EFFECT OF GAMMA IRRADIATION ON VEGETATIVE GROWTH AND BIOCHEMICAL CHANGES OF CUMIN PLANTS

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ABSTRACT
Gamma irradiation has emerged as a promising approach to enhance yield and quality of various crops. The aim of the current work was to assess the effect of various gamma irradiation doses (0, 100, 150, 200, 250 and 300 Gy) on the vegetative growth, biochemical changes and oil yield of cumin plants during the two consecutive seasons of 2020/2021 and 2021/2022 at the Experimental Farm of Sakha Horticulture Research Station, Kafr El-Sheikh Governorate, Egypt in a randomized complete blocks design with three replications.

The results showed that low doses of gamma irradiation significantly enhanced vegetative growth and yield characteristics, photosynthetic pigments, antioxidant activity and oil yield. On the other hand, as the applied doses increased, plant height, number of branches, dry weight, chlorophyll content and oil yield were decreased, while carotenoid and phenolic content were increased as defence mechanism against elevated irradiation doses compared to the control. The genetic advance (GA%) and the high heritability (Hb%) values indicated that the second generations may show additional advancements.

Generally, gamma irradiation can be used to improve the growth, oil yield and biochemical content of cumin at 150 Gy as a seed treatment.

Keywords: Cumin; Gamma irradiation; Phenolic; Flavonoids; Essential oil

INTRODUCTION
Cumin (Cuminum cyminum L.) is a member of the Apiaceae family, originated from Egypt and the eastern Mediterranean. It is considered to be one of the most popular spices. It is widely distributed throughout temperate climate regions and is commonly used as a vegetable, spice, or medicine due to the presence of beneficial metabolites (Olle and Bender, 2010). Cumin seeds are extensively used as a traditional flavoring in a number of food industries, as well
as ethnic cuisines and for the extraction of cumin oil which possess antimicrobial activity against various pathogenic microbes (Li and Jiang, 2004).

Gamma radiation is an effective and reliable means of altering the physiological and biochemical processes in plants (Hanafy and Akladious, 2018). Moreover, it is one of the most important physical agents that improves the productivity and vegetative growth characteristics of various plants. Gamma irradiation has been broadly utilized in agriculture for enhancing the tolerance of plants to abiotic stress (Ahmed et al., 2011 and Aly et al., 2018), boosting nutritional status of plants (Hussein and Hamideldin, 2016 and Corrales-Lerma et al., 2022), inducing genetic variation in plant breeding programs (Alikamanoglu et al., 2011; Eze et al., 2022 and Haridy et al., 2022), shelf life extension (Bindu et al., 2018) and increasing food safety for medicinally valuable plants (Almeida et al., 2018), furthermore, gamma radiation can be used as an eco-friendly method to control stored-product pests (Nasr et al., 2022).

Several studies displayed the physiological impact of low doses of gamma irradiation on plant growth which may be ascribed to the reaction among gamma rays and molecules in plant cells that induce free radicals production (Masoud et al., 2018 and Mounir et al., 2022). Depending on the level of radiation, the free radicals produced can change the main components of plant cells, as well as their morphology, physiology, anatomy, and biochemistry in different ways. These changes may be summarized in the acceleration of cell expansion, cell proliferation, enzyme potential, stress resistance and enhancement of plant yield, therefore, seeds exposed to gamma rays were found to have a significant impact on plants development, yield components, as well as the concentration of plant phytochemicals (Vardhan and Shukla, 2017; Majeed et al., 2018 and Mounir et al., 2022). Also, it was noticed that low gamma irradiation doses have a simulative effect represented by enhancing vegetative growth and yield characters (Hamideldin and Eliwa, 2015 and Verma et al., 2017), photosynthetic pigment content as well as anthocyanin content (El Sherif et al., 2011). In addition, there was a considerable influences of gamma irradiation on boosting, alkaloid, phenolic compounds, flavonoid content and essential oil as well as antioxidant activity in various plants (Masoud et al., 2018; Almeida et al., 2018; Shala, 2019, Mounir et al., 2022 and Vasudevan et al., 2023). On the other hand, plants developed protective mechanisms to counteract oxidative damage resulted from excessive reactive oxygen species which may produce under high irradiation doses via both enzymatic and non-enzymatic antioxidants. Carotenoids, flavonoids and phenolic compounds are non-enzymatic plant antioxidants that have previously been shown to have a protective role against high irradiation...
doses in medicinal plants (Masoud et al., 2018; Hanafy and Akladious, 2018 and Shala, 2019).

To develop successful breeding programs, it is necessary to determine genetic factors, such as heritability, genetic advance, and phenotypic and genotypic coefficients of variability. The genotypic coefficient of variation (GCV) presented by plant attribute is measured the range of genetic variation. Nevertheless, the proportion of variation that is purely inherited cannot be ascertained using the GCV. Genetic advancement must be taken into consideration in addition to heritability estimations, as the former alone cannot provide insight into the predicted gain in the following generation. Knowing heritability is crucial as it indicates the extent to which a character may be inherited by subsequent generations (Wani et al., 2014).

