Phytochemical studies of *Eruca sativa* affected by gamma radiation or silicon

N. Hamideldin, and N. E. Eliwa.

Natural Product Department,
National Center for Radiation Research and Technology.
Atomic Energy Authority, P.O. Box 29, Nasr City, Cairo, Egypt.
*Corresponding author e-mail address: n.hamideldin@yahoo.comTel: 0022022748246
The second corresponding author e-mail address: nohaeliwa@hotmail.com

Abstract: Seeds of *Eruca sativa* were irradiated with different doses of gamma irradiation (0, 20, 40 and 60 Gy) or treated with different concentrations of sodium silicate (0, 2.5%, 5% and 7.5%) to study the effect of gamma irradiation and sodium silicate treatment on growth, seed oil composition and protein electrophoresis of the yield. The treatments induced significant enhancements of growth. The maximum increment observed with gamma radiation dose (60Gy). This enhancement was accompanied with an increase in photosynthetic pigments. Analysis of seed oil by gas chromatography – mass revealed that there was variation in concentration of fatty acids, aliphatic alcohol, carbonyl, phenol, sterol compounds, Sulphur and nitrogen compounds, alkaloids as well as alkane compounds. The results of SDS-PAGE electrophoresis of protein showed variation in appearance and disappearance of polypeptide bands in response to Na- silicate or gamma irradiation.
[N.Hamideldin, and N. E. Eliwa. **Phytochemical studies of *Eruca sativa* affected by gamma radiation or silicon.** *Researcher* 2015;7(8):94-104]. (ISSN: 1553-9865). http://www.sciencepub.net/researcher. 14

Keywords: Eruca sativa, Silicon, Foliar spray, fatty acid, SDS protein electrophoresis.

Introduction

Young plants of *Eruca sativa* (commonly known as rocket plant) are edible vegetables in salad, or as a green fodder among Europeans, because of its stimulant, stomachic, diuretic and antiscorbutic properties. Seeds of the plant have a large amount of thiofunctionalized glucosinolates along with erucic acid (cis-13-docosenoic acid) (Falk et al., 2004, Lazzeri et al., 2004)]. In industry, rocket seed oil has successful uses in soap making as lubricant, in massage as illuminating agent, in medicine, as well as in cooking (Miyazawa et al., 2002)**.** The oil contains a large amount of erucic acid that is an important industrial compound, so the plant is considered as a potential industrial crop. Another feature of erucic acid was its usage as adulterant for rapeseed or mustard oils. There is also a great demand for the amide of erucic acid, namely erucamide, for the production of cosmetics, detergents and polymers. Furthermore, the crude oil of the plant is used as an alternative mineral oil in industry because of its biodegradability property (Ahh et al., 2002).

The importance of silicon (Si) came from its involvement in metabolic, physiological, and/or structural activity in higher plants that are subject to abiotic and biotic stresses, although there is no evidence that the molecule is an essential plant constituent or metabolite (Shena et al., 2010). In this respect, silicon increase plant resistance to unfavorable conditions and stimulate plant immunity. Plants treated with silicon showed better performance and improved quality (Dębicz and Wróblewska, 2011). Silicon affects the yield of plant by deposition of the element under the leaf epidermis, which results in a physical mechanism of defense, reduces loading, increases photosynthesis capacity and decreases transpiration losses (Korndörfer et al., 2004). Many scientists studied the importance of silicon for plant growth and reported that reduced amount of silicon in plant developed necrosis, disturbance in leaf photosynthetic efficiency, growth retardation and reduced grain yield in cereals (Shashidhar et al., 2008). Silicon may be important in metabolic or physiological activities in higher plants, since treatment with silicon led to significantly increased protein contents, as compared to control plants (Hamid et al., 2012)**.**

Gamma rays are the most energetic form of electromagnetic radiation and possess energy levels from 10 keV (kilo electron volts) to several hundreds. They are considered the most penetrating radiation source, compared with other sources such as alpha and beta rays (Kovács and Keresztes, 2002). Gamma rays fall into the category of ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect the morphology, anatomy, biochemistry, and physiology of plants to different extents, depending on the irradiation level and plant species. Such effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system, and accumulation of phenolic

compounds (Kim et al., 2004; Wi et al., 2005). Gamma irradiation of seeds improves photosynthesis by modulating pigment system (Hegazi and Hamideldin, 2010). It also affects proteins by causing conformational changes, oxidation of amino acids, and formation of protein free radicals. Chemical changes in the proteins caused by gamma irradiation also include fragmentation, cross-linking, aggregation and oxidation by oxygen radicals that are generated through the radiolysis of water (Lee et al., 2005). The aim of this research was to investigate the effect of gamma irradiation and sodium silicate on *Eruca sativa* plant growth, photosynthetic pigments, seed oil composition and protein electrophoresis of the yield.

Materials and Methods

Seeds of *Eruca sativa* were obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Gamma irradiation treatments

Irradiation treatments were performed using Co-balt 60 source from unit Gamma Chamber 4000; at the National Center for Research and Radiation Technology, Nasr City, Cairo. The seeds were irradiated at 0, 20 40 and 60 Gy in the gamma cell (gamma facility PX-g-30 irradiator model, Russia) at room temperature and in the presence of air. Treated and untreated (control) seeds were sown in a randomized complete block experiment in sandy-loam soil. Agricultural practices such as irrigation, weeding, fertilization and pest control were carried out as recommended.

Sodium silicate treatments

Sodium metasilicate was obtained from Sigma Company, Egypt. After 15 days of sowing, the plants were sprayed with Na-silicate solution at either 2.5%, 5% or 7.5%, whereas the control plants were similarly sprayed with tap water. Foliar spraying was repeated every following 15 days until harvesting. Data were recorded (ten replications) after 60 days from sowing. The results were statistically analyzed using Multiplier Range test (Duncan, 1955). Different letters indicate significant variation.

