Effect of irradiation by gamma rays and the use of benzyl adenine to increase the production of cardiac glycoside compounds from *Digitalis lanata* *in vitro*

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**Abstract.** Seeds of fox glove *Digitalis lanata* were irradiated with gamma ray with the dose 25, 50 grey in addition to the treatment of the comparison 0 grey, BA was also used at the concentrations of 0, 1, 2, 3, 4 mg . L⁻¹ to determine the effectiveness of the gamma ray and BA and their interaction in stimulating the proportion, speed of seed germination and production of Cardiac glycosides, chlorophyll and carbohydrates in the vegetative branches of the plantlet implanted on the nutritional media. The results showed that the irradiation at the dose of 50 grey was significantly increased the rate of germination percent to 100% and the speed of germination by a rate of 5 seeds / day, compared with control group which gave 30% and 1.1 seeds / day respectively. The same treatment had a significant increase on the rate of production of Digoxin, Digitoxin and Gitoxin glycosides to 45.54, 91.87 and 68.70 μg . g⁻¹ respectively, compared to control group values of 6.40, 20.17 and 13.09 μg . g⁻¹ respectively. The same treatment achieved a significant increase in the total chlorophyll and carbohydrate production rate of 2.10 and 3.80 mg g⁻¹ respectively, compared with the control group values of 1.30 and 2.82 mg . g⁻¹ respectively. Also the benzyl adenine at a concentration of 3 mg . L⁻¹ increased the rate of the same glycoside compounds which to 58.57, 79.15 and 78.14 μg . g⁻¹ respectively, compared to the control values of 25.97, 56.02 and 40.90 μg . g⁻¹ respectively. Also, the concentration of total chlorophyll and carbohydrates was 2.74 and 4.39 mg . g⁻¹ respectively, compared to the control group values of 1.70 and 3.31 mg . g⁻¹ respectively. As for the effect of the interference of irradiation treatment at 3 mg . g⁻¹ concentration with adenine on Digoxin and Gitoxin the concentrations were 79.11 and 118.20 μg . g⁻¹, respectively, and the chlorophyll concentration was 3.65 mg . g⁻¹. The irradiation treatment at 4 mg . L⁻¹ achieved the highest concentration of Digitoxin (118.23 μg. g⁻¹) and the highest concentration of carbohydrates of (5.13 mg . g⁻¹).

1. Introduction
The trend that has taken place in the past few decades is to pay attention to wild and cultivated plants and to return to what is known as traditional medicine or herbal medicine. There are several reasons for this trend, the most important of which is that the plants are a laboratory for the production of natural
pharmaceuticals while the manufactured drugs may cause harmful side effects on the health of the patient. Digitalis is considered one of these plants rich in cardiac glycosides. Digitalis genus, which is from the Plantaginaceae family, has more than 20 plant species of perennials, annuals, herbal yearbook and bushes, Lanata is the most important of these species because of its biological effectiveness because it contains many of the glycosides, including Digitoxin, Digoxin, Gitoxin, which is known as cardiac glycosides [1]. Glycosides form an important part of the active substances in medicinal plants and may cover most of the different physiological effects; it is also the second largest group in terms of importance and distribution. After alkaloids, Cardiac glycosides compounds extracted from the *Digitals lanata* plant have been widely used as drugs for the treatment of various heart diseases, because of the high effectiveness of these substances and because the failure of the heart muscle sometimes cannot be controlled by other drugs [2], as well as indirect effects in the heart system, such as pacemaker and increased strength and speed of contraction of the heart muscle, which has a positive effect [3]. Because of the increasing use and the great tendency to use herbs and medicinal materials and the urgent need for them has put them under the threat of eradication and consequent damage to the environment, therefore tissue culture is an indispensable means for the production of plant derived pharmaceutical materials [4 and 5], also, some of these compounds cannot be prepared in laboratories. It is worth mentioning that economically some of these materials cannot be chemically or biologically prepared at industrial levels, therefore, the only solution is to extract them from the plants that contain them but in small quantities [6], Hence, there is necessity of this technique to produce these drugs and pharmaceuticals [7], and the quantity is more as the extraction of vehicles is easier and less expensive for the absence of quantities of tinctures [8 and 9]. Several studies have indicated that the use of catalysts is one of the effective strategies to increase the production of secondary metabolic compounds in plant tissues, including the study conducted by [10], who found a significant increase in the concentration of cardiac cycles and chlorophyll and starch, when cytokinein concentration added to the media growth of the plant Digitalis purpuria was increased. [11] Found an increase in polyphenolic compounds by increasing the cytokinein concentrations added to vegetable branches cultures of *Scutellaria alpine*.

