Effect of Different Doses of Gamma Irradiation on Vegetative Growth and Oil Yield of *Ocimum basilicum* L.

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ABSTRACT

Gamma irradiation is important agent used to improve the productivity and quality of many plants. The present study was carried out to evaluate the effectiveness of different doses of gamma rays (0, 5, 10, 15, 20, 25, 30 kR) on germination percentage, vegetative growth, photosynthetic pigments, oil yield, oil components and total phenolic content of *Ocimum basilicum* L during the two successive seasons of 2017 and 2018 at the Experimental Farm of Sakha Horticulture Research Station, Kafr El-Sheikh Governorate, Egypt. Results showed that low doses of gamma irradiation significantly increased germination percentage, oil yield per plant and per fed, chlorophyll a and chlorophyll b, essential oil percentage, oil yield per plant and per fed, chlorophyll a and chlorophyll b, essential oil percentage, vegetative growth characters and oil yield while total carotenoids content and total phenolic content significantly increased as a defensive effect to the increase in irradiation doses. The study suggested 10kR dose as seeds treatment for increasing basil plants production through enhancing germination percentage, vegetative growth, fresh yield, essential oil yield and essential oil components. **Keywords:** Irradiation, *Ocimum basilicum* L, Phenolic content, Essential oil

INTRODUCTION

Basil (Ocimum basilicum L.) is a member of the Lamiaceae Family, which is a widely cultivated aromatic crop grown either for the production of essential oil, dry leaves for the market or as an ornamental (Simon et al., 1990). Basil includes annual and perennial herbs, and shrubs native to the tropical and subtropical areas of Asia, Africa and South America (Darrah, 1988). Almost 60 species of basil are recognized all over the world and widely planted in France, Egypt, Hungary, Indonesia, Morocco, The United States, Greece and Israel (Srivastava, 1980). Both the fresh and dry leaves of the plant are consumed in the food and spice industries. Additionally, it is also has been used for headaches, coughs, worms, stomach-ache and kidney malfunctions in medical therapies (Simon et al., 1990). Furthermore, it is also deemed as a source of aroma combinations and accordingly, owns a range of biological properties such as insect repellent, nematocidal, antibacterial, antifungal agents and antioxidant properties (Deshpande and Tipnis, 1977, Simon et al., 1990 and Juliani and Simon, 2002).

Gamma radiation, more energetic than X-rays, is implemented from sources of radioactive isotopes, cesium-137 or cobalt-60, and it is specified by the World Health Organization as a food preservation technique that enhances food safety without modifying the toxicological, biological or nutritional quality of the food (Diehl, 2002, Datta, 2009 and Farkas and Mohácsi-Farkas, 2011). Furthermore, (Mokobia and Anomohanran, 2005) indicated that gamma irradiation were beneficial not only for sterilization of medicine but also for the preservation of food and cereals in nutrition and agriculture. Irradiation was successfully applied to solve many agricultural problems: reduction of post-harvest losses (Villavicencio et al., 2018) and enhancement salt stress tolerance (Pimonrat and Yi, 2017 and Aly et al., 2018). Additionally, It is one of the important physical agents utilized to enhance the quality of medicinally valuable plants, including their biomass production (Hamideldin and Eliwa, 2015, Verma et al., 2017 and Masoud et al., 2018) through prompting cytological, genetical, biochemical, physiological and morphogenetic variations in cells and tissues relying on the irradiation level (Datta, 2009). Due to the low wavelength with elevated penetrable power of gamma rays, its interaction with atoms or molecules to generate free

radicals in the cells. Free radicals have been informed to induce changes in cellular structure and metabolism, *e.g.* dilation of thylakoid membranes, alteration in photosynthesis, modulation of antioxidative system and accumulation of phenolic compounds (Wi *et al.*, 2007 and Masoud *et al.*, 2018 and Villavicencio *et al.*, 2018).