Enhancement of the plant growth, antioxidant system along with plant secondary metabolites (medicinally active substances) with low doses of gamma irradiation could be used for improving the quality of medicinally important plants. Thus, the aim of the current investigation was to assess the effect of different doses of gamma irradiation on the morphological characters, biochemical changes and oil yield of cumin plants for optimizing the appropriate dose of gamma rays to stimulate the growth, yield and activate the bioactive compounds to meet the growing demand of cumin.

MATERIALS AND METHODS

Cumin seeds were obtained from Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. Dry cumin seeds were exposed to gamma rays at 0, 100, 150, 200, 250 and 300 Gy gamma irradiation doses using cobalt-60 at a dose rate of 3.58 min/10 kR at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt.

The irradiated and non-irradiated (control) seeds were sown on 15th November for both growing seasons (2020/2021 and 2021/2022) on 25 cm×60 cm plant spacing at the Experimental Farm of Sakha Horticulture Research Station, Kafr El-Sheikh, Egypt in a completely randomized block design with three replications. The experiment was conducted in clay texture soil (55.33% clay, 26.09% silt and 18.58% sand), its electrical conductivity (EC) in the extraction of saturated soil paste was 1.85 dSm⁻¹ and soil pH was 7.61 as a mean of 0-60 cm depth, the evaluated, physical and chemical properties were performed according to Page et al.(1982). All agricultural practices were performed as
recommended for cumin by the Egyptian Ministry of Agriculture and Land Reclamation.

Estimation of photosynthetic pigments, chlorophyll a, b and total carotenoids contents were determined spectrophotometrically in fresh cumin leaves for two seasons by the method of (Lichtenthaler and Buschmann, 2001) and expressed as mg/g fresh weight of leaves.

Growth and yield characters, plants were harvested and plant height, number of branches, dry weight and seed yield/plant were determined from randomly selected plants.

Essential oil extraction, 50 g. of irradiated and non-irradiated seeds were subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus according to the method described by (Pharmacopoeia, 1963). The essential oil percentage of the plants seed was reported by a volumetric method (ml/100 g) then the oil yield per plant (ml) was calculated.

The content of total phenolics in seed extracts was estimated according to the Folin-Ciocalteu method of Abeysinghe et al. (2007) with slight modification. A 0.5 mL of cumin seed extracts was mixed with 5 ml Folin Ciocalteu reagent (1:10), then 4 ml of Na₂CO₃ 1M was added. The mixture was incubated for 5 minutes at room temperature and the absorbance was measured at 765 nm. The standard curve was obtained by using gallic acid standard solution with concentrations ranging from 60-150 μg ml⁻¹. All assays were performed in triplicate and the total phenolic content was expressed as mg gallic acid equivalents (GAE) /g D.W.

Total flavonoids content was assayed according to Chang et al. (2002). 0.5 mL of seed extract was mixed with 0.1 ml AlCl₃ 10%, 0.1 mL sodium acetate 1M and 2.8 mL aquadest. The mixture was diluted with 1.5 ml ethanol, and incubated for 15 minutes. Samples absorbance was measured at 415 nm. The analysis was performed in triplicate for each extract. Quercetin with a concentration of 40-100 μg ml⁻¹ was used as standard to obtain a calibration curve. Total flavonoids content was expressed as mg quercetin g⁻¹ D.W.

DPPH radical-scavenging activity, DPPH assay is known to provide reliable information on the antioxidant activity of extracts or specific compounds. The DPPH radical scavenging potential was assayed according the method proposed by (Brand-Williams et al., 1995). To prepare the stock solution, 2.5 mg of DPPH were dissolved in 50 ml of methanol and kept in refrigerator (4°C) until further use. For the control 5 ml of the DPPH stock solution was also mixed with methanol to get an absorbance of 0.91 ± 0.02 units, at 517 nm. Absorbance was measured spectrophotometrically at 516 nm after mixing equal volumes of cumin seed extracts and the DPPH stock solution and incubated for 30 min in the dark.
via Hewlett Packard 8435 spectrophotometer UV-Vis. Samples measurements were performed in triplicate and the data displayed as the mean of three values.

**Statistical analysis:**

The obtained data were subjected to statistical analysis of variance (ANOVA) via COSTAT software. Differences among treatment means were performed by Duncan’s multiple range test at the 0.05 significant level (Snedecor and Cochran, 1980).