The effect of radiation on plant growth has been known for a long time and most researchers have shown that stimulation usually occurs in the early stages of growth and detection. Many studies have found that the rate of seed germination of some crops increases when exposed to low doses of radiation as a result of the increase in radiation. The speed of cellular cell division [12 and 13] has been supported by [14] that energy is a prerequisite for seed germination, That low doses of gamma rays may increase enzyme activity, which stimulates the level of cell division throughout vegetative growth. A number of researchers also pointed out that the low doses of gamma radiation improve the physiological characteristics of the plant and the production of high amounts of secondary metabolites [15].The purpose of this study is to study the possibility of exploiting the properties of irradiation and Cytokinines and their interaction in increasing the concentration of cardiac glycoside compounds in the vegetal branches cultures of *Digitals lanata in vitro*.

2. Materials and methods

2.1. The seeds of *Digitals lanata* were irradiated with gamma ray (0, 25, and 50) gray in the gama-Cobalt-60 cell after their moisture content was modified to 11%. Irradiated and non irradiated seeds were sterilized with NaOcl solution at 4.5% concentration for 15 minutes and then washed three times in a sterilized distilled water to remove the remains of sterilizing material. The sterilized seeds were planted on the surface of the nutrient media [16] free of growth regulators and by ten replicates per dose of gamma radiation (10 seeds/replicate), as well as a comparison treatment. The seedlings were incubated in the growth chamber at a temperature of 25 ° C ± 2 ° and a light intensity of 1000 lux for 16 hours / day. The study parameters, which included the calculation of the proportion and speed of germination, measurement of glycosides
concentrations and chlorophyll and carbohydrate concentrations from the total number of seedlings after one month of planting, were calculated by using the following equations:

\[
\text{% Germination} = \frac{\text{seed germination number}}{\text{total seed number}} \times 100
\]

\[
\text{Rate of germination} = \frac{\text{Number of seed growing in the first count}}{\text{number of days to the first count}} + \frac{\text{Number of seed growing in the second count}}{\text{number of days to the second count}} + \ldots \ldots \text{etc.} \quad [17]
\]

2.2 In view of the superior results of the treatment of the 50 gray doses, this dose was selected as well as a comparison treatment was used in the implementation of the subsequent experiment. The shoot tip of the branches of 1 cm long obtained from the establishment stage at the age of one month, were planted on MS media at concentrations of (0, 1, 2, 3.4) mg . L\(^{-1}\) of BA and with 0.2 mg . L\(^{-1}\) NAA to stimulate multiplication of vegetation. The cultures were kept in the growth chamber under the same conditions. The results of the study were taken after one month of planting, which included measurement of the concentration of the compounds of the cardiac glycosides, as mentioned [18]. The shoots drying at laboratory temperature, and then one g dry matter from each sample was taken and crushed by ceramic eyeliner and then 40 ml of methanol concentration of 70\% were added, then ultrasonic device was put in the aqueous bath at 100 Hz frequency and at room temperature for 20 minutes, the samples were then filtered with a 0.13 mm filter paper, the process was re-activated and the samples were ready for reading. Cardiac glycosides were assessed by using the mass HPLC method [19]. The concentration of each sample was calculated according to the following equation.

\[
\text{Concentration of the unknown (μg . g}^{-1}) = \frac{\text{(The model package space)}}{\text{(Measurement Pack Area )} \times \text{Measurement concentration} \times \text{Number of dilutions}}.
\]

2.3. The chlorophyll dye was estimated by using the method of [20]. One gram of shoots grown from irradiated seeds with 50 gray as well as the comparison treatment were taken and placed in a ceramic vase and 9 ml of acetone 85\% were added and then the shoots were crushed until colorless residues were obtained. The solution was filtered to a volumetric tube using filter paper and completed to 10 ml by using the same acetone. One ml of solution was then taken and appropriate dilution was performed. The photosynthesis absorption of the solution by using the Spectrophotometer at wave lengths of 663 and 645 were measured and the total chlorophyll content was calculated according to the following equation:

\[
\text{Total chlorophyll mg .l}^{-1} = 20.2D (645) + 8.02D (663).
\]