Plant phenolics are commonly involved in protection versus ultraviolet radiation or attack by pathogens, parasites and predators, as well as contributing to plants (Dai and Mumper, 2010). It has been reported that total flavonoids as a group of phenolics responded significantly to gamma radiation doses particularly 64 k-rad in different dill plant parts (Said-al Ahl *et al.*, 2015).

Likewise, (Masoud *et al.*, 2018) stated that 40 Gy dose increased accumulation of total phenolic compounds in *Cichorium pumilum* Jacq. roots also, gamma rays at 600Gy produced the highest phenol content of *Abelmoschus moschatus* (Suneetha *et al.*, 2018). The importance of phenols for plants is due to a fair correlation among antioxidant/free-radical scavenging activity and its phenolic content. Furthermore, phenol compounds are thought to protect the plant against irradiation-induced oxidative stress (Masoud *et al.*, 2018).

Regarding *Ocimum basilicum*, no published literature exists about the impact of gamma irradiation treatments on changes in its phenolic content and essential oil yield. Accordingly, the aim of the current work is to study the influence of different doses of gamma irradiation on germination, vegetative growth, potential changes in the total phenolic content and oil percentage as well as oil components of *Ocimum basilicum* L. plants.

MATERIALS AND METHODS

Seeds of sweet basil (*Ocimum basilicum* L.) were acquired from Medicinal and Aromatic plants Research Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. 500 dry seeds were subjected to 0, 5, 10, 15, 20, 25, 30 krad (kR) gamma-irradiation doses using cobalt-60 with dose rate of 3.58 min /10 kR at the National Center for Radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt.

The irradiated and non-irradiated seeds were sown in the greenhouse on 1st and 3rd March 2017 and 2018 seasons respectively into a combination of vermiculite and peat moss (2:1), the seedlings were transplanted on 15th



and 17th April 2017 and 2018 respectively on 30 cm× 60 cm plant spacing at the Experimental Farm of Sakha Horticulture Research Station Kafr El-Sheikh, Egypt on a complete randomized blocks design with three replications.

The soil of the experimental site was clay texture and it contained 54.10% clay, 26.30% silt and 19.60% sand. The electrical conductivity (EC) and pH of the soil in the saturated soil paste were 2.65 and 8.15 dSm⁻¹, respectively as mean of 0- 60 cm depth all chemical and physical properties were determined according to Page *et al.* (1982). All agricultural practices were done according to the Ministry of Agriculture and Land Reclamation recommendation.

Seeds germination percentage, was recorded after one week from germination which estimated by using this formula

$$Germination(\%) \frac{Number of seeds germinated}{Total number of seeds sown for germination} \times 100$$

Determination of photosynthetic pigments, chlorophyll a, b and total carotenoids content were evaluated in fresh leaves for both cuts of two seasons by the spectrophotometric method recommended by (Lichtenthaler and Buschmann, 2001). The pigment contents were assessed as mg/cm² fresh weight

Plants were harvested on July 15th and September 15th in 2017 and 2018 seasons respectively then the following data were registered: plant height (cm), number of main branches, plant fresh weight (g), plant dry weight (g) and fresh herb yield (t/fed.)

Herb essential oil percentage, plants were harvested and 100 gm fresh herb was utilized to a 3 h hydrodistillation apparatus according to (Pharmacopoeia, 1963). The essential oil ratio of the plants was reported by a volumetric method (ml/100 g) then oil yield per plant as well as per fed(1) was calculated and the isolated essential oil was retained at 4 C° until the gas chromatography analysis which was performed at Medicinal and Aromatic Plants Research Department Lab., Horticulture Research Institute, Giza, Egypt that was examined by DsChrom 6200 Gas Chromatograph prepared with a flame ionization detector for separation of volatile oil constituents. The analysis conditions were as follows: The chromatograph apparatus was connected with capillary column BPX-5, 5% phenyl (equiv.) polysillphenylene -siloxane 30 m x 0.25mmID x 0.25 µm film. Temperature program ramp rise with average of 10 C°/min from 70 to 200 C°. Flow rates of gases were nitrogen at 1 ml/min, hydrogen at 30ml/min and 330ml/min for air. Temperatures of detector and injector were 300C° and 250C°, respectively. The acquired chromatogram and report of GC analysis were analyzed to evaluate the percentage of essential oils main components.