Phenotypic and Genotypic Coefficient of Variation (PCV and GCV)

As per (Singh and Chaudhary, 1977), the PCV and GCV estimates were calculated as follows:

\[
PCV = \sqrt{\frac{\sigma^2_p}{\bar{x}}} \times 100 \\
GCV = \sqrt{\frac{\sigma^2_g}{\bar{x}}} \times 100
\]

Where; \(\sigma^2_p\) represents the phenotypic variance, \(\sigma^2_g\) represents the genotypic variance and \(\bar{x}\) represents the trait mean. GCV and PCV values were classified as low (0–10%), intermediate (10–20%), and high (20% and above) by Sivasubramanian and Madhavamenon, (1973).

The estimate of broad sense heritability (H^2b %) was calculated according the following equation;

\[
H^2b\% = \frac{\sigma^2_g}{\sigma^2_p} \times 100
\]

The heritability percentage was classified as low (0–30%), moderate (30–60%), and high (≥60%) according to (Robinson and Comstock, 1950).

Expected and Estimated Genetic Advance (GA)

\[
GA = K \sqrt{\sigma^2_p} H^2b
\]

According to Ayele, (2011) where K, a constant that symbolizes the selection strength, the value is 2.06 when K is 5%. Where; \(H^2b\) represents the heritability and \(\sqrt{\sigma^2_P}\) represents the phenotypic standard deviation.

To estimate the genetic advance as proportion of the mean (GA%), the following equation was used: \(GA\% = \frac{GA}{\bar{x}} \times 100\)

According to (Johnson et al., 1955), it was categorized as low (0–10%), moderate (10–20%), and high (20% or greater).

**RESULTS AND DISCUSSION**

Vegetative growth characteristics

Low doses of gamma irradiation significantly enhanced vegetative growth characteristics, the highest values of plant height (41.33 and 42.00 cm/plant), number of branches (14.33 and 16.00) and plant dry weight (14.28 and 14.33g/plant) were significantly obtained from cumin plants exposed to 150 Gy in
comparison with other doses and control during both growing seasons (Table 1). On the other hand, increasing doses of gamma rays (200, 250 and 300) gradually diminished the vegetative growth characters, in this regard the highest reduction of the above-mentioned characters was obtained from plants irradiated with 300 Gy as compared with other doses of gamma irradiation as well as control for both growing seasons (Table 1).

**Table (1)** Effect of gamma irradiation doses on vegetative growth characteristics of *Cuminum cyminum* plants during two consecutive seasons of 2020/2021 and 2021/2022.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1(^{st}) season</th>
<th>2(^{nd}) season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plant height (cm)</td>
<td>Branches No.</td>
</tr>
<tr>
<td>Control</td>
<td>27.33c</td>
<td>11.00bc</td>
</tr>
<tr>
<td>100 Gy</td>
<td>32.00b</td>
<td>11.33bc</td>
</tr>
<tr>
<td>150 Gy</td>
<td>41.33a</td>
<td>14.33a</td>
</tr>
<tr>
<td>200 Gy</td>
<td>35.00b</td>
<td>12.00b</td>
</tr>
<tr>
<td>250 Gy</td>
<td>25.00cd</td>
<td>9.66cd</td>
</tr>
<tr>
<td>300 Gy</td>
<td>21.66d</td>
<td>8.66d</td>
</tr>
</tbody>
</table>

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

The simulative influences of low doses of gamma irradiation on the vegetative growth characteristics of cumin are in harmony with results obtained by (Hamideldin and Eliwa, 2015), who suggested that vegetative growth characteristics of *Brassica alba* were significantly boosted by increasing gamma irradiation doses from 10 to 50 Gy. Also, (Hussein and Hamideldin 2016) found that low gamma irradiation dose (60 Gy) was the most prominent dose among the gamma rays used and it was more effective in increasing plant weight, number of capsules and weight of seeds/plan of *Sesamum indicum* L., as compared to 30 and 90 Gy. Radiation doses ranging from 25 to 200 Gy enhanced growth and yield parameters (Hanafy and Akladious, 2018). A dose of 100 Gy increased plant height of irradiated wheat plants (Aly et al., 2018), on *Cicer arietinum* Mullainathan and Umavathi (2018) found that the highest plants were reported at 20 kr, while the lowest plants were at 60 kr. El-Azab et al., (2018) illustrated that 100 Gy and 200 Gy increased shoot, fresh, and dry weights of soybean plants which correlated with increased mitotic activity in the root tip meristems.

Recently, Eze et al.,(2022) pointed out that the highest plants of *Sphenostylis stenocarpa* were reported for 25 and 100 Gy as compared to the control treatment. Also, Haridy et al. (2022) reported that both Egyptian bean
cultivars exposed to gamma radiation (10, 15 and 20 kr) increased the number of branches/plant and plant height, especially at the dose 20 kr.

