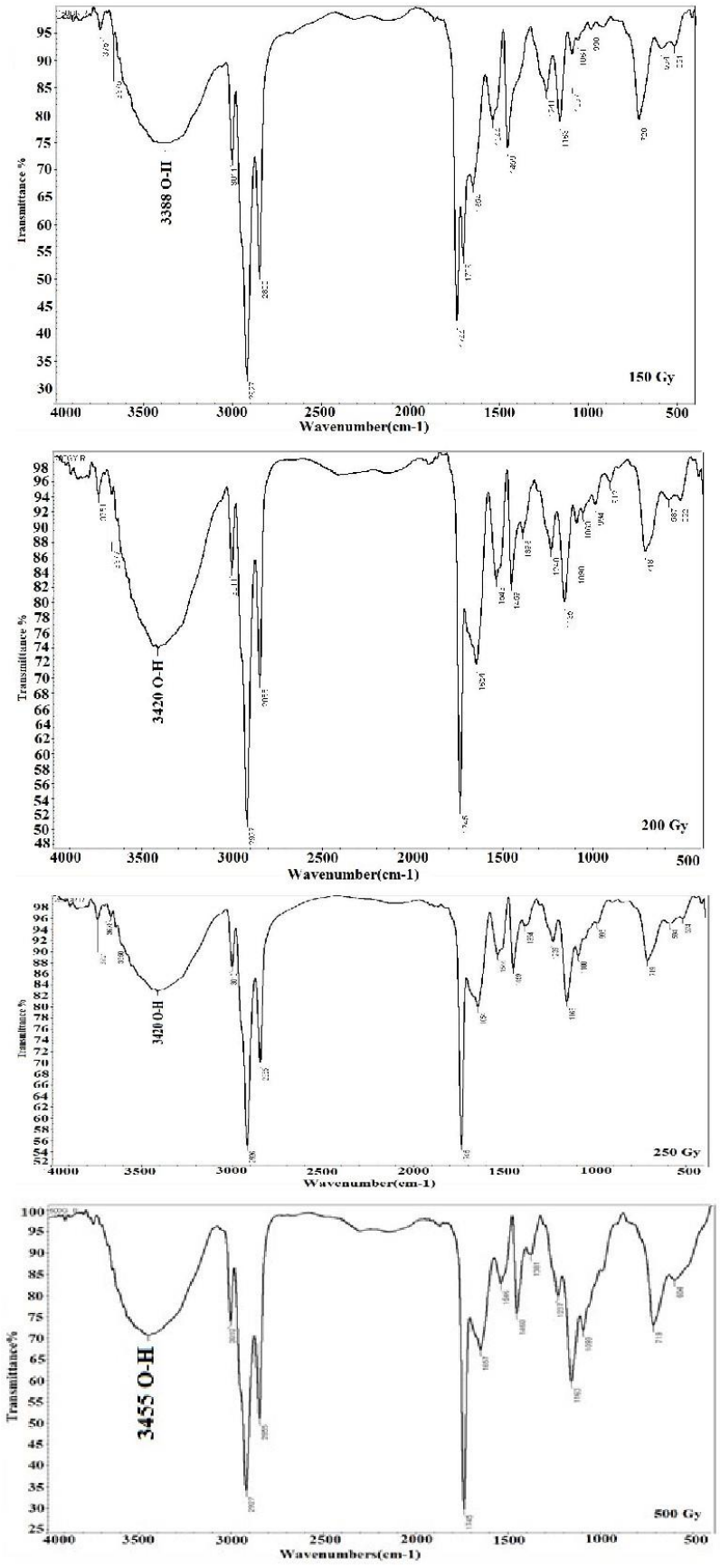


Figure 1. IR spectrum of Purslane seeds after exposure to gamma radiation.

Table 6. IR spectrum analysis of Purslane seeds after exposure to gamma radiation.

Samples doses/Gy	Position of O–H band	Transmission Intensity %
Non-irradiated	3420	80
50	3453	66.92
100	3451	82.3
150	3388	74.71
200	3420	73.9
250	3420	82.86
500	3455	70.55

3.6. Scanning electron micrographs (SEM)

Micrographs of Purslane seeds, (Figure 2), showed there is a greatly change in the interconnection forming dimensional network and benzene ring after exposed to gamma radiation at 500 Gy dose.