Growth criteria

Plant height (cm), number of leaves/ plant, root length, plant fresh weight and plant dry weight.

Photosynthetic pigment

Chlorophyll a, chlorophyll b and carotenoids were determined by the spectrophotometric method (Metzner et al., 1965).

Extraction of the seed oil

The powdered of the yielded seeds samples of *E. sativa* used to extract total oil content on Soxhlet apparatus by using ethanol as solvent for 16 hours

GC-MS analysis

Samples were analyzed on a Hewlett-Packard model 6890 Series GC System equipped with a HP 5973 MS detector (EI mode, 70 eV). A column type, HP-5 (5% phenyl dimethyl siloxane), 30 m length, inside diameter 0.25 mm and a film thickness of 0.25µm, was used. The temperature of the column was programmed to increase after 5 min from 70 to 150°C at the rate of 2°C/min and then after 5 min from 150 to 250°C at the rate of 1°C/min. Helium was used as a carrier gas at a flow rate of 1 ml/min. The injector and detector temperatures were 250 and 280°C respectively. The components in the sample extract were identified by comparing based on gas chromatographic retention indices (Adams, 1996), mass spectra from Wiley MS Chemstation Libraries (6 th ed., G 1034, Rev.C.00.00, Hewlett-Packard, Palo Alto, CA).

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Young full expanded leaf of 50-day-old (from the top) were collected from different treatments and taken for protein analysis. Sodium Dodecyl Sulphate (SDS)-polyacrylamide gel electrophoresis was performed in 12% acrylamide gel following the system of (Laemmli, 1970) to identify protein profiles. Protein extraction was conducted by mixing 0.3 g leaves then ground to fine powder using a mortar and pestle and homogenized with 1 M Tris-HCl buffer, pH 6.8, in a clean eppendorf tube and left in refrigerator overnight. This was followed by centrifugation at 10.000 rpm for 10 min. The supernatant of each sample (containing the protein extract) was kept in a deep-freeze until use for electrophoretic analysis. The extract was boiled for 5 minutes in a water bath before loading the gel. Gel preparation was carried out according to (Hames, 1981), where 15% resolving gel was used. Bromophenol blue (0.001%) tracking dye was used for marking the buffer front during electrophoresis. The gel was electophorized at 25-30 mA costant current, then at 200 V (for about 8-9 hours). Staining was done using coomassie brilliant blue, and a solution of 10% acetic acid, and 45% methanol was used for de-staining. Gels were photographed, scanned, and analyzed using Gel Doc Vilber Lourmat system.

Results and Discussion

Growth criteria

The changes in growth criteria of *Eruca sativa* irradiated with (0, 20, 40, 60 Gy) gamma rays or grown at (2.5, 5, 7.5%) sodium silicate are illustrated in Table (1).

The results showed that the growth criteria of *Eruca* plants irradiated with gamma rays were more positively affected, as compared with those of either the control or the groups treated with sodium silicate. Increasing the dose of gamma irradiation significantly increased all growth parameters studied (plant heights, leaf number, root length, fresh and dry weights).

Plants irradiated with 60 Gy gave the highest enhancement of growth. Some authors referred to that pre-irradiation of seeds with low doses of gamma rays increased germination and growth of roots and leaves (Thapa, 1999). In general, other workers showed that gamma - irradiation of plants with low or high dose showed enhancement or inhibition of germination, seedling growth and other physiological and morphological changes (Wi et al., 2005; Kim et al., 2009). Some explanations for the stimulation effects of low dose gamma -irradiation have been available. Thus, low doses of gamma irradiation were found to induce growth. Stimulation by changing the hormonal signaling network in plant cells or by increasing the antioxidative capacity of cells (Kim et al., 2004; Wi et al., 2007). The total yield of rocket plant, seed mass, and essential oil content were significantly increased in response to irradiation by 20 Gy gamma radiation, as compared with control (Moussa, 2006).

Table (1): Growth criteria of 50-day-old *Erucasativa* plants from seeds of the untreated control (c), those irradiated with gamma rays (20, 40 and 60 Gy) or plants sprayed with Na-silicate (2.5%, 5% and 7.5%) after 15 days from sowing. Data were statistically analyzed using the multiplier range test. Each value is the mean of ten replicates. Different letters indicate significant variation.

Treatments		Plant height (cm)	Leaf No.	Root length (cm)	Fresh weight (gm)	Dry weight (gm)
Control	C	26.7^{b}	13.5^{ab}	12.8^{ab}	4.38^{a}	0.524^{b}
Gamma rays	20 Gy	27.8^{b}	9.8^{c}	9.6^{c}	2.14^{cd}	$o.278^{b}$
	40 Gy	31.0^{ab}	13.2^{ab}	12.7^{ab}	2.54^{bc}	0.396^{b}
	60Gy	37.1^{a}	14.0^{a}	13.1^{a}	4.18^{a}	0.544^{b}
Na-silicate	2.5%	31.3^{ab}	11.8^{abc}	10.1^{bc}	1.18^{d}	0.426^{b}
	5%	33.1^{ab}	12.2^{abc}	11.3^{abc}	3.41^{ab}	0.970^{a}
	7.5%	27.1^{b}	10.4^{bc}	12.2^{abc}	2.3^{bcd}	0.306^{b}

Concerning the effect of sodium silicate, the results indicated increase of all growth parameters up to concentration 5%, while increasing sodium silicate concentration to 7.5% caused a significant decrease in all growth parameters studied (plant heights, leaf number, root length, fresh and dry weights per plant).

These results are in accordance with those of (Dębicz and Wróblewska, 2011) who demonstrated that spraying with silicon caused increased plant growth and development of *Verbena* 'Patio Blue' (i.e. increased shoot length, branches, plant diameter and lateral shoots. Growth stimulation of barley plants variety Preriya, especially root system, was observed in response to presowing treatment of seeds with silicon compounds (Lozhnikova and Slastya, 2010). High concentration of sodium silicate increased height, tiller number, leaf area and total dry weight of *Oryza sativa* L. plants. The increase of potassium silicate levels in stem and leaf tissue enhanced all morphological parameters in tillers (Gerami et al., 2012).