The above-mentioned results showed that the appropriate low dose of gamma irradiation can considerably enhance the vegetative growth characteristics of plants, which may be due to the stimulation of cell elongation or cell division and alteration of metabolic processes affecting the synthesis of nucleic acids or phytohormones (Hanan and Prusinkiewicz, 2008). In addition, the improvements in growth traits of plants exposed to low doses of gamma irradiation may be due to a positive mutational effects on genes controlling growth traits and rapid DNA repair mechanism, as well as activation of endogenous enzymes and hormones responsible for plant development (Majeed et al., 2018). In contrast, El Sherif et al. (2011) found that among the irradiation doses used, gamma irradiation at 600 Gy increased plant height, number of branches, fresh and dry weight of leaves, and stems/plant. Furthermore, Amir et al.(2018) observed that the tallest plants of Abelmoschus esculentus L. were obtained from 500 Gy as compared to other doses (0,100, 200, 300 and 400), while the highest number of branches was scored from 100 Gy as compared to other doses.

Previous studies have shown the adverse effects of high doses of gamma irradiation on morphological characteristics of Helianthus annuus L., Vigna radiata L., Glycine max L. (Merrill.), Trigonella foenum-graecum L., Hibiscus Sabdariffa L., Foeniculum vulgare Mill., and Linum usitatissimum (L.) derived from irradiated seeds as many workers observed reduction in plant height, number of branches and fresh and dry weight with increasing gamma irradiation doses which were in agreement with our outcomes on cumin plants and previously reported by (Alikamanoglu et al., 2011; El Sherif et al., 2011; Bhat et al., 2016; Verma et al., 2017; Hanafy and Akladious, 2018; Im et al., 2018 and Rifnas et al., 2022). Reduced cell division rate and respiration, as well as changes in physiological processes and decreased in auxin supply, could all contribute to the reduction in plant height under high gamma irradiation doses (Rifnas et al., 2022). In addition, El Sherif et al.(2011) assumed a reduction in plant fresh and dry weight due to decreasing moisture content caused by radiation stress resulting from exposure to high gamma irradiation doses. Also, irradiated seeds higher doses displayed negative impacts on protein synthesis, gas exchange, balance of endogenous hormones and enzyme activity (Hameed et al., 2008).

Photosynthetic pigments

Estimation of chlorophyll is one of the most important variables in determining the yield capacity. Low doses of radiation have noticeable effect on chlorophyll a and b contents in cumin leaves (Table 2), whereas plants irradiated
with 100 and 150 Gy led to produce higher chlorophyll a, b content than other gamma irradiation doses during both successive seasons without any significant variation with control. While the lowest contents of chlorophyll "a" (0.773 and 0.680 mg/g f.w.) and chlorophyll "b" (0.358 and 0.404 mg/g f.w.) were obtained from plants subjected to 300 Gy in both seasons respectively, as compared to the control treatment.

**Table (2)** Effect of gamma irradiation doses on chlorophyll A, chlorophyll b and carotenoid contents of *Cuminum cyminum* plants during two consecutive seasons of 2020/2021 and 2021/2022.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1st season</th>
<th>2nd season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ch. A (mg/g f.w.)</td>
<td>Ch. B (mg/g f.w.)</td>
</tr>
<tr>
<td>Control</td>
<td>1.312 a</td>
<td>0.555 a</td>
</tr>
<tr>
<td>100 Gy</td>
<td>1.168 ab</td>
<td>0.509 ab</td>
</tr>
<tr>
<td>150 Gy</td>
<td>1.214 ab</td>
<td>0.551 a</td>
</tr>
<tr>
<td>200 Gy</td>
<td>1.112 bc</td>
<td>0.451 bc</td>
</tr>
<tr>
<td>250 Gy</td>
<td>0.962 c</td>
<td>0.391 cd</td>
</tr>
<tr>
<td>300 Gy</td>
<td>0.773 d</td>
<td>0.358 d</td>
</tr>
</tbody>
</table>

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

The same outcomes of gamma irradiation influences on photosynthetic pigments, were presented by Alikamanoglu *et al.* (2011) who found that chlorophyll a and b contents were negatively decreased in leaves of *Glycine max* L. plants exposed to 400 and 500 Gy, while there were no significant variations in chlorophyll contents in control plants and 100 Gy dose. In addition, another study conducted on *Brassica alba* showed that low doses of gamma irradiation (10, 20, 30, 40 and 50 Gy) increased chlorophyll a and b (Hamideldin and Eliwa, 2015). In the same concern, Suneetha *et al.* (2018) observed that the highest chlorophyll content of *Abelmoschus moschatus* was obtained from plants exposed to 300 Gy followed by 100 and 200 Gy, while low chlorophyll content was obtained from higher irradiation doses (400,500 and 600 Gy). Also, Koutoua *et al.* (2021) suggested that leaf pigment accumulation depends on seed irradiation dose and found that the average of chlorophyll (a), as well as chlorophyll (b) content in maize plants was increased at 100 Gy and decreased at 300 grays. Low doses of gamma rays (5 Gy) increased chlorophyll a and b in *Jerusalem artichoke* leaves as compared with other doses (Mounir *et al.*, 2022). The stimulating role of gamma irradiation on chlorophylls may be attributed to promoting the biosynthesis of chlorophyll and/or delaying its degradation (Aly *et al.*, 2018).
Recently, Vasudevan et al. (2023) found that lower levels of photosynthetic pigments were observed in irradiated *Vigna radiata* seedlings compared to the control, and the maximum reduction of chlorophyll "a" and chlorophyll "b" was obtained at 800 Gy and the authors reported that higher doses of gamma irradiation gradually reduce the chlorophyll content due to the separation of chlorophyll from its protein complex via dephytolization.