Total phenolic content was evaluated quantitatively according to the method illustrated by (Jindal and Singh, 1975). A standard curve was made by using several concentrations of gallic acid and utilized for the determination of total phenolic content (mg/g d.wt).

Statistical analysis of variance (ANOVA) was conducted using COSTAT software. Differences between treatments means were carried out by Duncan's multiple range test (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Germination percentage

The lower gamma irradiation doses (5 and 10kR) noticeably stimulated basil seed germination while,

increased doses of gamma rays treatment (15 to 30kR) significantly reduced germination percentage as compared to control plants during both seasons (Fig., 1). These findings are consistent with the results presented by (Bhosale and More, 2014) on Withania somnifera, L., (Bhat et al., 2016) on Linum usitatissimum L., (Verma et al., 2017) on Foeniculum vulgare Mill. and Bhala and Verma, (2018) on Solanum lycopersicum that high levels of gamma irradiation had deleterious effects on seed germination percentage. The reduction in seed germination at raised doses of gamma irradiation could be due to the injury in seed tissue, chromosomes and subsequent mitotic retardation and the severity of the damage depend on the applied doses (Datta, 2009). Additionally disturbs the synthesis of protein, hormone balance, gas- exchange, water exchange and enzyme activity (Hameed et al., 2008).



Fig. 1. Effect of different doses of gamma irradiation on seeds germination percentage of *Ocimum basilicum* L. during both seasons of 2017 and 2018.

Vegetative growth characteristics

Increasing gamma irradiation doses significantly decreased plant height, the highest plants were reported for control and treated plants with 5kR respectively without significant differences between them in the first cut only while control plants recorded the greatest values for plant height in the second cut of both growing seasons (Table, 1). These remarks were in accordance with Bhosale and More (2014) who noticed a gradual decrease in Withania somnifera seedling height when gamma rays increased from 10 to 40kR, Khan et al.(2014) treated seeds of Brassica napus L. by gamma rays doses (0, 10, 15, 20, 25 and 30kR) and exhibited that elevated doses of radiation markedly reduced plant height compared with control. Similarly, Verma et al.(2017) stated that all levels of gamma rays (15, 17.5, 20, 22.5 and 25kR) reduced the fennel seedling height. The decline in plant height because of high irradiation doses can be attributed to minimize in mitotic activity of meristematic tissues and decreased seeds moisture content.

The maximum number of branches were noticed for plants irradiated with 10kR followed by 5kR without significant differences among them in the first cut of the first season only as compared with un-irradiated plants (Table, 1). These outcomes were in harmony with the observation of Hamideldin and Eliwa,(2015) in which branches number of mustard plants were progressively increased with rising gamma irradiation doses from 10 to 50Gy. Moreover, branches number were significantly diminished with raising gamma rays doses therefore, the least branches number was recorded for irradiated basil plants with 30kR. A reduction in branches number for several crops that were subjected to elevated gamma-ray doses had been previously reported (Khan *et al.*, 2014 and Verma *et al.*, 2017).

Table 1. Effect of various doses of gamma irradiation on plant height, main branches number, fresh and dry weight (g/plant) and fresh vield (t/fed.) of Ocimum basilicum L. during the two cuts of both seasons 2017 and 2018.