Photosynthetic pigments

The data in Table 2 recorded that gamma irradiation doses 20 and 60 Gy significantly increased the contents of chlorophylls a and b, carotenoids and total pigments as compared to control. The maximum increase was observed in treatment with 60 Gy. On the other hand, treatment with 40Gy decrease the contents of the studied photosynthetic pigments. The same results were observed in other works, Chlorophyll mutation of Roselle plant, which consequently increased all yield-related traits, raised strongly as a result of treatment with high doses of mutagens including gamma irradiation (El Sherif et al., 2011). Various gamma ray doses also stimulated chlorophyll parameters and consequently yield components (Rascio et al., 2001; Baser at al., 2007; Rejili et al., 2008).

Table (2): Photosynthetic pigments (mg/g) of 50-day-old *Eruca sativa* leaves from seeds of the untreated control (c), those irradiated with gamma rays (20, 40 and 60 Gy) or plants sprayed with Na-silicate (2.5%, 5% and 7.5%) after 15 days from sowing. Data were statistically analyzed using the multiplier range test. Each value is the mean of three replicates. Different letters indicate significant variation.

Treatments		Chlo.a	Chlo.b	Carotenoids	Chlo.a/Chlo.b	Total pigments
Control	C	4,76b	4.75^{a}	1.00^{c}	0.171^{cd}	9.10^{c}
Gamma rays	20 Gy	4.95^{b}	3.88^{bc}	1.28^{c}	0.267^{c}	9.16^{c}
	40 Gy	4.80^{b}	4.27^{ab}	1.13^{c}	0.074^{d}	8.87^{c}
	60 Gy	4.82^{b}	4.32^{ab}	1.17^{c}	0.253^{cd}	10.56^{ab}
Na-silicate	2.5%	6.37^{a}	2.66^{d}	2.39^{a}	1.082^{a}	10.18^{b}
	5%	6.07^{a}	3.51^{c}	1.71^{b}	0.696^{b}	10.45^{ab}
	7.5%	6.86^{a}	4.15^{b}	1.65^{b}	0.090^{cd}	11.08^{a}

Chl.a =Chlorophyll a Chl.b = Chlorophyll b

Table 2 shows the effect of sodium silicate on photosynthetic pigments. It is observed that chlorophyll a, chlorophyll b and total pigments increased with increasing sodium silicate concentration from 2.5% to 7.5 %, while carotenoids and chlorophyll a/b showed a significant decrease with increasing sodium silicate concentration. These results are in agreement with those of some workers (Lana et al., 2003; Donegá, 2009) which concluded that silicon supported plant structure and increased photosynthesis. The level of chlorophyll a in *Oryza sativa* plants enhanced when plant treated with both silicon and nitrogen (Ávila et al., 2010).Higher levels of sodium and potassium silicate fertilizer raised the amount of chlorophylls a and b and total chlorophyll by 5% in barley plants (Lozhnikova and Slastya, 2010).

GC-MS profile of seed oil

Fatty acid and esters content

Analysis of *Eruca* oil by GC-MS (Table 3) showed constituting fatty acid retention time and percentages. The fatty esters (4-pentanoic acid ethyl ester, nonanoic acid ethyl ester, 4D-methylhexanoicacid ethyl ester, butancioic acid diethyl ester) disappeared under gamma irradiation and sodium silicate treatments but appeared at 20 Gy gamma irradiation. Heptadecanoic acid (margarinic acid) also appeared under irradiation by dose 40 Gy.

Gamma irradiation by 60 Gy induced the appearance of propanedoic acid ethyl ester, butanedioic acid diethyl ester, vanilic acid ethyl ester and octanoic acid ethyl ester, but 4- pentanoic acid ethyl ester and pentanoic acid (aleric acid) were disappeared.

Gamma irradiation of seeds 20 and 40 Gy induced the appearance of 2propenoic acid ethyl ester, as compared with either the control or other treatments.

The percentage of alanoic acid methyl ester increased under gamma radiation or sodium silicate effect. The percentage of linolelic acid methyl ester decreased with gamma irradiation, but increased with sodium silicate treatment.

Table (3): Fatty acids and esters percentage of *Eruca sativa* seeds from seeds of the untreated control, or those irradiated with gamma rays (20, 40 and 60 Gy) or plants sprayed with Na-silicate (2.5%, 5% and 7.5%) after 15 days from sowing.

Fatty acid and esters	Retention Time	Control	Gamma rays			Na- silicate		
		C	20Gy	40Gy	60Gy	2.5%	5.0%	7.5
4-pentanoic acid ethyl ester	4.96	------	0.27	-------	-------	-------	------	------
Propanedoic acid ethyl ester	8.70	0.36	-------	------	0.18	-------	------	------
Alanoic acid ethyl ester	9.37	0.30	0.87	0.39	1.13	0.28	0.32	0.29
Butanedioic acid diethyl ester	10.86	------	-----	------	0.35	-------	-------	------
Nicotinic acid ethyl ester	11.56	0.35	-----	0.16	0.67	-------	--------	------
Nonanoic acid ethyl ester	12.96	----	0.43	-----	----	-----	-----	-----
Alanoic acid diethyl ester	14.44	0.21	0.28	----	-----	-----	0.27	0.18
4D- Methylhexanoic acid ethyl ester	14.68	-----	0.19	-----	------	-----	------	-----
Arboxylic acid methyl ester	14.79	0.43	-----	------	------	------	-----	-----
Heptanoic acid	15.35	-----	0.48	------	-----	-------	0.49	-----
4- Pentanoic acid ethyl ester	16.90	7.17	18.96	6.50	------	20.27	3.64	1.67
Butancioic acid diethyl ester	16.16	----	0.16	-----	-----	------	-----	------
Nonanoic acid	17.27	2.25	2.22	3.12	0.74	1.99	1.20	0.62
Vanilic acid ethyl ester	17.73	----	-----	-----	0.35	----	-----	-----
2-Propenoic acid	17.95	4.57	-----	-----	-----	------	------	-------
Pentanoic acid (Aleric acid)	18.30	2.58	4.53	1.04	----	5.36	0.63	0.82
Heptanoic acid (Enthic acid)	18.54	----	1.52	----	30.10	1.87	----	----
6Heptanoic acid ethyl ester	18.69	----	------	----	0.13	-----	----	-----
2-Butenoic acid	19.06	0.74	-------	------	-----	------	-------	------