In contrast, to our results the increased irradiation doses increased the chlorophyll a and b contents, and the results of El Sherif et al. (2011) stated that 700 Gy was the most effective dose for increasing the photosynthetic pigment content (chlorophyll a and b) compared to other doses (100, 200, 300, 400, 500, 600 and 800 Gy). Furthermore, Im et al., (2018) stated that the total chlorophyll values of irradiated Korean soybean cultivars were lower than the untreated control and the authors suggested that changes in chlorophyll pigments in irradiated plants depended on the plant cultivar and irradiation dose.

The carotenoid content in cumin leaves significantly increased with increasing gamma irradiation dose from 100 to 300 Gy, (Table 2). The highest carotenoid content was observed in plants treated with 250 and 300 Gy without significant differences between them as recorded (0.556, 0.597, 0.551 and 0.555 mg/g f.w.) for both seasons, respectively as parallel to control which recorded the least carotenoid content (0.338 and 0.358 mg/g f.w.) in the two growing seasons respectively. Increases carotenoids content in cumin are considered as a significant defensive approach to enhance plant resistance to high irradiation doses (Masoud et al., 2018). Similar results were formerly reported by Shala (2019), who found that the maximum carotenoid content in *Ocimum basilicum* was achieved from plants irradiated at 30 kr. On the other hand, Hamideldin and Eliwa (2015) found that carotenoid contents of *Brassica alba* decreased with increasing irradiation doses (10, 20, 30, 40 and 50 Gy). Likewise, Koutoua et al. (2021) reported that the highest carotenoid content in maize plants was resulted from 100 Gy. Also, Mounir et al. (2022) found that low doses of gamma rays (5Gy) increased carotenoid content in *Jerusalem artichoke* leaves as compared to other doses. In addition, gamma irradiation enhanced carotenoid content and 800 Gy was the most prominent dose for achieving the highest carotenoid content in *Vigna radiata* as compared to the control (Vasudevan et al., 2023).

**Plant yield**

Cumin yield was significantly influenced by gamma irradiation doses (Table 3), low dose of gamma radiation promoted cumin seed yield in this regard, the maximum seed yield per plant (2.07 and 2.11g/plant) was recorded in plants exposed to 150 Gy, followed by plants treated with 200 Gy, then seed yield
gradually declined with the increasing gamma irradiation doses, the highest seed yield reduction was achieved with 300 Gy as compared with unirradiated plants. The current study showed that it was possible to increase yield of cumin by using low doses of gamma irradiation which may be attributed to the stimulating role of irradiation on plant height, number of branches and photosynthetic pigments as compared to the non-irradiated plants which may have an impact on enhancing the photosynthetic process which results in carbohydrate accumulation and in turn increase the total seed yield.

**Table (3)** Effect of gamma irradiation doses on seed yield, oil percentage and oil yield of *Cuminum cyminum* plants during two consecutive seasons of 2020/2021 and 2021/2022.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1st season</th>
<th>2nd season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seed weight (g)/ plant</td>
<td>Oil (%)</td>
</tr>
<tr>
<td>Control</td>
<td>1.53 cd</td>
<td>1.74d</td>
</tr>
<tr>
<td>100 Gy</td>
<td>1.76 bc</td>
<td>2.53b</td>
</tr>
<tr>
<td>150 Gy</td>
<td>2.07 a</td>
<td>2.68a</td>
</tr>
<tr>
<td>200 Gy</td>
<td>1.94 ab</td>
<td>2.47b</td>
</tr>
<tr>
<td>250 Gy</td>
<td>1.36 d</td>
<td>2.16c</td>
</tr>
<tr>
<td>300 Gy</td>
<td>1.44 d</td>
<td>1.39e</td>
</tr>
</tbody>
</table>

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

Gamma irradiation had an enhancing effect on cumin seed yield which was similar to that previously published by Verma *et al.* (2017), who suggested that the seed fennel yield was increased at doses of 20 kr and 25 kr, followed by 22.5 kr and decreased at 17.5 kr and 15 kr compared to the control treatment. Similarly, El-Azab *et al.* (2018) reported that 100 Gy and 200 Gy increased the yield of soybean. Also, Mounir *et al.* (2022) found that Jerusalem artichoke tubers exposed to 5 Gy, followed by 2.5 Gy and 10 Gy resulted in higher plant yield than un-irradiated tubers. Recently, Haridy *et al.* (2022) reported that gamma irradiation at 20 kr increased seed yield/plant of both Egyptian bean cultivars compared with other doses used. The promoting role of gamma irradiation on yield may be ascribed to the stimulating role of irradiation on growth hormone and enzyme responsible for growth and yield (El Sherif *et al.*, 2011).