	1 st Season 2017								
	1 st cut			2 nd cut					
Dose (kR)	Plant	Branches	Fresh	Dry	Plant	Branches	Fresh	Dry	Fresh yield
	height (cm)	No.	weight (g)	weight (g)	height (cm)	No.	weight (g)	weight (g)	(t/fed.)
Control	70.66 a	13.66ab	221.53 b	71.32b	74.66 a	15.00c	300.00 c	95.41c	11.59b
5kR	70.66a	13.00abc	266.74a	87.75a	67.33 b	16.66b	347.90b	115.33b	13.66a
10kR	66.66b	14.33a	260.51a	82.11a	60.66 c	22.00a	377.20a	121.05a	14.17a
15kR	61.00 c	10.66bcd	182.86 c	62.43bc	58.00 d	11.33d	272.90d	72.62d	10.11c
20kR	58.00cd	9.66cd	168.40cd	58.61c	56.33 d	12.66d	266.00e	71.71d	9.65c
25kR	55.33de	9.66cd	165.85cd	48.73d	54.50 e	11.33d	242.04f	52.08f	9.64 d
30kR	54.00 e	7.66d	140.00 d	42.40d	51.00 f	9.33e	230.53g	57.54e	8.23e
2 nd Season 2018									
Control	71.63a	14.64ab	222.50b	70.37b	73.69a	15.95c	289.04c	93.41c	11.37b
5kR	71.63a	13.98abc	267.70a	86.81a	66.36b	17.61b	336.94b	113.32b	13.44a
10kR	67.63a	15.31a	261.48a	81.16a	59.69c	22.95a	366.24a	119.04a	13.95a
15kR	61.96b	11.64bc	187.70c	61.48bc	57.02d	12.28de	255.04d	70.61d	9.75d
20kR	58.97b	7.95 d	183.83c	57.66c	53.62e	13.61d	212.75e	67.95e	8.90d
25kR	53.96c	10.64 cd	161.82cd	49.45cd	50.53f	10.75e	213.91e	50.08g	8.34e
30kR	54.96c	8.64cd	140.97d	41.45d	50.02f	10.75e	201.72f	55.53f	7.62f

Means designed by the same letter at each column are not significantly different at the 5% level according to Duncan's multiple range test.

Fresh and dry weight (g/plant), as well as fresh vield (t/fed.) were significantly affected by gamma irradiation doses (Table, 1). The maximum values were recorded from plants irradiated with 5 and 10kR without significant variation between them in the first season only and 10 kR in the second season. The greatest impact of gamma irradiation doses on total yield was in conformity with the previous results of Hamideldin and Eliwa, (2015) in which total biomass of mustard plants was significantly enhanced when gamma rays dose increased from 10 to 50 Gy. The stimulatory effect of 5 and 10kR dose on fresh and dry weight were because gamma rays promote the role of enzyme as well as growth hormone responsible for growth and yield (Wi et al., 2007). As the dose of gamma rays increased from 15 to 30kR, there was a gradual decrease in the fresh, dry weight and total fresh yield of basil plants. These outcomes were in accordance with the observation of Bhat et al.(2016) who remarked that gamma rays at 15kR noticeably reduced dry weight of Linum usitatissimum L. plants. Likewise, Verma et al. (2017) noticed that about 50% reduction in fennel vegetative growth parameters were obtained at 20kR of gamma rays. The decrease in plants fresh and dry weights may be due to diminishing moisture content because of radiation stress especially when subjected to high doses of gamma rays.

Photosynthetic pigments

Irradiating plants with gamma rays at 10kR in the first cut and 15kR in the second cut for both growing seasons enhanced chlorophyll a and b contents as compared to control (Fig., 2). It was also observed that chlorophyll-a was greater than chlorophyll-b in each irradiated and non-irradiated plants. The present results are in conformity with the observations of Hamideldin and Eliwa, (2015) in which gamma irradiation increased photosynthetic pigments contents in the leaf of mustard plants also, Aly et al. (2018) reported that irradiated wheat plants with 100 and 200Gy of gamma rays improved photosynthetic pigment content under salt stress conditions. Increased chlorophylls by irradiation may be due to stimulation biosynthesis of chlorophyll and/or delaying of its degradation (Aly et al., 2018). Furthermore, alteration in photosynthesis in the irradiated plants maybe partly participates to increase growth (Wi et al., 2007). On the other hand, the higher dosage of gamma irradiation had recorded low chlorophyll content that was in conformity with the previous results of Suneetha et al.(2018) on Abelmoschus moschatus and Masoud et al. (2018) on Cichorium pumilum Jacq. Impact of gamma irradiation intensities on chlorophyll was assessed in terms of disturbed hormonal balance, leaf gas-exchange, water exchange and enzyme activity (Stoeva, 2002).