Octanoic acid ethyl ester	19.13	------	-------	------	1.59	-------	-------	-----
Tetradecanoic acid	20.05	1.28	------	------	0.20	-------	0.80	0.68
Z.7Hexadecanoic acid	22.39	0.58	0.50	0.51	1.80	0.75	0.46	0.80
Hexadecanoic acid (palmitic acid)	22.65	7.47	3.46	5.39	5.65	6.38	6.95	7.01
Hexadecanoic acid ethyl ester	22.99	0.25	1.15	1.26	3.58	1.19	1.99	2.56
Heptadecanoic acid (Margarinic acid)	23.79	0.25	-------	0.38	------	------	------	-----
Octadecenoic acid (Trans oleic acid)	24.78	14.86	14.16	15.17	11.23	14.54	14.24	16.09
Linoleic acid ethyl ester	24.97	--------	1.63	1.62	0.98	1.79	2.25	1.24
Ethyl oleate	25.02	4.41	-------	------	------	------	-------	2.13
2- propenoic acid ethyl ester	25.12	-----	0.99	1.63	------	------	-----	-----
Octadecanoic acid ethyl ester (stearic acid)	25.29	----	0.13	-----	0.97	-----	0.63	0.58
Octadecenoic acid (cis oleic acid)	26.88	1.49	0.52	0.28	0.51	0.42	0.74	0.72
Linolelic acid methyl ester (Eicosenoic acid)	27.15	1.45	0.90	1.24	1.40	1.71	1.77	1.87
Erucic acid	28.91	2.85	1.0	0.80	1.44	0.84	0.92	0.66
Erucic acid ethyl ester	29.15	3.69	2.93	4.30	3.45	2.52	5.19	7.50
Octadecanoic acid methyl ester	30.12	0.31	0.06	----	0.15	-----	-----	------
Pentadecyne (12- octadecadienoic acid methl ester)	30.86	0.23	----	-----	-----	------	0.31	-------
15-Tetracosenoic acid ethyl ester	30.02	-----	-----	------	0.35	------	0.47	0.46

It was also evident that both gamma radiation and sodium silicate decreased the percentage of octadecanoic acid ethyl ester and erucic acid. Meanwhile, gamma radiation and sodium silicate treatments caused disappearance of arboxylic acid methyl ester, 2-propenoic acid and 2- butenoic acid, as compared to the control.

Ethyoleate disappeared in response to gamma radiation or sodium silicate treatment except for 7.5% sodium silicate that showed only a decrease in its percentage.

Octadecanoic acid ethyl ester and linolelic acid methyl ester showed a decreased percentage than the control as affected by gamma radiation. In response to sodium silicate octadecanoic acid ethyl ester showed a decreased percentage, while linolelic acid methyl ester showed an increase. Sodium silicate caused disappear of octadecanoic acid methyl ester, as compared with control, whereas 5% Sodium silicate caused an increased content of petadecyne-12- octacadienoic acid methyl ester than the control, whereas it disappeared in all other treatments.

Aliphatic alcohol, carbonyl, phenol and sterol compounds

Table 4 illustrates the percentage of the aliphatic alcohol and carbonyl compounds in seeds of *Eruca sativa* analyzed by GC-MS. It could be observed that gamma irradiation at 20 and 60 Gy caused induction of 2- furanaldehyde, compared with control, while only 2.5% sodium silicate induced it. Gamma irradiation and 2.5% sodium silicate increased 2-furancarboxaldehyde above the control, but this compound was decreased with 5% sodium silicate and disappeared with 7.5% sodium silicate.

Undecenoic aldehyde appeared only on application of 60 Gy gamma radiation, whereas conifer alcohol and benzenamine appeared only on application of 20 Gy treatment. In addition, 4-Methoxy-2-hydroxy stibene decreased under the influence of 40 Gy gamma irradiation, but disappeared with the other treatments. Moreover, 20 and 40 Gy induced carbinoaxamin content, as compared with the control, but disappeared with all other treatments.

Table (4): Aliphatic alcohol , carbonyl , phenol and sterol compounds percentage of *Eruca sativa* seeds from seeds of the untreated control, or those irradiated with gamma rays (20, 40 and 60 Gy) or plants sprayed with Na-silicate (2.5%, 5% and 7.5%) after 15 days from sowing.