Carbohydrate concentration was estimated using method [21] called phenol-sulfuric acid method. 0.1 g of powder samples were taken and placed in a test tube and supplemented with 10 ml of ethyl alcohol concentration of 70\%. After good shaking, the tubes were placed in the centrifuge for 10 minutes and at a speed of 1500 rpm. 1 ml of filtrate was taken and put into a test tube and 1 ml of 5\% phenol reagent with 5 ml of 99\% sulfuric acid was added. The mixture was mixed thoroughly and placed in a water bath at 25-30 °C for 20 minutes, then the test tubes were left to cool and the concentration of carbohydrates by measuring the color intensity by the spectral device at the wavelength of 488 nm for the three replicates per treatment and the concentration was calibrated with the standard carbohydrate curve.

2.4. Statistical design: The experiment was carried out using the full randomized design (CRD), global experiments, and 10 replicates, comparing the averages by LSD and probability level 0.05 [22].

3. Results and discussion

3.1. The effect of gamma radiation on the percentage and speed of seed germination
The results of (table 1) and (figure 1) showed significant differences in the germination rate of the *Digitalis lanata* seeds, where the irradiation treatment of the dose 50 gray had achieved the highest germination rate of 100% after 20 days of planting the seed on MS medium at the rate of germination 5 seeds/day, followed by the treatment of 25 gray which achieved a germination rate of 85% after 24 days of planting. With a mean germination rate of 3.5 seeds/day. While the comparison treatment achieved the lowest germination rate of 30% with a speed of 1.1 seeds/day after one month.

**Table 1.** Effect of gamma radiation on the percentage and the speed of germination of the *Digitalis lanata* seeds after one month of planting on MS medium

| Concentration of radiation dose (gray) | Percentage of germination (%) | Speed of germination (seed.day⁻¹) |
|----------------------------------------|-------------------------------|----------------------------------|
| 0                                      | 30                            | 1.1                              |
| 25                                     | 85                            | 3.5                              |
| 50                                     | 100                           | 5                                |
| L.S.D (0.05)                           | 13.44                         | 1.12                             |

**Figure 1.** Effect of gamma radiation in the germination of the *Digitals lanata* seeds after one month of planting (color on line).

3.2. **Effect of gamma radiation on the concentration rate of cardiac glycosides compounds in Digitalis lanata shoots after one month of culturing**

The results in table 2 and figures 2, 3 and 4 showed a clear increase in the concentration of the cardiac glycosides Digoxin, Digitoxin and Gitoxin by increasing the concentration of the irradiation dose. The comparison treatment achieved the lowest concentration of 6.40, 20.17, 13.09 μg . g⁻¹ respectively, followed by the treatment of irradiation 25 gray which gave a concentration rate of 32.08, 80.13, 57.22 μg . g⁻¹ respectively, while the irradiation treatment of 50 gray was significantly higher and gave the highest concentration of cardiac glycosides with 45.54, 91.87 and 68.70 μg . g⁻¹ respectively.
Table 2. Effect of gamma radiation in the concentration of cardiac glycosides compounds (μg / g) Dry weight in *Digitalis lanata* shoots after one month of planting on MS medium.

| Concentration of radiation dose (gray) | Cardiac glycosides compounds |  |
|---------------------------------------|-------------------------------|---|
|                                       | Digoxin | Digitoxin | Gitoxin |
| Retention time of Cardiac glycosides compounds | | | |
| 0          | 2.45    | 3.33      | 4.11    |
| 25         | 6.40    | 20.17     | 13.09   |
| 50         | 32.08   | 80.13     | 57.22   |
| LSD(0.05)  | 2.10    | 2.19      | 2.08    |
3.3. The effect of gamma radiation on the concentration of chlorophyll and carbohydrates in *Digitalis lanata* shoots after one month of planting.