Regarding the adverse effect of high irradiation doses on plant yield, El-Azab *et al.* (2018) found that high doses (300 Gy to 600 Gy) negatively diminished the soybean yield compared to low doses. Also, increasing gamma ray doses (20, 30, 40, 50 and 60 kr) decreased the yield of *Cicer arietinum* plants,
which may be related to meiotic disruption affecting the frequency of typical microspores and megaspores, thus directly affecting the trait (Mullainathan and Umavathi, 2018).

**Essential oil yield**

Essential oil percentage, as well as essential oil yield were significantly influenced by gamma irradiation treatments (Table 3). The highest percentage of essential oil (2.68 and 2.55%), as well as oil yield (0.055 and 0.054 ml) were reported from plants irradiated with 150 Gy, followed by plants treated with 100 Gy without significant differences in between only in the second season than control treatment in both growing seasons. While the lowest oil percentage values (1.39 and 1.33%) and oil yield (0.020 and 0.019 ml) were produced from irradiated plants with 300 in both growing seasons. 

These results are in harmony with the results obtained by Khan et al. (2014) on *Brassica napus* who found that increasing irradiation doses (10, 15, 20, 25 and 30 kr) decreased oil content, and the lowest oil content was obtained from irradiated plants with 30 kr. Similarly, Shala (2019) observed that oil percentage and oil yield of *Ocimum basilicum* were considerably enhanced with low irradiation dose (10 kr), while irradiated plants with 25 and 30 kr produced the lowest essential oil percentage compared to the control in both growing seasons. Gamma rays enhanced the yield of secondary metabolites via activating certain key biosynthetic enzymes and inducing the gene responsible for secondary metabolite production (Vardhan and Shukla, 2017).

**Total phenolic, flavonoid contents and antioxidant activity**

Plants accumulate flavonoids and phenolic compounds as a defense mechanism against irradiation stress due to their antioxidant properties. Gamma irradiation induced changes in phenolic content as compared to the unirradiated control (Fig 1). Exposure of cumin seeds to lower doses of gamma irradiation (100, 150 and 200 Gy) significantly improved the phenolic content as compared to the control and its greatest content was recorded at 200 Gy. Meanwhile, the highest dose of irradiation (300Gy) resulted in a slight decrease in total phenolic content in both growing seasons (Figure 1).
Figures 1 and 2. Effect of gamma irradiation doses on total phenolic content of *Cuminum cyminum* plants during the two consecutive seasons of 2020/2021 and 2021/2022.

Flavonoids are a class of secondary plant metabolites that are generally distributed in plants and mitigate the damage caused by irradiation stress (Hanafy and Akladious, 2018). Regarding the flavonoid content, the results of the current study showed that different doses of gamma irradiation gradually increased the flavonoid content by compared to the control plants during both growing seasons (Figure 2). Plants grown from seeds irradiated at 200 Gy significantly reached the maximum flavonoid content, followed by plants raised from seeds irradiated at 150 Gy without significant differences in the second season only as compared to unirradiated plants. In contrast, the total flavonoid content was reduced in plants that produced from seeds irradiated at the highest dose (300 Gy).

DPPH radical scavenging activity considered a great indicator of antioxidant potential. A lower IC$_{50}$ value indicates a higher antioxidant activity. Our results clearly indicated that increasing gamma irradiation dose up to 150 Gy significantly increased the DPPH radical scavenging activity in cumin seeds to (7.62% and 7.48%) as compared to untreated plants which resulted in the least DPPH radical scavenging activity (21.34 and 21.10%) for both growing seasons, respectively (Figure 3).
Figure (2) Effect of gamma irradiation doses on total flavonoid content of *Cuminum cyminum* plants during the two consecutive seasons of 2020/2021 and 2021/2022.