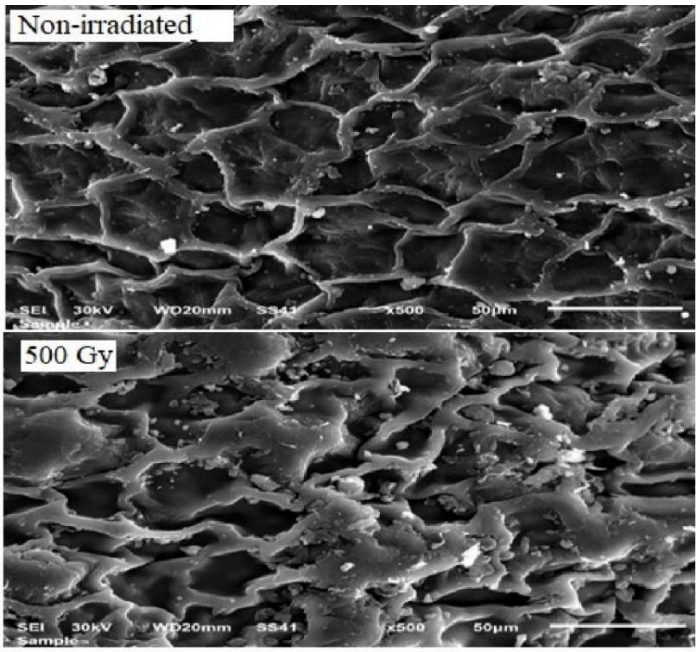


Figure 2. SEM images of non-irradiated Purslane seeds and after exposure to 500 Gy.

4. Discussion

The ionizing radiation due three effects, first is ionization, second is dissociation and third is excitation. Ionization and dissociation lead to a strong interaction while excitation leads to a weak interaction. Atoms or molecules in the biological cell interacted with ionizing radiation during penetrating, mainly water, to produce free radicals which diffuse and damage many important compounds of cell, and the ejection of electrons from atoms also caused chemical and physical changes in the constituents of cell.

Gamma radiation has a highly effect on seed viability, irradiation at a dose of 500 Gy resulted in declining of seed bio-properties compared with non-irradiated sample. Some bio-properties for seeds

decreased in contrast increased other after exposure to radiation. Some changed that effected the seed physiology whether physical, physiological and biochemical happen after exposed seed to radiation [7]. Gamma irradiation at 500 Gy caused a negative effect on the biological properties of the seeds [5,6]. The production of free radicals caused harmful effects on the important components of the plant such as enzyme activity, protein, water exchange and hormone balance, which depending on the irradiation dosage [19].

Phenolic compound content decreased after exposure to gamma radiation because it court responded to gamma rays doses [20]. The change in phenolic content directly linked to protein synthesis. Gamma radiation removing the free radicals from the cells, which effect on phenolic content and this agree with other studies [3,21]. Also the stress produced by gamma radiation caused a reduction of the protein bands, decreased in flavonoids content.

Ascorbic acid decreased after exposure to gamma radiation because the enzymes activity was enhanced which caused rapid degradation of ascorbate or a partial conversion of ascorbate to dehydroascorbic [22].

During ionizing process by gamma radiation may decomposed water and other chemical compounds induced oxidative stress with overproduction of reactive oxygen species disturbance of cellular metabolism. Some protein bands disappeared or degraded during exposure to gamma radiation, dependent on stress intensity [23,24]. Gamma radiation interacted with the bio-content caused a quantity change in it, and as that the emergence of free radicals resulting from gamma radiation damaged the proteins of the cell membrane.

From IR analysis there is a change in peak position and transmittance intensity for hydroxyl band after exposure to gamma radiation because has enough energy to modify or destroy some bonds. Also arrangement or size or accumulation or interconnections of some molecules (Benzene ring and molecules or atoms around them) were changed after exposure to gamma radiation.

From scanning electron micrographs, there is a change in internal structure of matrix, because gamma ray collided with almost all structural and functional organic molecules, resulting destroying or modifying some in it.

5. Conclusions

High significant change on total phenolic content, ascorbic acid and super oxidase dismutase for Purslane seeds occurred after exposed by gamma radiation, with changed chemical composition, molecular and internal structure of it. Gamma irradiation at a dose 500 Gy resulted in more change in all measured properties compared with non-radiated seeds.

Conflict of interest

The authors declare no conflict of interest.

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