It was noted from the results of Table 3 that the different doses of gamma radiation had a significant effect on the rate of production of chlorophyll and carbohydrates in the shoots. The irradiation treatment 50 gray was significantly higher than the other treatments by giving the highest concentration of 2.10 and 3.80 mg g\(^{-1}\) respectively which recorded the lowest rate of the same compounds of 1.30 and 2.82 mg g\(^{-1}\) respectively.

| Concentration of radiation dose (gray) | Concentration of chlorophyll (mg g\(^{-1}\)) | Concentration of carbohydrates (mg g\(^{-1}\)) |
|---------------------------------------|---------------------------------------------|---------------------------------------------|
| 0                                     | 1.30                                        | 2.82                                        |
| 25                                    | 1.60                                        | 3.35                                        |
| 50                                    | 2.10                                        | 3.80                                        |
| L.S.D(0.05)                           | 0.12                                        | 0.12                                        |

It is clear from the results presented in (Tables 1, 2 and 3) and the (Figures 1, 2, 3 and 4) that the dose of radiation of 50 gray which was found within the levels of low radiation doses has a stimulating effect in the rate of studied traits, which included the rate and speed of seed germination, the rate of production of cardiac cyclic compounds, as well as the rate of chlorophyll and carbohydrate production, and were significantly different from other radiotherapy treatments. Therefore, the study relied on the choice of this dose as well as the comparison treatment to achieve the subsequent stimulation experiment. The cause of the stimulation when exposing the seeds to activated radiation doses may be due to increased efficacy in RNA synthesis and protein obtained in the early stages of germination. Or the cause of stimulation in the speed of seed germination when exposed to low doses of radiation may be due to increase in the speed of cell division of
embryonic cells [23 and 24]. This has supported by [25] that energy is a prerequisite for seed germination, but low doses of gamma ray may increase enzyme activity which stimulates cell division. These results have agreed with the results of [26] when the radiation dose of 50 gray has stimulated germination of *Pisum sativum* seeds by 85% compared with the comparison treatment which achieved a germination rate of 30%, as well as an increase in the efficiency of the process of photosynthesis and the production of metabolic compounds has been attributed to the stimulation and the increase in the amount of internal growth regulators or the increase in the manufacture of exposure to low doses of gamma radiation, The result as found with [27] When the seeds of *Lens culinaris* were exposed to different doses of gamma radiation, the low levels resulted in a significant increase in germination rate and improved appearance and physiological characteristics of the plants.

3.4. Effect of cytokinein and irradiation and their interferon in the production of cardiac glycosides compounds.

The results of (Table 4) and (Figures 5, 6, 7, 8, 9, 10, 11, and 12) showed significant differences in the concentration of the studied cardiac glycosides compounds when different concentrations of cytokinein were added to the MS medium, the concentration of 3 mg .L⁻¹ was significantly higher and gave the highest concentration rat of the cardiac glycoside compounds when Digoxin, Digitoxin, Gitoxin were 58.57, 79.15 and 78.14 μg . g⁻¹ dry weight respectively, The response was then reduced by the increased concentration of cytokinein to the medium, The concentration of 4 mg . L⁻¹ of benzyl adenine gave a concentration rate of cardiac glycosides compounds, and they were 50.60, 75.21 and 66.29 μg . g⁻¹ dry weight, respectively, while the lower comparison ratio for the same compounds were 25.97, 56.02 and 40.90 μg . g⁻¹ dry weight respectively. The irradiation treatment showed a significant increase in the concentration of the cardiac glycosides compounds with levels of 63.64, 102.33 and 93.34 μg . g⁻¹ dry weight, respectively, compared to the comparison treatment which achieved a concentration rate levels of 26.79, 34.64 and 29.56 μg . g⁻¹ dry weight, respectively. As for the effect of interference between irradiation and concentrations of benzyl adenine in the rate of the concentrations of cardiac glycoside compounds, the irradiation treatment at 3 mg. L⁻¹ concentration gave a significant increase in the concentration of the Digoxin and the Gitoxin concentrations wit levels of 79.11 and 118.20 μg . g⁻¹ dry weight respectively, and the concentration of 4 mg.L⁻¹ gave the highest concentration of Digitoxin with a level of 118.23 μg . g⁻¹ of dry weight. While the irradiation treatment in free medium of benzyl adenine gave the lowest rate of the cardiac glycoside compounds Digoxin, Digitoxin and Gitoxin with levels of 45.54, 91.87 and 68.70 μg . g⁻¹ dry weight, respectively. As for the interaction effect between non-irradiated treatment and benzyl adenine concentrations in the concentration rate of the cardiac glycoside compounds, the concentration of 3 mg . L⁻¹ was the highest concentration of Digoxin concentration was 38.03 μg . g⁻¹ dry weight, while the highest rate of Digitoxin and Gitoxin at 2 mg . L⁻¹ was 50.71 and 40.31 μg . g⁻¹ dry weight respectively, while the comparison treatment gave the lowest rate of the cardiac glycoside compounds Digoxin, Digitoxin and Gitoxin at levels of 6.40, 20.17 and 13.09 μg . g⁻¹ respectively.
Table 4. Effect of gamma radiation and benzyl adenine and there interaction in the concentration of cardiac glycosides compounds (μg. g⁻¹) Dry weight in Digitalis lanata shoots after one month of planting on MS medium.