Concerning carotenoids content, increasing gamma irradiation levels from 5 to 30kR markedly increased carotenoids content of basil leaves (Fig., 2). The highest carotenoids content was achieved from plants irradiated with 30kR without significant differences between high irradiation dosages as compared with non-irradiated plants which recorded the lowest carotenoids content in both growing seasons. Increases in basil carotenoids content are a significant way to increase plant resistance to abiotic stresses (Shala and Mahmoud, 2018). Similar results were earlier reported by Masoud et al. (2018) in which carotenoids content increased as a defensive impact to the increase of gamma doses and the highest carotenoids content produced from irradiated chicory plants with 80Gy. Essential oil percentage and oil yield

Oil percentage and oil yield/plant (ml) and/fed (l) were increased gradually at low doses of gamma irradiation the highest values were produced from irradiated plants with 10kR as compared with control treatment (Table, 2). Meanwhile, the least values for oil percentage and oil yield were obtained from irradiated plants with 25 and 30kR without significant differences among them in both seasons. Similar observations were formerly reported by Khan et al. (2014) who detected that oil content of Brassica napus L. considerably declined with high radiation doses (30kR). Likewise, Hamideldin and Eliwa, (2015) demonstrated that fatty acids contents of mustard seeds were changed by gamma irradiation doses and Masoud et al. (2018) suggested that gamma irradiation at 40Gy was an effective dose to stimulate the metabolism and accumulation of active compounds of chicory plants. Gamma ray increase secondary metabolites yield by enhancing the activity of certain key biosynthetic enzymes as reported by Vardhan and Shukla, (2017).



Fig. 2. Effect of different doses of gamma irradiation on chlorophyll a, b and carotenoids content (mg/cm²F.W.) of Ocimum basilicum L. during the two cuts of both seasons (2017/2018).

Table 2. Effect of different doses of gamma irradiation on oil percentage,	oil yield/plant (ml) and oil yield /fed (l)							
of Ocimum basilicum L. during the two cuts of both seasons 2017 and 2018.								

	1 ²² Season 2017							
	1 ^s	^t cut	2 ⁿ					
Dose (kR)	Oil percentage	Oil yield /plant(ml)	Oil percentage	Oil yield /plant(ml)	Oil yield (l/fed.)			
Control	0.10 d	0.22cd	0.15c	0.45d	14.93d			
5kR	0.16 b	0.43b	0.19b	0.66b	24.16b			
10kR	0.19 a	0.50a	0.21a	0.79a	28.61a			
15kR	0.13c	0.24c	0.20ab	0.54c	17.39c			
20kR	0.13c	0.22cd	0.13d	0.35e	12.55e			
25kR	0.10d	0.17de	0.10e	0.24f	9.06f			
30kR	0.11d	0.15e	0.11e	0.25f	9.06f			
2 nd Season 2018								
Control	0.14d	0.31b	0.14d	0.40d	15.92c			
5kR	0.18a	0.48a	0.20b	0.67b	25.67b			
10kR	0.18a	0.47a	0.23a	0.84a	29.20a			
15kR	0.16b	0.29b	0.18c	0.46c	16.75c			
20kR	0.15c	0.28b	0.11f	0.23e	11.46d			
25kR	0.12e	0.20c	0.12ef	0.26e	10.25e			
30kR	0.13e	0.18c	0.13de	0.26e	9.90e			

Means designed by the same letter at each column are not significantly different at the 5% level according to Duncan's multiple range test.

Essential oil components

Gas chromatographic analysis for basil essential oil detected the existence of 22 compounds of them 14 components were distinguished by the retention times acquired from pure reference compounds (Table, 3). The defined components were linalool and eugenol as a main components followed by 1.8 cineole, borneol, camphor, α -terpineol, methyl chavicole, terpinene-4-ol, terpinolene, β -pinene, bornyl-acetate, α -pinene, camphene and β -caryophyllene while rest of the compounds were unknown.