Figure (3) Effect of gamma irradiation doses on antioxidant activity of *Cuminum cyminum* plants during the two consecutive seasons of 2020/2021 and 2021/2022.
Gamma irradiation had diverse influences on total phenolic content, flavonoid content and DPPH radical scavenging activity in cumin plants, which was in accordance with the results obtained by El Sherif et al. (2011), who revealed that anthocyanin content as phenolic component increased at 600 Gy. Also, Akshatha et al. (2013) found that the highest concentration of phenolic content and DPPH radical scavenging activity were obtained from plants irradiated at 25 and 150 Gy in *Terminalia arjuna* Roxb seedlings. In addition, Aly et al. (2018) stated that the total phenolic content was significantly enhanced at all irradiation doses (100, 200 and 300 Gy) in bread wheat and Hanafy and Akladious, (2018) showed that gamma rays (25, 50, 100 and 200 Gy) increased the total phenolic and flavonoid content of *Trigonella foenum-graecum* and the highest induction was obtained for the 100 Gy dose level, as well as Masoud et al. (2018) observed that gamma irradiation at 40 Gy increased the total phenolic accumulation in roots of *Cichorium pumilum* Jacq., while the total phenolic content was inhibited by increasing the gamma dose to 80 Gy. Gamma irradiation treatment at 600Gy increased the phenolic content of *Abelmoschus moschatus* while the lowest phenolic content was recorded at 100Gy (Suneetha et al., 2018). Recently, Mounir et al. (2022) observed that Jerusalem artichoke tubers treated with 5 and 10 Gy of gamma irradiation showed an increase in flavonoids content as well as, total soluble phenolics compared to unirradiated plants. The stimulating effect of gamma rays on phenolics and flavonoids accumulation could be due to the activity of phenylalanine ammonia-lyase enzyme. It is considered to be one of the enzymes responsible for phenolic compounds biosynthesis and it has been shown that this enzyme is stress sensitive and is activated by oxidative stress (Gitz et al., 2004). It has been shown that phenylalanine ammonia-lyase activity increased by 80% in irradiated treatments. Furthermore, there is a positive correlation with phenylalanine ammonia-lyase activity that is directly proportional to the irradiation dose and the total phenolic content in the plant extract. Additionally, gamma irradiation enhanced the chalcone synthase enzyme as the key enzyme for flavonoid formation (Vardhan and Shukla, 2017). The authors also stated that the increase in flavonoids and phenolics in response to gamma irradiation may help to mitigate the damage induced by the irradiation stress.

**Genetic parameters**

To successfully breed any plant species, it is required to understand its genetic potential, heritability, the genotypic and phenotypic variance structure and functional trait dependence. The genetic parameter estimates for M2 (Cultivated plants produced from the harvested seeds of the M1 generation) are shown in (Table 4). There was a difference between the genotypic and phenotypic
coefficients of variability (GCV and PCV). The genotypic coefficient of variability (GCV) was smaller than the phenotypic coefficient of variability (PCV). The greatest PCV values were recorded for dry weight/plant, oil yield/plant and DPPH IC$_{50}$ (51.59%, 46.23% and 45.94%, respectively). However, the moderate (10-20) estimates were for total flavonoids (14.94%) and seed weight/plant (18%). While the other traits have higher PCV values up to (20%) and there are not any treats have PCV values (0-10%) (Table 4).

Table (4) Estimates of genetic parameters for all the studied traits in the M2 generation of cumin

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean(x)</th>
<th>PCV</th>
<th>GCV</th>
<th>H$^b$ (%)</th>
<th>GA</th>
<th>GA%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height (cm)</td>
<td>29.33</td>
<td>27.59</td>
<td>27.11</td>
<td>96.56</td>
<td>16.10</td>
<td>54.89</td>
</tr>
<tr>
<td>Branch No.</td>
<td>11.33</td>
<td>24.82</td>
<td>23.78</td>
<td>91.78</td>
<td>5.32</td>
<td>46.94</td>
</tr>
<tr>
<td>Dry weight/ plant (g.)</td>
<td>7.37</td>
<td>51.59</td>
<td>50.07</td>
<td>94.20</td>
<td>7.38</td>
<td>97.8</td>
</tr>
<tr>
<td>Total flavonoids mg/g D.W.</td>
<td>6.38</td>
<td>14.94</td>
<td>14.85</td>
<td>98.90</td>
<td>1.94</td>
<td>30.44</td>
</tr>
<tr>
<td>Total phenol mg GAE/g DW</td>
<td>4.15</td>
<td>29.82</td>
<td>29.76</td>
<td>99.60</td>
<td>2.54</td>
<td>61.19</td>
</tr>
<tr>
<td>DPPH IC$_{50}$(mg/ml)</td>
<td>14.15</td>
<td>45.94</td>
<td>45.92</td>
<td>99.92</td>
<td>13.38</td>
<td>94.57</td>
</tr>
<tr>
<td>Ch-A (mg/g f.w.)</td>
<td>0.999</td>
<td>20.75</td>
<td>19.24</td>
<td>86.04</td>
<td>0.367</td>
<td>36.78</td>
</tr>
<tr>
<td>Ch-B (mg/g f.w.)</td>
<td>0.469</td>
<td>20.95</td>
<td>19.83</td>
<td>89.65</td>
<td>0.181</td>
<td>38.69</td>
</tr>
<tr>
<td>Carotenoid (mg/g f.w.)</td>
<td>0.399</td>
<td>21.46</td>
<td>19.94</td>
<td>86.36</td>
<td>0.152</td>
<td>38.18</td>
</tr>
<tr>
<td>Oil percentage</td>
<td>2.08</td>
<td>23.70</td>
<td>23.55</td>
<td>98.76</td>
<td>1.00</td>
<td>48.22</td>
</tr>
<tr>
<td>Seed weight/ plant</td>
<td>1.65</td>
<td>18.00</td>
<td>17.27</td>
<td>92.13</td>
<td>0.56</td>
<td>34.16</td>
</tr>
<tr>
<td>Oil yield/plant</td>
<td>0.035</td>
<td>46.23</td>
<td>31.65</td>
<td>46.88</td>
<td>0.015</td>
<td>44.64</td>
</tr>
</tbody>
</table>