| Concentration of BA (mg.l⁻¹) | Cardiac glycoside compounds(μg. g⁻¹)dry weight | Retention time of Cardiac glycosides compounds | LSD(0.05) |
|------------------------------|-----------------------------------------------|-----------------------------------------------|-----------|
|                              | Digoxin | Digitoxin | Gitoxin | 2.45 | 3.33 | 4.11 | 1.18 | 1.26 | 1.20 | 1.70 | 1.73 | 1.72 |
| 0                            | 25.97   | 56.02     | 40.90   | 24.22 | 46.75 | 51.97 | 1.70 | 1.73 | 1.72 |
| 1                            | 38.18   | 55.08     | 52.68   | 25.97 | 56.02 | 40.90 | 24.22 | 46.75 | 51.97 | 1.70 | 1.73 | 1.72 |
| 2                            | 52.76   | 76.99     | 69.25   | 37.06 | 63.70 | 47.01 | 24.22 | 46.75 | 51.97 | 1.70 | 1.73 | 1.72 |
| 3                            | 58.57   | 79.15     | 78.14   | 40.90 | 56.02 | 40.90 | 24.22 | 46.75 | 51.97 | 1.70 | 1.73 | 1.72 |
| 4                            | 50.59   | 75.21     | 66.29   | 32.18 | 36.27 | 32.18 | 1.70 | 1.73 | 1.72 |
| Irradiation treatment        |         |           |         |       |       |       |      |      |      |
| Irradiation (50)gray         | 63.64   | 102.33    | 93.34   | 1.70 | 1.73 | 1.72 |
| Non- irradiation             | 26.79   | 34.64     | 29.56   | 1.70 | 1.73 | 1.72 |
| LSD(0.05)                    | 0.73    | 1.20      | 0.89    | 1.70 | 1.73 | 1.72 |
| Irradiation interaction      |         |           |         |       |       |       |      |      |      |
| 0- Irradiation               | 45.54   | 91.87     | 68.70   | 1.70 | 1.73 | 1.72 |
| 1- Irradiation               | 58.13   | 83.12     | 85.30   | 1.70 | 1.73 | 1.72 |
| 2- Irradiation               | 70.32   | 103.27    | 98.18   | 1.70 | 1.73 | 1.72 |
| 3- Irradiation               | 79.11   | 115.18    | 118.20  | 1.70 | 1.73 | 1.72 |
| 4- Irradiation               | 65.08   | 118.23    | 96.31   | 1.70 | 1.73 | 1.72 |
| 0- non- irradiation          | 6.40    | 20.17     | 13.09   | 1.70 | 1.73 | 1.72 |
| 1- non irradiation           | 18.23   | 27.03     | 20.07   | 1.70 | 1.73 | 1.72 |
| 2- non irradiation           | 35.20   | 50.71     | 40.31   | 1.70 | 1.73 | 1.72 |
| 3- non irradiation           | 38.03   | 43.11     | 38.08   | 1.70 | 1.73 | 1.72 |
| 4- non irradiation           | 36.11   | 32.18     | 36.27   | 1.70 | 1.73 | 1.72 |
Figure 5. Effect of BA 1 (mg L\(^{-1}\)) and gamma ray (0) gray in cardiac glycosides production

Figure 6. Effect of BA 2 (mg L\(^{-1}\)) and gamma ray (0) gray In cardiac glycosides production
Figure 7. Effect of BA 3 (mg L\(^{-1}\)) and gamma ray (0) gray in cardiac glycosides production

Figure 8. Effect of BA 4 (mg L\(^{-1}\)) and gamma ray (0) gray in cardiac glycosides production

Figure 9. Effect of BA 1 (mg L\(^{-1}\)) and gamma ray (50) gray in cardiac glycosides production

Figure 10. Effect of BA 2 (mg L\(^{-1}\)) and gamma ray (50) gray in cardiac glycosides production
3.5. Effect of benzyl adenine and irradiation and their interaction in the concentration of chlorophyll and carbohydrates