Compounds	Retention Time	Control	Gamma rays			Na- silicate		
			20Gy	40Gy	60Gy	2.5%	5.0%	7.5%
Aliphatic alcohols &carbonyl compounds								
2-Furanalaldehyde	4.07	-----	0.55	----	0.61	0.67	------	------
2-Furancarboxaldehyde	11.99	0.55	9.17	0.59	0.39	8.40	0.86	------
Undecenoic aldehyde	14.14	----	-----	-----	0.14	----	-----	------
Coniferyl alchol	18.98	----	0.96	----	---	---	-----	------
Benzenamine	20.39	----	0.53	----	------	-----	------	------
4-Methoxy-2-hydroxy stibene	21.15	0.51	-----	0.15	----	-----	-----	------
Carbinoaxamin	28.09	0.42	1.59	1.76	-----	-----	-----	------
Phenols and carbonyl compounds								
Benzaldhyde	6.47	----	-----	------	0.15	-----	-----	----
Cyclopentanol	7.29	----	----	----	0.26	----	-----	----
Methylene- cyclo pentanol	12.37	----	0.16	0.31	0.86	------	-------	0.46
4- Vinylguaiacol	13.37	0.67	0.89	0.93	0.04	0.53	0.67	0.24
Benzene acetaldehyde	13.50	-----	0.37	----	-----	0.33	-----	-----
Undecenoic aldehyde	14.14	----	-----	-----	0.14	----	------	-----
4-methyl-2,5 Dimethyl	17.40	2.25	2.67	3.12	------	1.99	1.20	0.62
Syringaldehyde	18.86	-----	0.64	----	-----	-----	------	--------
Cyclohexanon	26.67	------	0.41	0.58	-----	----	------	------
2 Benzenedicarboxilic acid (phathalat)	28.98	----	0.23	0.59	-----	------	------	8.11
Sterol compounds								
Gamma Tocopherol Vitamin A	34.54	1.28	1.27	3.09	-----	2.22	5.02	3.15
Cholest-5-en-3-ol Provitamin	35.71	1.47	1.16	2.17	2.72	-----	2.26	1.71
Crinosterol	36.56	2.59	2.19	4.46	1.22	2.66	3.55	3.46
Ergosta -5-en-3-beta-ol Campesterol	37.80	6.59	4.70	9.98	3.63	6.38	9.62	8.42
Stigma sterol	38.50	-----	-----	-----	0.49	------	------	0.69
Gamma sitosterol	39.88	17.41	14.01	28.24	9.80	17.82	25.69	23.67
Fucosterol	40.30	-----	----	0.70	----	-----	1.10	0.68

Phenols and carbonyl compounds (Table 4) showed occurrence of benzaldhyde, cyclopentanol and undecenoic aldehyde only under irradiation by 60 Gy but Syringaldehyde appeared under irradiation by 20 Gy. All doses of Gamma irradiation and the dose 7.5% of sodium silicate induced the appearance of methylene- cyclopentanol.

Benzene acetaldehyde appeared only under treatments by low doses of gamma irradiation (20Gy) and sodium silicate (2.5%). Cyclohexanon and 2

benzenedicarboxilic acid (phathalat) appeared under irradiation by 20 and 40 Gy also2 benzenedicarboxilic acid (phathalat) appeared under treatment by 7.5% of sodium silicate.

Concerning sterol compounds, Table 4 shows that gamma irradiation using 40 Gy and 5.0% sodium silicate were accompanied with a marked enhancement in the percentage of γ-tocopherol (Vitamin A), cholest-5-en-3-ol (Provitamin), crinosterol, ergosta-5-en-3-beta-ol (Campesterol) and γ-sitosterol. The high dose of gamma irradiation (40 Gy) and concentration of sodium silicate (7.5%) induced the appearance of stigma sterol, that disappeared in either the control or with all other treatments. Fucosterol appeared only in treatment with 40 Gy or 5.0% and 7.5%sodium silicate solutions.

Derivatives of sulphur, nitrogen, alkaloid, and alkane compounds

The effect of gamma radiation or sodium silicate appeared clearly on sulphur and nitrogen compounds (Table 5). Gamma radiation only at 20 Gy induced the occurrence of 5- methylmethanethoiosulphinate compound, compared to the control and other treatments. Hexanedinitrile compound was also induced at 60 Gy.

On the other hand, alkaloid compounds were also affected by gamma radiation or sodium silicate. Gamma irradiation at 60 Gy showed appearance of N-methoxymethoxylamine, compared with the control and other treatments. Furthermore, 20 Gy dose showed appearance of benzenamine that did not appear in the control and other treatments.

Table (5) : sulphur and nitrogen compound, alkaloids, and alkane compounds percentage of *Eruca sativa* seeds from seeds of the untreated control, or those irradiated with gamma rays (20, 40 and 60 Gy) or plants sprayed with Na-silicate (2.5%, 5% and 7.5%) after 15 days from sowing.

Compounds	**Retention Time**	**Control**	**Gamma rays**			**Na- silicate**		
			20 Gy	**40 Gy**	**60 Gy**	**2.5**	**5.0**	**7.5**
sulphur&Nitrogen compound								
5-Methyl methanethiosulphinate	6.84	----	0.25	-----	-----	-----	----	------
Methyl sulfanyl -pentanetrile	11.31	----	2.63	0.77	----	0.53	5.70	-----
Hexanedinitrile	18.51	-----	-----	-----	30.10	-----	-----	------
Alkaloid								
N-methoxy- methoxylamine	3.74	-----	-----	-----	0.81	-----	-----	------
isoglutamine	15.60	0.68	0.84	-----	1.10	-----	-----	------
Benzenamine	20.39	-----	0.53	------	------	------	------	------
4- methoxy2-hydroxy stilbene	21.15	0.51	----	0.15	----	-----	-----	------
trimethylstilbene	22.20	0.34	----	0.22	----	------	----	------
carbinoxamine	28.09	0.96	1.59	1.76	-----	------	------	------
Alkanes								
Propane	4.49	5.09	----	-----	-----	2.11	----	------
Butane	6.29	-----	0.21	----	------	-----	-----	------
Hexane	9.13	0.59	0.34	0.15	-----	-----	----	------
2-phenyl-1-methylen-ecyclopropane	10.49	-----	-----	----	0.85	----	----	----
1,1 Diethoxyhexane	18.16	----	0.55	----	-----	----	-----	-----
hexadecane	24.41	0.44	0.31	0.42	-----	-----	0.45	1.10
Nonacosane	32.32	----	0.48	----	0.55	----	1.12	0.34
1-octadecane	24.05	----	-----	-----	-----	----	----	0.22
1(cyclohexanon) methane	26.67	----	0.41	0.58	-----	------	------	-----
Nonadecane	28.37	-----	0.05	-----	------	------	0.06	0.04

It is valuable to note that sodium silicate has a negative effect on all alkaloid compounds of *Eruca sativa* since the alkaloid compounds disappeared under the influence of all concentrations of sodium silicate.