Our obtained data pointed out that the relatively low doses of gamma rays (10kR) markedly increased α pinene, camphene, β - pinene, 1.8 cineole, linalool and eugenol as compared to control but some components like methyl chavicole was decreased as compared to all treatments that might nominate 10kR to be the optimum dose for increasing basil essential oil components. While, the highest percentage from terpinene-4-ol, borneol, α terpineol and β -caryophyllene were obtained from plants irradiated with 15kR as compared to un-irradiated plants however, camphor disappeared at the same dose and 20kR but increased at 5kR. On the other hand, terpinolene declined at all doses as compared to control. These results are supported by the previous study on *Brassica alba* (Hamideldin and Eliwa, 2015) who noticed that low doses of gamma radiation (10 and 20Gy) increased oleic and linoleic acid contents, whereas a decrease was obtained in response to seed treatments with the relatively higher doses (30, 40, 50Gy) as compared to control. On contrary, Erucic acid increased in response to the highest dose of gamma radiation (50Gy) and decreased particularly at lower doses.

Components name	Control	5kR	10kR	15kR	20kR	25kR	30kR
α-Pinene	0.39	0.27	0.73	0.18	0.16		0.23
Camphene	0.22	0.35	0.40	0.12	-	0.39	0.56
β- Pinene	1.11	0.89	1.11	0.85	0.82	1.07	0.98
1.8 cineole	12.64	12.36	12.56	11.69	10.34	10.61	10.01
Terpinolene	1.21	0.62	1.14	0.86	0.85	0.66	0.69
Linalool	27.53	27.24	28.25	24.10	24.83	26.59	26.37
Camphor	3.90	5.18	3.87			3.79	3.42
Borneol	7.79	5.31	7.59	11.85	9.90	6.10	6.90
Terpinene-4-ol	1.71	1.58	1.52	1.77	1.36	1.48	1.26
α-Terpineol	2.48	5.38	5.04	5.70	5.00	5.17	3.88
Methyl chavicole	2.26	5.49	2.12	7.04	5.58	5.38	4.16
Bornyl-acetate	1.34	1.47	1.18	1.57	1.51	1.84	2.57
Eugenol	28.27	23.23	28.76	25.93	24.62	22.27	18.43
β-caryophyllene	0.61	-	0.69	2.37	0.30	1.01	0.33

 Table 3. Effect of different doses of gamma irradiation on basil essential oil components in the second season of 2018

Total phenolic content

The changes in phenolic content would play functional roles in the irradiation stress response of basil plants. Plants exposed to elevated γ -irradiation doses significantly increased total phenolic content, the highest content was reported for irradiated plants with 30kR while the lowest content was obtained under 5kR and control without significant variation between them especially in the second season (Fig., 3). The importance of phenol



evaluation is due to the phenolic compounds perform significant physiological and ecological roles, being participated in resistance to various types of stresses (Masoud *et al.*, 2018). Increases in total phenolic content is considered a common response for high irradiation doses which were earlier confirmed by the findings of several workers in different plants (Said-al Ahl *et al.*, 2015, Masoud *et al.*, 2018 and Suneetha *et al.*, 2018).



Fig. 3. Effect of various doses of gamma irradiation on total phenolic content (mg/gm) of *Ocimum basilicum* L. in the two cuts of both seasons (2017/2018).

CONCLUSION

From the current investigation, it is clear that low doses of gamma rays increased germination percentage, vegetative growth, chlorophyll a, b, and yield characters of *Ocimum basilicum* L. On the other hand, high gamma irradiation doses significantly decreased germination percentage, vegetative growth, chlorophyll a, b, oil yield and oil components while, carotenoids and total phenolic content were increased as compared to non-irradiated plants in both growing seasons. It can be concluded that treating basil seeds by gamma rays at 10kR can be successfully utilized for enhancing vegetative growth and oil yield of basil plants.

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