The results of Table 5 indicate that the addition of benzyl adenine and its different concentrations had significantly affected on the production rate of chlorophyll and carbohydrates. The concentration of 3 mg . L\(^{-1}\) exceeded and gave the highest rate of chlorophyll of 2.74 mg . g\(^{-1}\) compared to the comparison treatment which gave the lowest rate of 1.70 mg . L\(^{-1}\), as for carbohydrates, the highest concentration was achieved at a dose of 3 mg . g\(^{-1}\) of 4.39 mg . g\(^{-1}\), which was not significantly different from the concentration of 4 mg . L\(^{-1}\), which achieved a concentration rate of 4.37 mg . g\(^{-1}\), while the comparison treatment achieved the lowest rate of 3.31 mg . g\(^{-1}\), as for the irradiation effect. The same table data showed that the irradiation ratio was significantly higher in the chlorophyll and carbohydrate concentrations, which was 2.85 and 4.70 mg . g\(^{-1}\) respectively, compared with irradiated treatment, which gave a rate of 1.65 and 3.41 mg . g\(^{-1}\) respectively. As to the effect of interference, the irradiation treatment at 3 mg / L was significantly higher and gave the highest concentration of chlorophyll value of 3.65 mg . g\(^{-1}\). In addition, the treatment of overlap with the concentration of 2 mg . L\(^{-1}\) BA gave the highest rate of carbohydrates with a value of 5.13 mg . g\(^{-1}\) which did not differ significantly from the treatment of interference with the concentration of 3 mg . L\(^{-1}\), which achieved a concentration rate of 5.08 mg . g\(^{-1}\), compared to the lowest rates recorded for chlorophyll and carbohydrate concentrations in the comparison treatment with values of 1.30 and 2.82 mg . g\(^{-1}\), respectively.
Table 5. Effect of benzyl adenine and irradiation and their interaction in the concentration of chlorophyll and carbohydrates in the shoot of *Digitalis lanata* after four weeks of planting on MS medium.

| Concentration of BA (mg L⁻¹) | Chlorophyll concentration rate (mg L⁻¹) | Carbohydrate concentration rate (mg L⁻¹) |
|-----------------------------|---------------------------------------|----------------------------------------|
| 0                          | 1.70                                  | 3.31                                   |
| 1                          | 2.00                                  | 3.94                                   |
| 2                          | 2.51                                  | 4.28                                   |
| 3                          | 2.74                                  | 4.39                                   |
| 4                          | 2.29                                  | 4.37                                   |
| L.S.D(0.05)                | 0.10                                  | 0.10                                   |

Irradiation treatment

| Irradiation (50)gray | 2.85 | 4.70 |
| Non-irradiation      | 1.65 | 3.41 |
| LSD(0.05)             | 0.08 | 0.07 |

Interaction

| 0- Irradiation       | 2.10 | 3.80 |
| 1- Irradiation       | 2.40 | 4.68 |
| 2- Irradiation       | 3.30 | 5.13 |
| 3- Irradiation       | 3.65 | 5.08 |
| 4- irradiation       | 2.78 | 4.81 |
| 0- non-irradiation   | 1.30 | 2.82 |
| 1- non irradiation   | 1.60 | 3.20 |
| 2- non irradiation   | 1.71 | 3.42 |
| 3- non irradiation   | 1.83 | 3.70 |
| 4- non irradiation   | 1.80 | 3.92 |
| LSD(0.05)             | 0.14 | 0.13 |