Gamma radiation showed also a great effect on the detected alkane compounds (Table 5). Thus, the dose 20 Gy induced the appearance of butane and 1, 1 diethoxyhexane and 2- phenyl-1-methylenecyclopropane appeared only in response to 60 Gy gamma. On the other hand, 2.5% sodium silicate induced the appearance of propane, compared to the control and other treatments. Gamma irradiation of oil seeds could produce chemical changes in different constants, especially lipids, by catalyzing their reaction with molecular oxygen causing auto-oxidation or by direct oxidation of the unsaturated site of fatty acids with free radicals (Afify et al., 2013). The fatty acid linoleic is considered to be most affected by radiation, where it showed over 2% decrease, on gamma irradiation with 7.5 kGy, followed by oleic, linolenic

and palmitoleic acids. Application of 2.5, 4.0, 5.5, and7.0 kGy doses of gamma irradiation to the clary sage (*Salvia sclarea* L.) seeds caused significant differences in composition by affecting all fatty acids except palmitic, palmitoleic, and eicosenoic acids (Yalcin et al., 2011).

It was also apparent that the accumulation of phenolic and flavonoid compounds was considerably affected by different levels of gamma irradiation (0, 10, 15, and 20 Gy) in *Curcuma alismatifolia* (Zingiberaceae) leaves and induce changes on C18:3n-3 in (Taheri et al., 2014)**..** Different doses of silicon were also found to increase phenolic acid content (Shetty et al., 2011).

Gamma irradiated melon plant led to appearance of new alcholol (nonyl alcohol) and carbonyl compound (nonylaldehyde, nonanoe) and alteration of their relative contents; changes that might be responsible for the formation of off- flavor in the melon juice (Ma et al., 2007).

Some workers (Fan and Mastovska, 2006; Harrison and Were, 2007) suggested that gamma irradiation might increase total phenolic compounds through release of phenolic compounds from glycosidic components and degradation of larger phenolic compounds into smaller ones.

SDS-Protein electrophoresis

The results of SDS-PAGE of soluble protein extracted from *Eruca sativa* leaves treated untreated or after treatment with gamma radiation or sodium silicate are shown in Figure 1 and Table 6. The molecular weights of polypeptide bands ranged from 5 to 2952 kDa.

The results obtained showed that the band with molecular weight 1319kDa disappeared under all treatments, but the band with molecular weight 172 kDa disappeared under gamma radiation treatments only. Gamma irradiation with 60 Gy induced the appearance of a band having a molecular weight 386 kDa, whereas a disappearance of the bands with molecular weights 410, 535 and 609 kDa was obtained. In other work, gamma irradiation was also found to cause changes in protein patterns of okra plant (Hegazi and Hamideldin, 2010). Gamma irradiation induced the appearance of the protein patterns ά, α and β with molecular weights 79, 70 and 50 kDa, respectively that corresponded to β-conglycinin of soybean protein, as compared to control (Afify et al., 2011).

High dose of sodium silicate (7.5%) induced the appearance of bands having molecular weights 1121, 1600 and 1923 kDa and disappearance of bands with molecular weights 5, 1464, 1670 and 2952 kDa. Sodium silicate treatment change the protein content of the plant that agree with other work which concluded that, application of 0.50% silicon (Si2) gave the maximum grain protein of *Oryza sativa* L. (Ahmad et al. , 2013).

Table (6): Protein pattern of 50-day-old *Eruca sativa* leaves from seeds of the untreated control (c), or those irradiated with gamma rays (20, 40 and 60 Gy) or plants sprayed with Na-silicate (2.5%, 5% and 7.5%) after 15 days from sowing.

Band No.	M.W. KDa	Control	Gamma rays			Na- silicate			Polymorphism
			20Gy	40Gy	60Gy	2.5%	5%	7.5%	
1	2952	1	1	1	1	1	1	0	Unique
2	1923	0	0	0	0	0	0	1	Unique
3	1886	1	1	1	1	1	0	0	Polymorphism
4	1670	1	1	1	1	1	1	0	Unique
5	1600	0	0	0	0	0	0	1	Unique
6	1464	1	1	1	1	1	1	0	Unique
7	1319	1	0	0	0	0	0	0	Unique
8	1269	0	1	1	1	1	1	0	Polymorphic
9	1121	0	0	0	0	0	0	1	Unique
10	1065	1	1	1	0	0	1	0	Polymorphic
11	985	0	0	0	1	1	0	0	Polymorphic
12	780	1	1	1	1	1	1	1	Monomorphic
13	609	1	1	1	0	1	1	1	Unique
14	535	1	1	1	0	1	1	1	Unique
15	490	0	0	0	0	0	0	1	Unique
16	410	1	1	1	0	1	1	1	Unique
17	386	0	0	0	1	0	0	0	Unique
18	300	1	1	1	1	1	1	1	Monomorphic
19	220	1	1	1	1	1	1	1	Monomorphic
20	172	1	0	0	0	1	0	1	Polymorphic

21	122	1	1	1	1	1	1	1	Monomorphic
22	80	1	1	1	1	1	1	1	Monomorphic
23	50	1	1	1	1	1	1	1	Monomorphic
24	30	1	1	1	1	1	1	1	Monomorphic
25	20	1	1	1	1	1	1	1	Monomorphic
26	15	1	1	1	1	1	1	1	Monomorphic
27	5	1	1	1	1	1	1	0	Unique
Total bands		21	20	20	18	21	19	18	