Phenotypic Coefficient of Variation (PCV), genotypic Coefficient of Variation (GCV), broad sense heritability (H$^b$%), expected genetic advance (GA %)

The highest estimates of GCV (>20%) were recorded for dry weight/plant, oil yield/plant and DPPH IC50 (50.07%, 31.65% and 45.92% respectively), while the moderate estimates were those of total flavonoids (14.85%), Ch-A (19.24%), Ch-B (19.83%), carotenoid (19.94%) and seed weight/plant (17.27%). Meanwhile, the other traits have higher PCV values up to (20%). While the genotypic coefficient of variation indicated the amount of genetic diversity present in the genotypes for various phenotypes, it did not provide enough information to identify how much of the variance was heritable. For this reason, crop enhancement requires knowledge of heredity. According to the results, all the traits showed high heritability values. It was found that features with heritability in the broad sense ranged from 86.04% (Ch-A) to 99.92% (DPPH IC$_{50}$) as shown in Table 4.
For genetic advance (GA%) the all treats showed higher genetic advance (>20%) where the estimates ranged from 30.44 percent (total flavonoids) to 97.8 percent (dry weight/plant). For yield traits, many studies revealed that heritability in the broad sense was moderate to high estimates (Rohini et al., 2017 and Amer, 2018). High $H^2_b$ associated with high GA was observed for plant height, branches number, dry weight/plant, DPPH IC$_{50}$, total flavonoids, total phenolics and essential oil percentage (Table 4).

**Conclusion**

The present results showed that irradiated cumin seeds displayed higher values for morphological characteristics such as plant height, number of branches, dry weight and chlorophyll a and b contents, as well as oil content up to 150 Gy then gradually declined with higher doses. On the other hand, carotenoids increased at higher irradiation doses. Biochemical characteristics, such as phenolic and flavonoid concentrations, as well as DPPH scavenging activity were induced with increased doses. As a result, these outcomes proposed that low gamma irradiation doses could be utilized for oil yield enhancement, as well as in the planning for cumin breeding programs. Additionally, the simulative effects of low doses of irradiation on vegetative characters of cumin which reflects on the improvement of total yield and biochemical constituents which gave the opportunity to cultivate irradiated cumin seeds under marginal soils that are considered a great obstacle for the expansion of cumin cultivation with enhanced its secondary metabolites.

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تأثير أشعة جاما على النمو الخضري والتغيرات الكيميائية لنبات الكمون

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يعتبر استخدام أشعة جاما من الطرق الوعائية لزيادة إنتاجية وجودة النباتات المختلفة. تم إجراء التجربة بهدف دراسة تأثير جرعات مختلفة من أشعة جاما (0، 50، 100، 150، 200، 250، 300 جراك) على النمو الخضري والكيميائيا وحجم الزيت للنبات الكمون خلال موسمين النمو 2020/2021 و2021/2022. الالزرونة التجريبية بمحطة بحوث البساتين بمحافظة كفر الشيخ. لم يتم تصميم التجربة في صورة قطاعات كاملة العشوية.

وقد أظهرت النتائج أن الجرعات المنخفضة من أشعة جاما أدت إلى زيادة معتنوبة في النمو الخضري والمحصول وصباغة التمثيل الضوئي وزيادة الزيت والفلافونيدات وقدرة النباتات كعصاب أكسدة. بينما أدت زيادة جرعات الإشعاع إلى إنخفاض طول النباتات وعدد الأفرع والوزن الحاصل ونسبة الكرفون الهيدروكيك لزيت النباتات، بينما تم زيادة محتوى النباتات من الكراتينويدات والفينولات ككلة دفاع للنبات ضد حساسية الشعاع المرتفعة. تم قياس القيم العالية لدرج التوريث بالممارسات H2b (GA %) ونسبة التقدم الوراثي 150% إلى أنه يمكن إجراء مزيد من التحسين الوراثي في الأجيال القادمة. النتائج: تأثيرات الدراسة أن يمكن استخدام أشعة جاما لمراجعة البذور بمعدل 150 جرار تحسين نمو الكمون ومحصول الزيت والكتويت الكيميائهن لنباتات تربة

كلمات مفتاحية: الكمون – أشعة جاما – الفلافونيدات – الفينولات – الزيوت العطرية