From above, the results in (Table 4) and (Figures 5, 6, 7, 8, 9, 10, 11, and 12) showed that the addition of BA to the MS medium with different concentrations resulted in a significant increase in the mean concentrations of cardiac glycoside compounds, the result may be due to its role in the process of cell division and increase in size and differentiation, which has a role in the various processes of growth and development and the result will be more progress in the conduct of various metabolic processes primary and secondary, including the formation of cardiac glycoside compounds [28]. This is also confirmed by the study of [29 and 6] that the differentiation at the level of the cell and the formation of the organs is important in the formation of therapeutic compounds, so it was observed in this experiment that the presence of BA in the medium led to a significant increase in the amount of cardiac glycoside compounds compared with the comparison treatment. As for the other properties, total chlorophyll content and carbohydrates, the results shown in (Table 5) showed a significant increase in total chlorophyll concentration by increasing the concentrations of BA added to the medium, the reason may be attributed to the role of cytokines known in the formation of chloroplast, which has a role in the composition of Crane, Stoma lamella and chlorophyll [30 and 31]. Thus, the degree of progress in the differentiation of chloroplast was one of the reasons that led to an increase in the amount of cardiac glycoside compounds. The table also showed the positive role of BA in the concentration of carbohydrates measured in the shoots of digitalis plant, which is an important and very important factor in the formation of cardiac glycosides, as the source of energy and carbon necessary
to form the large carbon structure of these compounds. So it was noted that the presence of BA in the medium had a positive role in the proportion of metabolic compounds mentioned, where the explants with the presence of a strong catalyst for growth and development will draw the components of the medium to take advantage of them in various growth and development, in other words, the cultivated part of the plant will become the drainage area of the nutrient medium [32]. The effect of overlapping between the components of the medium and the added BA will be a reflection on the various growth and development processes that will have an effect on the degree of differentiation of the cell, the tissue and the formation of the organs and its natural reflection on the process of primary and secondary metabolic processes, such as carbohydrate formation and cardiac glycoside compounds. This result agreed with the conclusion reached by [33] when BA was used in the medium for the shoot culture of the *Hypericum hirsutum* plant as a catalyst that encouraged the accumulation of Hypericin and Pseudohypercin by four times compared with the comparison treatment, as well as with the study carried out by [34], when the concentration of BA and cholesterol added to the medium of the shoot culture of *Digitalis purpurea* increased the production of Digitoxin compared with the treatment of comparison. The result has shown that interference has stimulated the regulation of genes directly responsible for metabolizing secondary compounds, as well as with[35] when adding different concentrations of BA to the plant branches medium of the plant *Mentha piperita* led to a significant increase was 35% of the composition of Menthol compared to the comparison treatment, and with [36] when the adding of BA to the shoot culture medium of the *Digitalis lanata*, stimulated the accumulation of Digitoxin, Digoxin and Gitoxin glycosides to levels of 89.5, 73.5 and 57.8 μg / g respectively compared to the comparison treatment, and with [37] when BA stimulated the production of secondary metabolites in the vegetative branches culture of *Stevia rebaudiana*, and the result was a significant increase by about 50% in the production of phenols and flavonoids compared with the comparison treatment. The data of the same tables and forms indicated that irradiation had an effective role in stimulating the increase in the rate of studied traits, which included the concentration rate of cardiac glycoside compounds and the concentration of chlorophyll and carbohydrates. There were significant differences in the concentration of these compounds in the treatment of irradiation compared with non irradiated treatment, the reason may be that radiation has stimulant, inhibitory and deadly effects. The effect type is related to the dose at which the plant is exposed. The low dose stimulated the growth of most plants [38], the cause of the stimulus may be due to the fact that gamma ray photons have enough energy to increase the elasticity or break of the chemical bonds of the material, thereby causing photochemical reaction and thus obtaining biologic effects [26], or activation may be due to the direct effect of radiation in the genetic material of the nucleus, based on the many cases that indicate the transmission of the activation effect of radiation over generations, as well as the low doses of radiation lead to the removal of some enzymes inhibiting certain life processes in the plant [39]. Another thought is that the activation of radiation causes a change in the physiological properties of cytoplasm, which causes an increase in physiological processes and life interactions. It is supported by[40] that the low doses of gamma radiation improve the physiological characteristics of plants and produce high quantities of secondary metabolites, this is in line with [41] that the low doses of gamma ray were the best in the accumulation of total phenols, total flavonoids, anti-oxidant defense, and amino acids in callus of *Rosmarinus officinalis*, and with [42] When irradiating *Coriandrum sativum* seeds with a different dose of gamma radiation, the low doses of gamma ray was the best in stimulating the production of plant hormones, amino acids and aromatic oils in the plant. And with [43] showed that low doses of gamma ray gave the best results in terms of rate and speed of *Terminalia arjuna* seed germination, as well as increased plant growth and metabolites such as proline and phenolic compounds and their chlorophyll content.
4. Conclusion
The uses of gamma rays and BA can stimulate the increase in the production of cardiac glycosides compounds, as well as chlorophyll and carbohydrates in the leaves of digitals, therefore, this technology can be adopted in increasing these compounds, which have an important role in medicinal drugs for the treatment of heart disease.

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