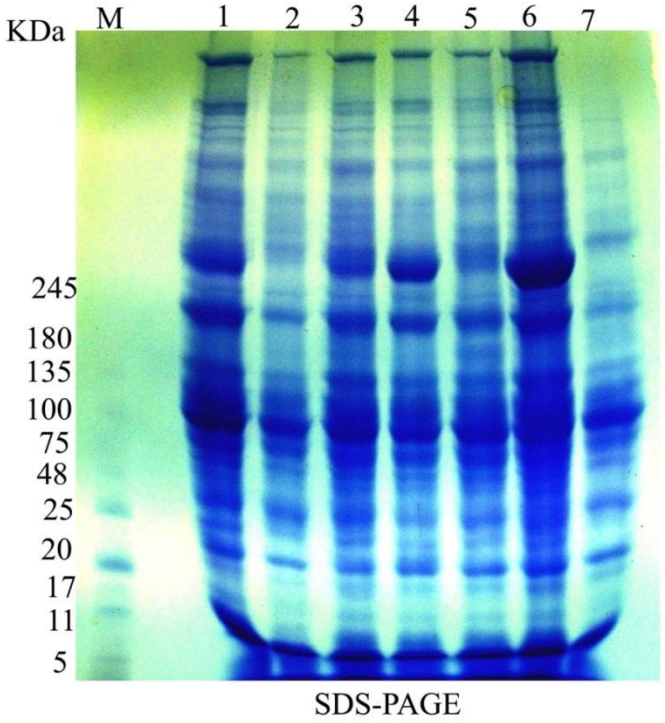

Figure (1): Protein pattern of 50-day-old *Eruca sativa* leaves.
1= untreated control (c)
2, 3, 4= irradiated with gamma rays (20, 40 and 60 Gy)
5, 6, 7= plants sprayed with Na-silicate (2.5%, 5% and 7.5%)
M= Marker

Conclusion

Gamma irradiation or sodium silicate induced enhancement of growth and photosynthetic pigments. The maximum increment observed with gamma radiation dose (60Gy). While, they showed change in oil composition and variation in protein bands when compared with untreated plants (control).

References

1. Adams, R.P. 1996. Identification of essential oil components by gas chromatography and mass spectroscopy. Biochem. Syst. Ecol. 24(6): 594.
2. Afify, A.M.R., El-Beltagi, H.S. Fayed, S.A., Shalaby, E.A., 2011. Acaricidal activity of successive extracts from *Syzygium cumini* L. Skeels (Pomposia) against Tetranychus urticae Koch. Asian Pac. J. Trop Biomed. 1(5).359-364.
3. Afify, A.M. R., Rashed, M.M., Ebtesam, A.M., El-Beltagi, H.S.2013. Effect of gamma radiation on the lipid profiles of soybean, peanut and sesame seed oils. Grasasy Aceites. 64 (4), 356-368.
4. Ahh, A., Khalil, M.K., Farrag, A.M., 2002. Nitrate accumulation, growth, yield, and chemical composition of rocket (Eruca sativa) plant as affected by NPK fertilization, kinetin and salicylic acid. Ann. Agric. Sci. (Cairo) 47, 1–26.
5. Ahmad, A., Afzal, M., Ahmad, A.U.H., Tahir, M., 2013.Effect of foliar application of silicon on

yield and quality of Rice (*Oryza sativa* L). Cercetari Agron. Moldova. 46 (3), 21-28.
6. Ávila, F.W., Baliza. D.P., Faquin, V., Araujo, J., Ramo, S.J., 2010. Silicon -nitrogen interaction in rice cultivated under nutrient solution. Revista Ciencia Agron. 41, 184–190.
7. Baser, I., Bilgin, O., Korkut, K.Z., Balkan, A., 2007.Improvement of some quantitative characters by mutation breeding in durum wheat. Tarim-Bilimleri-Dergisi. 13(4), 346-353.
8. Dębicz, R., Wróblewska, K., 2011. The effect of silicon foliar napplication on the development of seasonal ornamental plants. PART I: *Sanvitalia speciosa* 'Sunbini', Verbena 'Patio Blue' and *Portulaca umbraticola* 'Duna Red'. Acta. Agro. Botan. 64 (4), 99–106.
9. Donegá, M.A., 2009. Ratio K: Ca and application of silicon in the nutrient solution for the hydroponic cultivation of coriander. [MSc.n Dissertation] Piracicaba High School of the Agriculture, Luiz de Queiroz. 1–62.
10. Duncan, B.D., 1955. Multiple range and multiple F tests; Biometrics 11, 1.
11. El Sherif, F., Khattab, S., Ghoname, E., Salem, N., Radwan, K.H., 2011. Effect of Gamma Irradiation on Enhancement of Some Economic Traits and Molecular Changes in *Hibiscus sabdariffa* L. Life Sci. J. 8(3).
12. Falk KL, Vogel C, Textor S, Bartram S, Hick A, Pickett JA. 2004. Glucosinolate biosynthesis: Demonstration and characterization of the condensing enzyme of the chain elongation cycle in *Eruca sativa*. Phytochem. 65, 1073–84.
13. Fan, X., Mastovska, K., 2006. Effectiveness of ionizing radiation in reducing furan and acrylamide levels in foods. J Agric. Food Chem. 54, 8266-8270.
14. Gerami, M., Fallah, A., Khatamimoghadam, M., 2012. Study of potassium and sodium silicate on the morphological and chlorophyll content on the rice plant in pot experiment (*Oryza sativa* L.). Inter. J. Agri. Crop. Sci. 4(10), 658-661.
15. Hames, B.D., 1981. An introduction to polyacrylamide gel electrophoresis. IN: Gel Electrophoresis of Proteins. PP. 1-91. B.D. Hames and D. Richwool (Eds.). A Prractical Approach.IR Press. Oxford Ltd. (Pnb.). UK.
16. Hamid, N., Naz, B., Rehman, A., 2012 Effect of Exogenous Application of Silicon with Drought Stress on Protein and Carbohydrate Contents of Edible Beans (*Vigna radiate & Vigna unguiculata*). Pak. J. Chem. 2(2), 99-105.
17. Harrison, K., Were, L.M., 2007. Effect of γ-irradiation on total phenolic content yield and antioxidant capacity of Almond skin extracts. Food Chem. (102), 932 – 937.
18. Hegazi, A.Z., Hamideldin N., 2010. The effect of gamma irradiation on enhancement of growth and seed yield of okra [*Abelmoschus esculentus* (L.) Moench] and associated molecular changes. J. Hort. Forestry. 2(3), 38-51.
19. Kim, J.H., Baek, M.H., Chung, B.Y., Wi, S.G., Kim, J. S., 2004.Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (*Capsicum annuum* L.) seedlings from gamma-irradiated seeds. J. Plant. Biol. 47, 314-321.
20. Kim, J.H., Lee, M.H., Moon, Y.R., Kim, J.S., Wi, S.G., Kim, T.H., Chung, B.Y., 2009. Characterization of metabolic disturbances closely linked to the delayed senescence of Arabidopsis leaves after gamma-irradiation. Environ. Exp. Bot. 67(2), 363-371.
21. Korndörfer, G.H., Pereira, H.S., Nolla, A., 2004. Silicon analysis in soil, plant and fertilizers. Boletim. Técnico. (2), 34.
22. Kovács, E., Keresztes, A,. 2002. Effect of gamma and UV-B/C radiation on plant cells. Micron. 33(2), 199-210.
23. Laemmli, U.K. 1970. Clearage of structural proteins during the assembly of the head bacteriophage T4. Nature 227, 680-685.
24. Lana, R.M.Q., Korndorfer, G.H., Zanão Júnior, L.A., Silva, A.F., Lana, A.M.Q., 2003.Effect of calcium silicate on the productivity and silicon accumulation in the tomato plant. Biosci. J. 19, 15–20.
25. Lazzeri, L., Errani, M., Leoni, O., Venturi, G., 2004. *Eruca sativa* spp.Oleifera: A new non-food crop. Ind. Crop. Pro. 20, 67–73.
26. Lee, S., Lee, M., Song, K., 2005. Effect of gamma-irradiation on the physicochemical properties of gluten films. Food Chem. 92, 621-925.
27. Lozhnikova, V. N., Slastya, I.V., 2010. Growth of spring barley and activity of endogenous phytohormones under the influence of silicon compounds. Agr. Biol. 3, 102-107.
28. Ma, Y. X., Chen, J., Wu, J., Zhao, G., 2007. Effect of freezing modes and frozen storage on aroma, enzyme, and micro-organism in Hami melon juice. Food Sci. and Techno. Inter. (13), 259−267.
29. Metzner, H., H. Rau, and H. Senger, 1965. "Untersuchungen zur Synchronisierbarkeit einzelner Pigmentmangel Mutanten von Chlorella". Planta (65): 186-189.
30. Miyazawa, M., Maehara, T., Kurose, K., 2002. Composition of the essential oil from the leaves of *Eruca sativa*. Flav. Frag. J. 17,187–90.
31. Moussa, H.R., 2006 Role of gamma irradiation in regulation of NO3 level in rocket (*Eruca*

vesicaria subsp. sativa) plants. Rus. J. Plant Physiol. 53(2), 193-197.
32. Rascio, A., Russo, M., Mazzucco, L., Plantani, C., Nicastro, G., Difonzo, N., 2001. Enhanced osmo-tolerance of a wheat mutant selected for potassium accumulation. Plant Sci. 160, 441-448.
33. Rejili, M., Telahigue, D., Lachihe,b B., Mrabet, A., Ferchichi, A., 2008. Impact of gamma radiation and salinity on growth and K^{+}/Na^{+} balance in two populations of *Medicago sativa* (L.) cultivar Gabès. Prog. Nat. Sci 18(9), 1095-1105.
34. Shashidhar, H.E., Chandrashekhar, N., Narayanaswamy, C., Mehendra, A.C., Prakash, N.B., 2008. Calcium silicate as silicon source and its interaction with nitrogen inaerobic rice. Silicon in Agriculture: 4th International Conference 26-31October, South Africa: 93.
35. Shena, X., Zhoua, Y., Duana, L., Lia, Z.A., Enejib, E., 2010. Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. J. Plant Physio. 167 (15), 1248–1252.
36. Shetty, R., Frette, X. , Jensen, B., Shetty, N. P., Jensen, J. D., Jorgensen, H. J. L., Newman, M., Christensen, L. P., 2011. Silicon-Induced Changes in Antifungal Phenolic acids,Flavonoids, and Key Phenylpropanoid Pathway Genes during the Interaction between Miniature Roses and the Biotrophic Pathogen *Podosphaera pannosa*. Plant Physiol. 157, 2194–2205.
37. Taheri, S., Abdullah, T. L., Karimi, E., Oskoueian, E., Ebrahimi, M., 2014. Antioxidant Capacities and Total Phenolic Contents Enhancement with Acute Gamma Irradiation in *Curcuma alismatifolia* (Zingiberaceae) Leaves Int. J. Mol. Sci, 15, 13077-13090.
38. Thapa, C.B., 1999. Effect of acute exposure of gamma rays on seed germination of *Pinus kesiya* Gord *and P. wallichiana* A.B. Jacks. *Botanica Orientalis*. J. Plan. Sci. 2,120-121.
39. Wi, S.G., Chung, B.Y., Kim, J.H., Baek, M.H., Yang, D.H., Lee, J.W., Kim, J. S., 2005. Ultrastructural changes of cell organelles in Arabidopsis stem after gamma irradiation. J. Plant. Biol .48(2), 195-200.
40. Wi, S.G., Chung, B.Y., Kim, J.S., Kim, J.H., Baek, M.H., Lee, J.W., Kim, Y.S., 2007. Effects of gamma irradiation on morphological changes and biological responses in plants. Micron. 38(6), 553-564.
41. Yalcin,H., Ozturk, I., Tulukcu E., Sagdic, O., 2011. Effect of γ-irradiation on bioactivity, fatty acid compositions and volatile compounds of clary sage seed (*Salvia sclarea* L.). J. Food Sci. 76(7), 56-61.

8/23/2015